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Leptosphaeria spp., phoma stem canker and potential spread of *L. maculans* on oilseed rape crops in China

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In China, the incidence of phoma stem canker observed in pre-harvest surveys from 2005 to 2012 was greater on winter oilseed rape in provinces in central China (in May) than on spring oilseed rape in north China (in August). In all 742 cases when the causal pathogen was isolated from stem cankers, it was identified as *Leptosphaeria biglobosa* by morphology in culture and/or by species-specific polymerase chain reaction. Both *L. biglobosa* and *Leptosphaeria maculans* were detected on crop debris and seed in shipments of oilseed rape seed imported into China through Shanghai or Wuhan ports in 2009–2011. Descriptions of the observed spread of *L. maculans* into areas previously colonized by *L. biglobosa* across a spring oilseed rape growing region (Alberta, Canada, westwards, 1984–1998) and across a winter oilseed rape growing region (Poland, eastwards, 1984–2004) were used to estimate the potential westward spread of *L. maculans* in China across spring oilseed rape growing regions (north China) and winter oilseed rape growing regions (central China, generally provinces along the Yangtze River), respectively. The rates of spread were estimated as 47 km per year across spring oilseed rape in north China and 70 km per year across winter oilseed rape in central China. Dispersal modelling suggested that the rate of spread of *L. maculans* across Alberta, Canada (*c.* 17 km per year) could be explained by windborne dispersal of ascospores.

Keywords: ascospore dispersal modelling, epidemiology, food security, invasive species, plant quarantine, spatial disease spread

Introduction

Phoma stem canker (blackleg), caused by *Leptosphaeria* species, is a serious disease of oilseed rape (*Brassica napus*, canola) that produces considerable worldwide losses, worth more than £1000 million per cropping season at a price of £400 per tonne (Barnes *et al.*, 2010). Severe epidemics occur on both winter (autumn-sown) and spring crops of oilseed rape in many areas of the world, including Europe, Australia and North America (Fitt *et al.*, 2006a). The severity of epidemics differs from cropping season to season, from region to region and from crop to crop. The pathogen populations frequently comprise two coexisting, closely related species, *Leptosphaeria maculans* and *Leptosphaeria biglobosa*: *L. biglobosa* is generally less damaging in these regions, where it is often associated with upper stem lesions (Jedryczka *et al.*, 1999; West *et al.*, 2002), whereas *L. maculans* is more damaging and often associated with stem base cankers (West *et al.*, 2002). Both *L. maculans* and *L. biglobosa* are often spread from affected stem debris of previous oilseed rape crops onto new crops by airborne ascospores (Fig. 1a) produced in pseudothecia (Fig. 1b) on the debris (Fig. 1c). These pathogens infect the leaves of new crops through stomata by means of hyphae produced from the germinating ascospores (in autumn on winter oilseed rape crops in Europe; Toscano-Underwood *et al.*, 2001). From the resulting phoma leaf spots, the pathogens then grow along the leaf petioles to the stems where stem base cankers or upper stem lesions develop several months later (in spring/summer on winter oilseed rape crops in Europe). Such airborne ascospores generally pose a risk to new crops sown near previous oilseed rape crops (Marcroft *et al.*, 2004; Huang *et al.*, 2005). Dispersal over longer distances may occur through transport of infected crop debris, which is a potent source of *Lep-*

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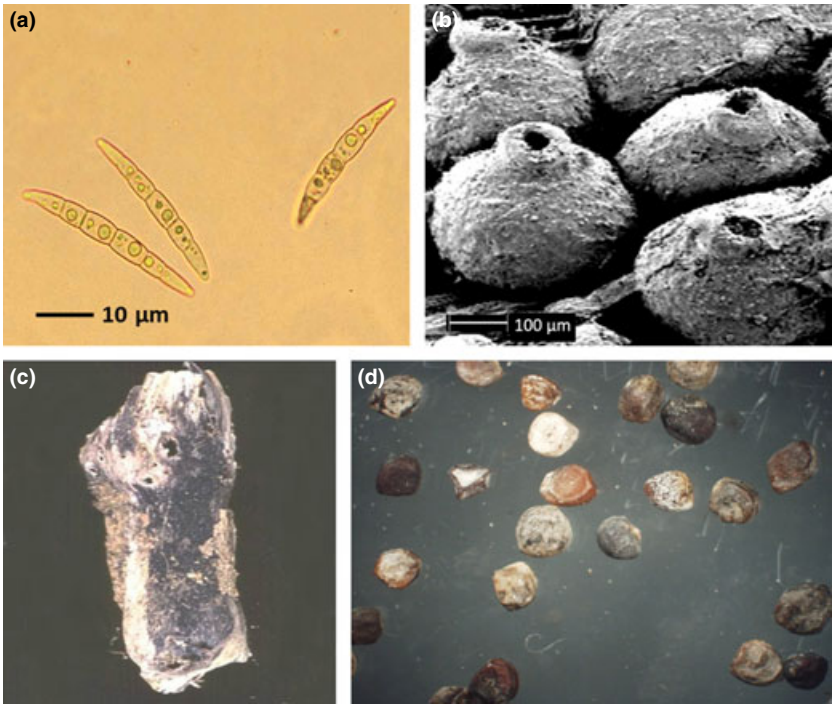

Figure 1 Airborne ascospores of *Leptosphaeria maculans* or *Leptosphaeria biglobosa* (a) produced in pseudothecia (b) on the stem debris (c) after harvest of oilseed rape crops affected by phoma stem canker and the abnormal, wrinkled, discoloured oilseed rape seed (d) infected by *L. maculans* or *L. biglobosa*.

tosphaeria ascospore inoculum (Fitt *et al.*, 2008). Both pathogens are seedborne (Fig. 1d; Chigogora & Hall, 1995; Chen *et al.*, 2010) but long-distance transport of infected seed poses a much smaller risk of spreading the pathogens than transport of infected crop debris because there is little evidence that these pathogens are effectively transmitted from infected seeds to seedlings.

In China, phoma stem canker on oilseed rape (Fig. 2a, b,c) has not generally been a serious problem and only the less damaging *L. biglobosa* has been isolated from diseased crops previously (Mendes-Pereira *et al.*, 2003; Fitt *et al.*, 2006a; Hao *et al.*, 2012). However, it is possible that the more damaging *L. maculans* may spread to China because it has been spreading into areas of the world where only *L. biglobosa* had been present, such as Canada or Poland (Liu, 2007; Fitt *et al.*, 2008). Furthermore, the climatic and agronomic conditions in China may be favourable for *L. maculans* because these two related *Leptosphaeria* species occupy similar ecological niches (West *et al.*, 2002; Fitt *et al.*, 2006b). Moreover, some Chinese oilseed rape cultivars have been very susceptible to *L. maculans* when grown as crops in countries where *L. maculans* is widespread such as the UK (e.g. Ningyou 7 in 2006; Fitt *et al.*, 2008), France (36 Chinese cultivars, 1999; Baocheng Hu, Anhui Academy of Agricultural Sciences, Hefei, China, personal communication), Poland (four Chinese cultivars, 2003; M. Jedryczka, unpublished data) or Australia (15 out of 20 Chinese *B. napus* lines, 2005, 2006; Li *et al.*, 2008) or tested against *L. maculans* in controlled environment experiments (28 out of 30 Chinese cultivars; Qiang-Sheng Li, Anhui Academy of Agricultural Sciences, Hefei, China and Jon West, Rothamsted Research, Harpenden, UK, personal communications). However, some recent Chinese cultivars have shown some resistance in field experiments in Canada (2012; Dilantha Fernando, University of Manitoba, Canada, personal communication).

Publication of a paper that discussed strategies to prevent spread of *L. maculans* into China (Fitt *et al.*, 2008) contributed to concerns in China that serious economic consequences could result if *L. maculans* was to become established in China. It was considered that imported seed might pose a risk because China had begun to import oilseed rape seed for crushing for cooking oil in 1994 and has been importing an average of 1·25 Mt of seed from Canada, Australia or the Ukraine since 1998. These imports were generally through the ports of Lianyungang, Nanjing, Nantong, Shanghai, Shenzhen, Taizhou, Tianjin, Wuhan, Zhangjiagang and Zhoushan (Fig. 3), several of which are situated in areas with intensive oilseed rape cultivation. For example, China imported 2·9 Mt of seed and 0·4 Mt of oil from Canada in the year from August 2008 (Fig. 4) and imported 0·4 Mt of oilseed rape seed from Australia in June 2009. Import into China of oilseed rape meal remaining after extraction of the oil, used for feeding livestock, from Canada began in the year from August 2009 (0·43 Mt).

Officials from the Chinese General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ) did a Pest Risk Analysis (PRA) for *L. maculans* (http://www.fas.usda.gov/info/factsheets/reports.asp). After the PRA assessment, in November 2009, China announced a transitional period restricting import of oilseed rape seed from countries where *L. maculans* is present until further safeguards were put in place to protect the oilseed rape

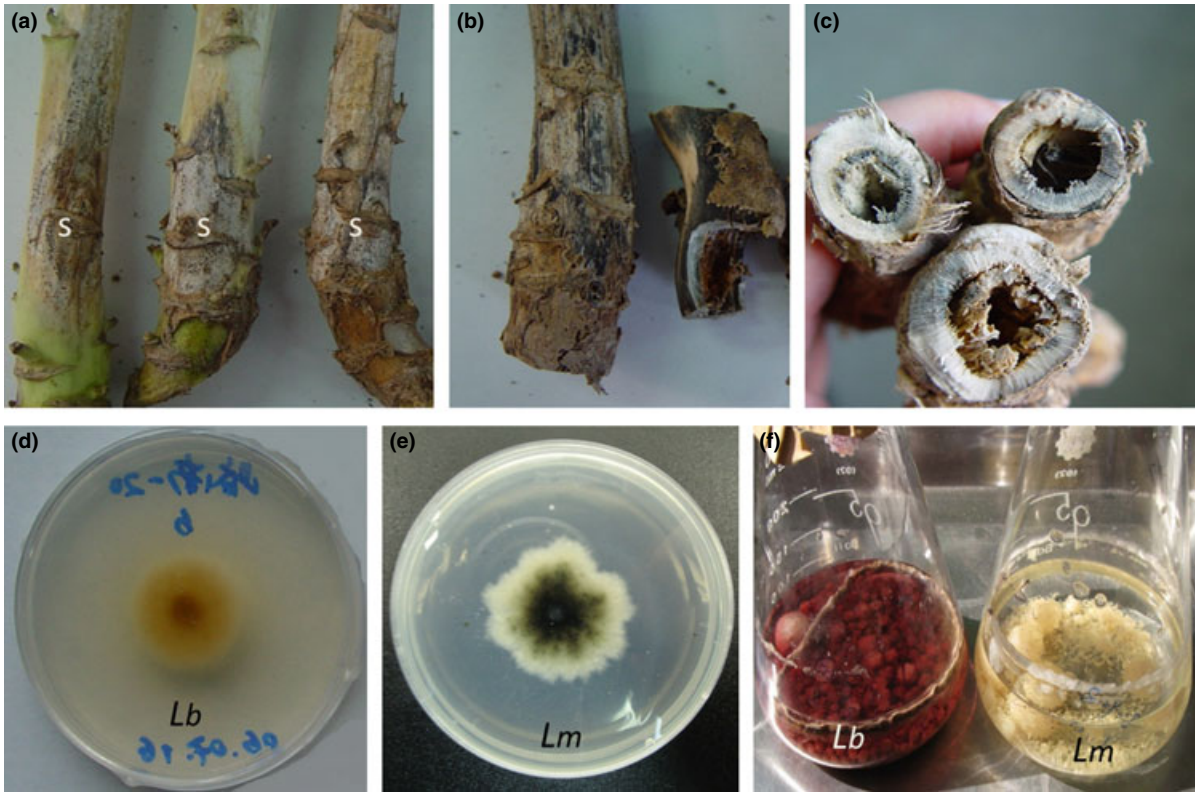

Figure 2 Phoma stem canker on oilseed rape crops in China and identification of causal *Leptosphaeria* species. External canker symptoms (s) observed on upper stems (a) and stem bases (b) of winter oilseed rape sampled before harvest (black dots are pycnidia of *Leptosphaeria biglobosa*) with cross-sections showing internal necrosis (c). Tests on potato dextrose agar, showing *L. biglobosa* (*Lb*) (d) but not *Leptosphaeria maculans* (*Lm*) (e) producing pigment, and in Czapek–Dox broth (f), showing *L. biglobosa* but not *L. maculans* producing pigment, done by the Chinese quarantine administration, were used to confirm the presence of *L. maculans* or *L. biglobosa* in seed imported from Canada, Australia or the Ukraine.

crop in China from *L. maculans* (http://www.canola-council.org/). This led to a decrease (to 0·9 Mt) in imports of oilseed rape seed into China from Canada in the year 2010/2011, although imports of oil and meal were unaffected (Fig. 4), and to a cessation of imports of seed from Australia (Trent Potter, Yeruga Crop Research, Naracoorte, Australia, personal communication). However, as a result of an interim trade agreement with Canada that was extended in June 2010 (Clinton Jurke, Canola Council of Canada, Winnipeg, Canada, personal communication), imports were allowed through ports in areas where little oilseed rape is grown, such as Zhanjiang in Guangdong province, Xiamen in Fujian province and Fangchenggang in Guangxi province (Fig. 3) for immediate crushing near to the port. Subsequently, imports of seed from Canada returned to 2·5 Mt in the year 2011/2012. A similar agreement was reached with Australia in 2012 and imports of seed from Australia into China were more than 0·5 Mt in the year 2012/2013 (Steve Marcroft, Marcroft Grains Pathology, Horsham, Australia, personal communication).

Although the pathogen causing phoma stem canker in China since 2000 has been considered to be *L. biglobosa* (then known as B-type *L. maculans*; West *et al.*, 2000; Mendes-Pereira *et al.*, 2003; Hao *et al.*, 2012), there has been no systematic disease survey of Chinese winter oilseed rape crops (*c.* 7 million hectares, mostly in provinces along the Yangtze River) or spring oilseed rape crops (*c.* 2 million hectares in north China; Fig. 3) to determine if *L. maculans* is also present. Whilst *L. maculans* has been detected on oilseed rape seed imported into China (Chen *et al.*, 2010), no survey has been made of the incidence of infection by *L. maculans* or *L. biglobosa* on the oilseed rape seed being imported into Chinese ports. Furthermore, Fitt *et al.* (2008) applied a model describing the spread of *L. maculans* across spring oilseed rape in Canada (estimated rate of spread 0·152 degrees (i.e. 17 km) per year) to winter oilseed rape in China, but the rate of spread of *L. maculans* through winter oilseed rape growing regions in China might differ from the rate of spread through spring oilseed rape growing regions in Canada. This paper reports results of surveys of pathogens associated with phoma stem canker in China over the period 2005–2012, estimates of infection by *L. maculans* and *L. biglobosa* on seed imported into China and application to China of descriptions of the spread of *L. maculans* through spring oilseed rape growing regions (Canada, applied to north China) and winter oilseed rape growing regions (Poland, applied to central China).

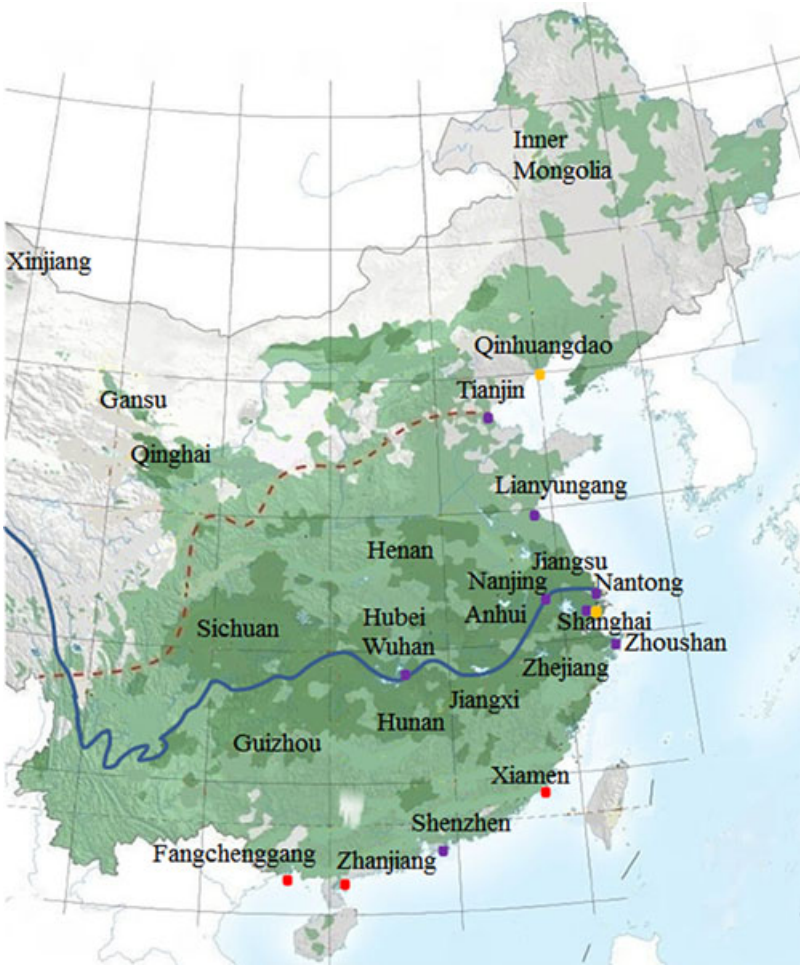

Figure 3 Distribution of oilseed rape cropping in China. Arable land on the Chinese mainland where there is widespread cultivation of oilseed rape (the green area, including winter oilseed rape to the south and spring oilseed rape to the north of the red dotted line; the dark green shows the areas intensely cropped with oilseed rape). The blue line shows the Yangtze River. This map shows locations of provinces where phoma stem canker was observed on oilseed rape crops. It also shows locations of ports (Lianyungang, Nanjing, Nantong, Shanghai, Shenzhen, Taizhou, Tianjin, Wuhan, Zhangjiagang and Zhoushan, shown by the purple dots, where Taizhou and Zhangjiaguang are close to Nantong) through which oilseed rape seed was imported into China until November 2009 and ports (Shanghai and Qinhuangdao, shown by the yellow dots) which were assumed to be the points of the entry into China for *Leptosphaeria maculans* when projecting the potential spread of the pathogen through central or northern regions of China growing winter oilseed rape or spring oilseed rape, respectively. It also shows the ports (Fangchenggang, Xiamen and Zhanjiang, shown by the red dots) through which most oilseed rape seed is now imported into China.

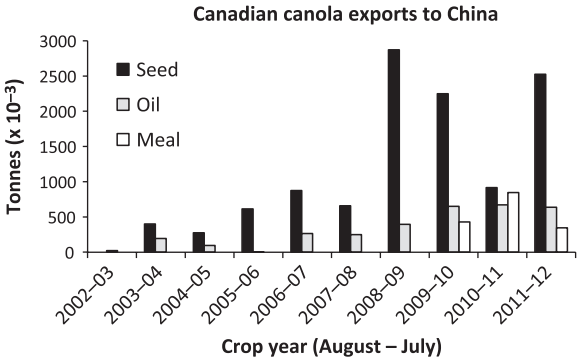

Figure 4 Annual imports into China from Canada of oilseed rape seed (black), oil (grey) or meal (white) for the years (August to July) from 2002–2003 to 2011–2012. Data were supplied by the Canola Council of Canada and obtained from the Statistics Canada international trade database (http://www.statcan.gc.ca/bsolc/olc-cel/olc-cel?lang = eng&catno = 65F0013X). Imports in the period 2002–2009 were generally through Lianyungang, Nanjing, Nantong, Shanghai, Shenzhen, Taizhou, Tianjin, Wuhan, Zhangjiagang and Zhoushan, whereas after November 2009 they were generally through Fangchenggang, Xiamen and Zhanjiang (Fig. 3).

Materials and methods

Survey of phoma stem canker on oilseed rape crops in China, 2005–2012

Surveys of pathogens associated with phoma stem canker in China have been done since 2005 in provinces with oilseed rape crops. Recently, the survey structure has included date of sampling, site (latitude, longitude), type of oilseed rape (winter or spring), number of crops assessed, total number of plants sampled and number of plants with phoma stem canker symptoms. When phoma stem canker symptoms were observed in crops, a small number of the stems sampled were taken to the laboratory for pathogen isolation and identification. Diseased stem tissues were surface-sterilized to obtain pathogen isolates using the method described by West *et al.* (2002). These isolates were characterized as *L. maculans* or *L. biglobosa*, initially by colony morphology/pigment production on potato dextrose agar (PDA) medium (West *et al.*, 2002), then by species-specific polymerase chain reaction (PCR) (Liu *et al.*, 2006).

Winter oilseed rape, central China

In central China, winter oilseed rape is generally sown in October and harvested the following May. Before harvest, in May 2005, 2006 and 2008, specific disease surveys were done on winter oil-

seed rape crops in Anhui, Hubei and Guizhou provinces (Fig. 3). The surveys were initially done by searching for plants with phoma stem canker symptoms in different crops at different locations in each province. Once diseased plants were observed, they were uprooted and the stems were taken for pathogen identification in the laboratory. In May 2012, more systematic surveys were done in some provinces along the Yangtze River, including Sichuan, Jiangsu, Shanghai and Hubei (Fig. 3). In Sichuan, Jiangsu and Shanghai provinces, the survey was done in two crops in two districts in each province. In each crop, 30 plants were sampled from each site in a 'W' route (i.e. five sampling sites in each crop). In total, 150 plants per crop were sampled from each location. In Hubei province, the survey was done in 17 crops in seven districts. In each crop, 100 plants were sampled from each site in a 'W' route (i.e. five sampling sites in each crop). In total, 500 plants were sampled from each crop. The distance between two districts sampled was *c.* 10 km and the distance between two crops sampled was more than 1 km. Each stem was assessed externally for upper stem lesions (Fig. 2a) and stem base cankers (Fig. 2b) and some were cut to examine internal necrosis (Fig. 2c) and then used for identification of the pathogen by isolation, morphological identification and species-specific PCR.

In addition, a large-scale survey was done by the Anhui Academy of Agricultural Sciences during the period 2008–2012, with samples collected in May before harvest from winter oilseed rape crops in 49 districts across 11 provinces (Anhui, Guizhou, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shanghai, Shanxi, Sichuan, Zhejiang; Li *et al.*, 2013). Some of the stems with phoma stem canker symptoms were brought to the laboratory for pathogen identification. Small pieces of stem tissues were sampled from stems with canker symptoms and then divided into two sets. One set was used for pathogen isolation and identification by morphology/pigment observation and PCR and the other set was used for direct DNA extraction and identification by PCR without isolation.

Spring oilseed rape, north China

In north China, where winter oilseed rape cannot survive the cold winter, spring oilseed rape is usually sown in April and harvested in September. In Inner Mongolia, north China (Fig. 3), the main spring oilseed rape growing area, surveys for phoma stem canker started in August 2005 and initially involved searching for plants with phoma stem canker symptoms at the Hailar farm (49·2°N, 119·7°E). Stems showing phoma stem canker symptoms were sampled before harvest for identification of the causal pathogens in the laboratory. In August 2009 (two crops), 2010 (two crops), 2011 (three crops) and 2012 (12 crops), more detailed surveys were also done at the Labudalin farm (49·2°N, 119·8°E), Sanhe farm (50·3°N, 120·3°E) and Yakeshi farm (49·3°N, 120·7°E). In 2009, 2010, 2011 and 2012, 20 plants were sampled from each site, with five sites in each crop in a 'W' route (i.e. 100 plants were sampled from each crop). The distance between two crops sampled was about 2 km. The stems were assessed for external symptoms of phoma stem canker and some were then used for identification of the pathogen. In addition, during the period 2008–2012 in August, stem canker surveys were done by the Anhui Academy of Agricultural Sciences on spring oilseed rape crops in 11 districts across another five provinces (Gansu, Inner Mongolia, Qinghai, Tibet and Xinjiang; Li *et al.*, 2013).

Disease severity testing

Nineteen *L. biglobosa* isolates obtained from winter oilseed rape and three *L. biglobosa* isolates obtained from spring oilseed rape, whose identities had been confirmed by both morphology/pigment production and PCR, were chosen for disease severity testing (Li *et al.*, 2013). To investigate differences between regions and cultivars in the aggressiveness of these isolates, cotyledons of one Chinese cultivar (Zhoungyou 821) and three European cultivars (Hearty, Bristol and Courage) were inoculated with conidial suspensions (10^7 conidia mL^{-1}) of these 22 isolates using standard cotyledon test methods and lesions were scored on a 0–9 scale (Yu *et al.*, 2008). One Polish *L. biglobosa* isolate (PL-Lb) was used as a control.

Survey for presence of *L. biglobosa*/*L. maculans* on oilseed rape seed cargoes imported into China

Oilseed rape seed ship cargoes imported into China were tested for presence of *L. biglobosa* and *L. maculans*, using both cultural and molecular methods, by the Entry–Exit Inspection and Quarantine Bureau in the ports of Shanghai and Wuhan (on the Yangtze River; Fig. 3). The tests were done on seeds imported from Australia, the Ukraine or Canada in 2009 at Shanghai port and on seeds imported from Canada in 2010 and 2011 at Wuhan port. Researchers from the Quarantine Bureau sampled imported seed cargoes at Shanghai port (Zhou *et al.*, 2010, 2011; Yi *et al.*, 2010). Seed cargoes were sampled randomly from several places and the samples were mixed, to give a final sample of 2–3 kg seed. The seed samples were cleaned, first using a 2·5-mm pore diameter sieve to collect pieces of stem and pod debris ('dockage') for testing and then using a 1·5-mm pore diameter sieve to separate out small and broken seeds. From the cleaned seeds, the abnormal, wrinkled and discoloured seeds were selected (Yi *et al.*, 2010; Zhou *et al.*, 2011). A subsample of 1 g of the selected seeds was used for DNA extraction and PCR detection (Yi *et al.*, 2010; Zhou *et al.*, 2010) with the primers described by Liu *et al.* (2006). If this subsample was positive for *L. maculans* by PCR, another subsample (500–1000 seeds) from the selected seeds was used for pathogen isolation. To obtain *Leptosphaeria* isolates, the selected seeds were washed with tap water then soaked in sterile distilled water for 16 h, then placed at –20°C for 1 day. The seeds were then surface-sterilized with sodium hypochlorite solution (NaOCl; 1% available chlorine) for 5 min and subsequently rinsed in sterile distilled water three times.

The surface-sterilized seeds were placed on PDA plates amended with 40 units mL^{-1} streptomycin. The plates were incubated at 20°C in darkness for 5 days. Plates with fungal growth were selected and small pieces of agar with mycelium were transferred to new PDA plates for identification by pigment and spore production (West *et al.*, 2002). To promote spore production, the new plates were incubated at 20°C with alternating 12 h light/12 h darkness for 10–14 days. Pycnidia with pinkish, creamy cirrhi (composed of conidia) were selected and slides were made to examine the morphology of the conidia under a microscope. Isolates showing similar morphological characters to reference *L. maculans* isolate 8129-5 (obtained and identified by Shanghai Quarantine Bureau from seed imported into China from Australia) or *L. biglobosa* isolate Gs 5-5 (obtained from Anhui Academy of Agricultural Sciences) were subcultured to obtain mycelium for DNA extraction and PCR using the species-specific primers described by Liu *et al.* (2006). Some isolates confirmed as *L. maculans* or *L. biglobosa* were subcultured for conidial production and pathogenicity testing using a cotyledon inoculation method (Balesdent *et al.*, 2001). To confirm the identification of *L. maculans* from imported oilseed rape seeds, the ITS region of some isolates was

amplified and sequenced, then compared with that of available *L. maculans* isolates in GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

The incidence (%) of seed infected by *L. maculans* was also estimated. From each 2–3 kg sample from a ship cargo, a random sample of 300 g of oilseed rape seed (*c.* 90 000 seeds) was taken. Then 1000 seeds were selected randomly from this sample for pathogen isolation and identification. These seeds were surface-sterilized with sodium hypochlorite (NaOCl, 1% available chlorine) before placing them on streptomycin-amended PDA plates. The incidence (%) of seed infected by *L. maculans* was estimated from the number of seed infected by *L. maculans* divided by the total number of seeds sampled.

Researchers from the Hubei Quarantine Bureau sampled imported seed cargoes at Wuhan port in 2010 and 2011 (Wang *et al.*, 2010, 2011). A total of 2–5 kg seed was sampled from each seed cargo. The samples were processed by the method used at Shanghai port. Because *L. maculans* is the main threat to Chinese oilseed rape production, the investigation of imported seeds focused on detection of *L. maculans*. Seed samples were first tested for *L. maculans* using species-specific PCR as described by Taylor (1993). If *L. maculans* was detected by PCR, the samples were used for isolation of *L. maculans* (Wang *et al.*, 2010, 2011). Stem and pod debris separated from the seed sample using a 2·5-mm pore diameter sieve, and meal sampled after oil extraction at facilities near to the port, were also tested to detect and isolate *Leptosphaeria* pathogens. Identification of isolates as *L. maculans* by PCR was confirmed by inoculation onto cotyledons (Balesdent *et al.*, 2001) and by sequencing the ITS region of three isolates per sample and comparing the sequence with that of *L. maculans* ITS sequences available in GenBank.

Models of spread of *L. maculans* across Alberta, Canada (spring oilseed rape) and Poland (winter oilseed rape)

The model that had been developed to describe the westward spread of *L. maculans* across Alberta, Canada by Fitt *et al.* (2008), based on data from disease surveys in spring oilseed rape, was unsuitable for use with the Polish data from disease surveys in winter oilseed rape, which had been collected in a different way from the Canadian survey data. Therefore, alternative methods were developed to describe spread of *L. maculans* into both regions previously colonized by *L. biglobosa*. The Canadian data were used to predict potential westward spread of *L. maculans* across north China where spring oilseed rape is grown. The Polish data were used to predict potential spread of *L. maculans* across central China in provinces along the Yangtze River where winter oilseed rape is grown.

In Alberta, Canada, stem canker survey data had been collected from more than 700 spring oilseed rape crops (sown in April/May and harvested in August, different numbers of crops in different years) during the period with harvest years from 1984 to 1998. These crops had not been sprayed with fungicide for control of phoma stem canker. Plant pathologists examined 25–50 plants per crop sampled from five sites *c.* 100 m apart along a 'W'-shaped route in the crop. Plants were uprooted and lower leaves and stems, particularly the stem base region, were examined for the presence of pycnidia of *L. maculans* (which generally produces more pycnidia in cankers than *L. biglobosa*). Disease incidence (% plants affected) data were collected. Samples were taken to the laboratory to confirm the presence of *L. maculans* by isolation onto culture medium. In certain cases, presence of *L. maculans* was confirmed by inoculating cotyledons of a susceptible cultivar (McGee & Petrie, 1978). The Canadian data set included data on latitude and longitude of crops for the period 1984–1998 together with the binary dependent variable indicating the presence of *L. maculans* (value 1) or *L. biglobosa* (value 0) in each crop. These data provided 4488 records for describing westward spread of *L. maculans* across Canada (Fitt *et al.*, 2008).

In Poland, stem canker survey data had been collected from more than 200 winter oilseed rape crops (sown in September of one year and harvested in July of the following year, different numbers of crops in different years) during the period with harvest years from 1984 to 2004. These crops had not been sprayed with fungicide for control of phoma stem canker. The presence/absence of phoma stem canker was recorded. Confirmation of the causal pathogen as *L. maculans* or *L. biglobosa* was done by isolation. The survey data has been summarized in a monograph on phoma stem canker in Poland (Jedryczka, 2007). More crops were sampled after 2000 than before 2000. The latitude and longitude for each crop were recorded, together with the binary dependent variable indicating the presence of *L. maculans* (value 1) or *L. biglobosa* (value 0). These data provided 1453 records for describing the eastward spread of *L. maculans* across Poland.

After removing crops with some missing values, the total numbers of observations in the Canadian data set decreased from 4488 to 4235. Out of 4235 observations in the Canadian data set, 3871 crops were identified as crops infected by *L. biglobosa* and 364 crops as infected by *L. maculans*. All methods (e.g. classification tree, K-nearest neighbour (KNN)) were tested by estimating their sensitivity and specificity scores (Giudici, 2003) using the full Canadian data set. Sensitivity was estimated by comparing values predicted by model against observed data for crops infected by *L. maculans* (value 1) to calculate the proportion of these crops that were correctly classified by the model. Specificity was estimated by comparing values predicted by model against observed data for crops infected by *L. biglobosa* (value 0) to calculate the proportion of these crops that were correctly classified by the model. The results were very poor, with 100% specificity and 0% sensitivity, i.e. the model correctly predicted all crops infected by *L. biglobosa*, whereas all crops infected by *L. maculans* were predicted incorrectly. This was a clear indication that the data were unbalanced.

To resolve this problem, a statistical process known as 'balancing rare events' (Hanson, 2006) was used. Thus, *c.* 15% of records of crops infected by *L. biglobosa* (i.e. 559 crops) were selected at random from the total of 3871 infected crops. Similarly, the number of crops infected by *L. maculans* was decreased from 364 to 335. This then provided a balanced dependent variable with similar numbers of crops infected by *L. biglobosa* or *L. maculans*, i.e. 559 or 335 crops infected by *L. biglobosa* or *L. maculans*, respectively (894 observations). The Canadian description was constructed using these 894 observations. After removing crops with some missing values, the total numbers of observations in the Polish data set decreased from 1453 to 1169. The Polish data set had a balanced dependent variable (i.e. 595 crops infected by *L. biglobosa* and 574 crops infected by *L. maculans*) and therefore no random sampling was required.

Year and the natural logarithm (ln) of the minimum distance between two crops were derived and used as independent variables to predict the binary dependent variable (presence of *L. maculans*, value 1, or *L. biglobosa*, value 0). For the Canadian data, year 0 was 1984 and year 14 was 1998. For the

Polish data year 0 was 1984 and year 20 was 2004. The minimum distance (D) (km) between the current crop (*Lat1*, *Long1*) and the nearest crop with *L. maculans* in the previous year (*Lat2*, *Long2*) was calculated from the latitudes and longitudes of the two sites using the formula (Blue, 2007):

$$D = \text{acos}(\cos(\text{radians}(90 - Lat1)) \times \cos(\text{radians}(90 - Lat2)) + \sin(\text{radians}(90 - Lat1)) \times \sin(\text{radians}(90 - Lat2)) \times \cos(\text{radians}(Long1 - Long2))) \times 6371.$$

To describe the spread of *L. maculans* from one crop to another in the next year, it was assumed that the pathogen could spread only from the nearest crop with *L. maculans* in the previous year. The spread of *L. maculans* was described by four statistical methods: logistic regression, classification tree, neural network and K-nearest neighbour (KNN); all these methods are described in detail by Giudici (2003). All this work was done using SPSS 19 (IBM Corporation, 2010).

The logistic regression model was defined as:

$$\text{Logit}(\pi_i) = a + b_1 x_1 + b_2 x_2 \text{ for } i = 1, 2, 3, \ldots, n$$

where π_i was the probability that *L. maculans* had spread from one infected crop to another given crop in the next year. b_1 and b_2 were the coefficients of the two independent variables, natural logarithm of the minimum distance (ln (minimum distance)) between the current crop and the nearest crop infected by *L. maculans* in the previous year ($x_1 = \ln D$) and year (x_2). The classification tree divided the binary data for crops infected by *L. maculans* (value 1) or *L. biglobosa* (value 0) into sets of homogeneous groups with similar variability using the two independent variables. Therefore, the probability of spread of *L. maculans* from one infected crop to a given crop in the next year was calculated using the set of branches determined successively by the two independent variables. These data based on the two independent variables were represented in a tree-like structure, where the branches were labelled as nodes. Node 0 was the trunk node (parent node) and the branches were the terminal nodes (child nodes). For instance, if a homogeneous group had a year after 1994 (i.e. year >10 since year 0 = 1984) and ln (minimum distance) between the current crop and the nearest crop infected by *L. maculans* in the previous year >3, then the estimated probability that a crop was infected by *L. biglobosa* for this group was 0·64 (Fig. S1). Therefore, if a new observation was in this group, then the outcome was categorized as *L. biglobosa*, because the estimated probability of infection by *L. biglobosa* was >0·5.

The neural network algorithm first computed a set of coefficients (i.e. for relative importance) through an iterative process (known as backward propagation) for year and ln (minimum distance). Then it used these coefficients to calculate the probability that a crop was infected by *L. maculans* at a given site in a given year. The KNN method (Giudici, 2003) classified a new observation x (e.g. a new crop observed to be infected by *L. maculans* in year 10 [i.e. 1994]) by identifying the K crops closest (based on minimum distance between the data points) to crop x in the data set for the previous year (i.e. year 9). Thus, assuming that four out of the five crops closest to crop x were infected by *L. maculans* in year 9, then this particular observation at crop x was classified as infected by *L. maculans* because the majority of the five crops were classified as infected by *L. maculans*. A similar procedure was followed with the other independent variable [ln (minimum distance)] (Giudici, 2003).

The predictive accuracies of the four statistical methods were compared (Giudici, 2003). Sensitivity, specificity and the overall classification accuracy were used to evaluate the four methods. Sensitivity was estimated by comparing values predicted by the method against observed data for the crops infected by *L. maculans* (value 1) and specificity was estimated by comparing values predicted by the method against observed data for the crops infected by *L. biglobosa* (value 0) to estimate the proportions of these crops that were classified correctly. Overall classification accuracy indicated the overall proportion of crops that were correctly classified as infected by *L. maculans* or infected by *L. biglobosa*. These three measures were used to evaluate the predictive accuracy of each method. The method that produced the greatest overall accuracy for the three measures was selected.

Investigating ascospore dispersal as a potential mechanism for observed spread of *L. maculans* across Canada

Phoma stem canker is a monocyclic disease that can spread from infected stem debris of previous oilseed rape crops onto new, distant crops by windborne dissemination of ascospores (Fitt *et al.*, 2006a). To investigate if a single dispersal event per year could account for the observed rate of spread of *L. maculans* across Canada (17 km per year), a Gaussian plume model was used to calculate dispersal of *L. maculans* ascospores under different climatic conditions. A worst-case scenario was assumed, with a 1 ha crop debris source releasing 10^4 ascospores cm^{-2} of debris surface (Huang *et al.*, 2005), making a total of 10^{12} ascospores for the source site. *Leptosphaeria maculans* ascospores are forcibly ejected from pseudothecia on infected stem debris lying on the soil surface, so an ascospore release height of 2 cm was assumed. Lateral and vertical plume spread was expressed in terms of downwind distance from the source and atmospheric turbulence using Briggs interpolation formulae for Pasquill–Gifford stability classes A to D. These stability classes are defined for different meteorological situations and categorize the capacity of the atmosphere for dispersal. Stability class A is the most unstable or turbulent class and is typical of a calm, sunny spring day with considerable convective air movement that mixes and dilutes spore plumes. Stability class D is the least turbulent daytime class, representing windier and more overcast conditions that inhibit plume dilution. Wind speed and solar radiation values that spanned these stability classes were chosen to cover the range of likely daytime atmospheric transportation conditions. Although *L. maculans* ascospores are small (≤30 μm in length; Fig. 1a), they have mass and a shape that affects their dispersal through the air. This was accounted for using Stokes' law to calculate the downward movement of ascospores under gravity and a dynamic shape factor was applied to simulate the effect of ascospore shape (approximately cylindrical) on particle dispersal. The Gaussian plume model was used with a 'source-depletion method' for the dry deposition of spores. The source-depletion method accounted for the deposition of airborne spores by appropriately decreasing the source strength as a function of downwind distance. The 'deposition velocity,' or average rate at which the airborne ascospores returned to the ground surface, was estimated as three times the settling velocity. The full model was implemented on a raster lattice with an extent of 20 × 20 km and a spatial grain (cell size) of 1 ha. All dispersion formulae and parameterization schemes are given by Arya (1999).

Projection of the spread of *L. maculans* across China

To describe the westward spread of *L. maculans* across north China or central China, the values produced by the best methods for Canada and Poland, respectively, were used to predict the probabilities of infection by *L. maculans* for successive years for all crops. The Canadian description, constructed with spring oilseed rape data, was used for projection of the spread of *L. maculans* across north China over a *c.* 16-year period, assuming entry into China through Qinhuangdao port (119·6°N, 40·0°E) and spread across the area where there is widespread cultivation of spring oilseed rape (Fig. 3). The Polish description, constructed with winter oilseed rape data, was used for projection of the spread of *L. maculans* across central China over a *c.* 16-year period, assuming entry into China through Shanghai port (121·4°N, 31·2°E) and spread along Yangtze River valley where there is widespread cultivation of winter oilseed rape.

To estimate spread of *L. maculans* across the two regions in China, rectangular grids with 0·2 degree intervals between grid points (north–south and east–west) were constructed for each region using GenStat v. 14 (Payne *et al.*, 2011). For each year, a set of pseudo-randomly uniform probabilities between 0 and 1 for each point on the grid was generated. Expected probabilities of infection by *L. maculans* were evaluated from the classification tree and a grid point (crop) was considered to be infected by *L. maculans* if its expected probability was less than the randomly generated uniform probability of infection. This was a form of the envelope rejection technique for creating a random sample for a given distribution (Knuth, 1997). The average probability of infection, as determined by the classification tree, was formed as the average expected probability of infection by *L. maculans* after a few years. Infection was considered established in a patch (5 × 5 grid points, 1 degree × 1 degree) when sufficient crops, determined from the classification accuracies of the classification trees for Canadian and Polish data, were infected. Otherwise the patch was assumed to be infected by *L. biglobosa*. To produce disease maps on a degree scale, the values for crops at grid points were tabulated by summing over the 25 values in each patch and these values were used to produce contour maps using SigmaPlot (www.sigmaplot.com).

Results

Survey of phoma stem canker on oilseed rape crops in China, 2005–2012

In the period from 2005 to 2012, phoma stem canker was found on winter or spring oilseed rape crops in 14 out of 16 provinces sampled, including Anhui, Guizhou, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shanghai, Sichuan and Zhejiang in central China (Table 1), and Inner Mongolia (Table 2), Gansu, Qinghai and Xinjiang in north China. No phoma stem canker was found in Shanxi or Tibet provinces.

Winter oilseed rape, central China

In the area where winter oilseed rape is grown, plants with phoma stem canker symptoms were found in 2005 in Hubei province and sampled for isolation of pathogens associated with internal necrosis (Fig. 2c). Stem canker symptoms were usually (>95%) on upper stems (Fig. 2a) and few stem base cankers (<5%; Fig. 2b) were observed. Only *L. biglobosa* isolates were obtained from these diseased stems (Fig. 2d, Table 1). In May 2006, plants with phoma stem canker symptoms were found in Anhui province and 10 *L. biglobosa* isolates were obtained. *Leptosphaeria maculans* (Fig. 2e) was not detected by either cultural isolation or PCR. In May 2012, phoma stem canker symptoms were found in four provinces along the Yangtze River. However, the incidence of phoma stem canker was small (Table 1). In 2012, 91·3% of crops of winter oilseed rape sampled and 12·5% of stems assessed had symptoms of phoma stem canker. Diseased stems were used for pathogen isolation and only *L. biglobosa* was identified from these stems.

In the large-scale 2008–2012 survey, phoma stem canker disease was observed on winter oilseed rape in 37

Table 1 Occurrence of phoma stem canker and identification of the causal *Leptosphaeria* species on winter oilseed rape in Chinese provinces along the Yangtze River, 2005–2012

Month/year	Province	No. crops inspected/ no. with phoma stem canker	No. stems assessed/ no. with phoma stem canker	Identification of *Leptosphaeria* species		
				No. diseased stems used for isolation	No. isolates identified by culture[a]	No. isolates identified by PCR[b]
May 2005	Hubei	–	–[c]	30	26 *Lb*, 0 *Lm*	26 *Lb*, 0 *Lm*
May 2006	Anhui	–	–[c]	10	10 *Lb*, 0 *Lm*	10 *Lb*, 0 *Lm*
May 2008	Anhui	–	–[c]	2	2 *Lb*, 0 *Lm*	2 *Lb*, 0 *Lm*
	Guizhou	–	–[c]	3	3 *Lb*, 0 *Lm*	3 *Lb*, 0 *Lm*
May 2012	Hubei	17/17	8500/1158[d]	208	118 *Lb*, 0 *Lm*	118 *Lb*, 0 *Lm*
	Jiangsu	2/2	300/4	2	2 *Lb*, 0 *Lm*	2 *Lb*, 0 *Lm*
	Shanghai	2/0	200/0	0	0	0
	Sichuan	2/2	300/2	2	2 *Lb*, 0 *Lm*	2 *Lb*, 0 *Lm*
2008–2012[e]	10 provinces	281/281	42150/11046	580	525 *Lb*, 0 *Lm*	361 *Lb*, 0 *Lm*

[a]*Leptosphaeria biglobosa* (*Lb*) produces a yellow pigment in culture on PDA, whereas *L. maculans* (*Lm*) does not (Williams & Fitt, 1999; West *et al.*, 2002; Fig. 2d,e).

[b]PCR primers used for identification of *L. maculans* (LmacF/LmacR) or *L. biglobosa* (LbigF/LmacR) are described by Liu *et al.* (2006).

[c]No systematic survey was done in these years but diseased stems were sampled from crops in these provinces in May before harvest.

[d]The number of stems sampled per crop assessed was 500, 150 or 100, depending on the province.

[e]During the period 2008–2012, stem canker was found on winter oilseed rape crops in 37 districts in 10 provinces: Anhui, Guizhou, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shanghai, Sichuan, Zhejiang (Li *et al.*, 2013).

Table 2 Occurrence of phoma stem canker and identification of the causal *Leptosphaeria* species on spring oilseed rape in Inner Mongolia, north China, 2005–2012

Month/year	No. crops inspected/ no. with phoma stem canker	No. stems assessed/ no. with phoma stem canker	Identification of *Leptosphaeria* species	
			No. diseased stems used for isolation	No. isolates identified by PCR[a]
August 2005	–[b]	–	20	20 *Lb*, 0 *Lm*
August 2009	2/2[c]	200/3	3	3 *Lb*, 0 *Lm*
August 2010	2/1	200/1	1	1 *Lb*, 0 *Lm*
August 2011	3/2	300/2	2	2 *Lb*, 0 *Lm*
August 2012	12/5	1200/8	8	5 *Lb*, 0 *Lm*
2008–2012[d]	23/23	340/102	30	23 *Lb*, 0 *Lm*

[a]PCR primers used for identification of *L. maculans* (LmacF/LmacR) (*Lm*) or *L. biglobosa* (LbigF/LmacR) (*Lb*) are described by Liu *et al.* (2006).
[b]No systematic survey was done in 2005 but diseased stems were sampled from crops in August before harvest.
[c]150 stems per crop were assessed.
[d]During the period 2008–2012, stem canker was found on spring oilseed rape crops in five districts in four provinces: Inner Mongolia, Gansu, Qinghai and Xinjiang (Li *et al.*, 2013).

out of 49 districts in 10 out of 11 provinces, with the disease incidence ranging from 1 to 92% stems affected (Li *et al.*, 2013; Table 1). In some crops in Henan and Sichuan provinces, severe stem canker caused death of up to 5% of plants. A total of 525 isolates were obtained from diseased stems and 361 of them were tested by PCR; only *L. biglobosa* was detected. When an additional 468 diseased stem samples were used directly for DNA extraction and PCR, only *L. biglobosa* was identified.

Spring oilseed rape, north China

In the area where spring oilseed rape is grown, plants with phoma stem canker symptoms were found at Hailar farm in 2005. Stem canker symptoms were mainly on upper stems and very few stem base cankers were observed. From these diseased stems, 20 *L. biglobosa* isolates were obtained and no *L. maculans* was detected (Table 2). Spring oilseed rape in Inner Mongolia has been surveyed for phoma stem canker symptoms every year since 2009. In August 2009, out of the 200 stems in two crops at Labudalin farm investigated, only three stems had phoma stem canker symptoms. Both cultural characteristics and PCR identification showed that the disease was caused by *L. biglobosa* not *L. maculans* (Table 2). Similar results were observed at farms in Inner Mongolia in 2010, 2011 and 2012. Although phoma stem canker symptoms were observed each year, the incidence of disease was very small. In the survey of spring oilseed rape from 2009 to 2012, 52·6% of crops sampled and 0·7% of stems assessed had phoma stem canker symptoms.

In the large-scale 2008–2012 survey, phoma stem canker disease was identified on spring oilseed rape in five out of 11 districts in four out of five provinces. The disease incidence ranged from 1 to 5% stems affected, which was less than that in winter oilseed rape crops (Li *et al.*, 2013). A total of 23 isolates were obtained from diseased stems and tested by PCR; only *L. biglobosa* was detected.

Disease severity testing

The 22 *L. biglobosa* isolates (19 from winter oilseed rape and three from spring oilseed rape) were similarly aggressive to the Chinese cultivar Zhongyou 821 (mean disease score 6·3) and the European cultivars Hearty (6·2), Bristol (5·9) and Courage (6·1). However, there were differences in aggressiveness between isolates collected from different regions. The most aggressive isolate was from Shanghai (7·4) and the least aggressive isolate was from Inner Mongolia (4·6). All the Chinese *L. biglobosa* isolates except for one isolate from Inner Mongolia were more aggressive than the Polish *L. biglobosa* isolate.

Survey for presence of *L. biglobosa*/*L. maculans* on oilseed rape seed cargoes imported into China

At Shanghai port in June 2009, *L. maculans* was detected by initial PCR in both seeds and stem/pod debris separated from the original imported seed cargoes from Australia (Table 3; Yi *et al.*, 2010). Similarly, *L. maculans* was detected in another cargo of imported seed from Australia in August 2009. Both *L. maculans* and *L. biglobosa* were detected in seed cargoes imported from Canada and Ukraine in August 2009 (Zhou *et al.*, 2010, 2011). The incidence of *L. maculans* detected in the seed imported into China was *c.* 2·4% seeds infected from Australia, 0·2% from the Ukraine and 0·4% from Canada. At Wuhan port in 2010, *L. maculans* was detected in oilseed rape seeds and debris in cargoes from Canada (Table 4; Wang *et al.*, 2010, 2011). However, the pathogen was not detected in the oilseed rape meal after extraction of oil.

These results were confirmed by pathogen isolation, identification by pigment production on agar plates (Williams & Fitt, 1999) or in liquid media (Fig. 2f) and PCR at both Shanghai (Table S1) and Wuhan (Table S2). When the ITS sequences of some of these *L. maculans* isolates were compared with those of known *L. maculans* isolates (Tables S1 & S2), the sequence of one *L. maculans* isolate from seed from Australia at Shanghai port showed 99·8% similarity to that of a *L. maculans* isolate (GenBank accession no. DQ458907) and 99·5% similarity to sequences of 21 *L. maculans* isolates

Table 3 Detection using PCR of *Leptosphaeria maculans* (*Lm*) or *Leptosphaeria biglobosa* (*Lb*) in seed or debris samples from shiploads of oilseed rape seed cargoes imported into China (Shanghai port) from Australia, Canada or the Ukraine

Date of import	Source of seed	No. of samples	Amount sampled	Subsample used for PCR[a]	Initial PCR results	Reference
Jun 2009	Australia	1	2–3 kg	1 g debris[b]	1 *Lm*	Yi *et al.*, 2010
				1 g seed	1 *Lm*	
Aug 2009	Australia	1	2–3 kg	1 g debris[b]	1 *Lm*, 1 *Lb*	Zhou *et al.*, 2010
				1 g seed	1 *Lm*, 1 *Lb*	
Aug 2009	Canada	4	2–3 kg	1 g seed	4 *Lm*	Zhou *et al.*, 2011
Aug 2009	Ukraine	10	2–3 kg	1 g seed	10 *Lm*	Zhou *et al.*, 2011

[a]PCR primers used for detection of *L. maculans* (LmacF/LmacR) or *L. biglobosa* (LbigF/LmacR) in seed or debris samples are described by Liu *et al.* (2006).
[b]The 2–3 kg samples were sieved (sieve pore diameter 2·5 mm) to separate out the stem and pod debris, and the abnormal, wrinkled and discoloured seeds were separated from the normal seed (Yi *et al.*, 2010; Zhou *et al.*, 2010, 2011). Then 1 g of the abnormal seed and 1 g of oilseed rape stem and pod debris obtained after sieving were sampled for DNA extraction and PCR.

Table 4 Detection using PCR of *Leptosphaeria maculans* (*Lm*) in seed, debris or meal samples from shiploads of oilseed rape seed cargoes imported into China (Wuhan port on the Yangtze River) from Canada or after processing for extraction of oil

Date of import	No. of samples	Amount sampled (type of sample)	Subsample used for PCR	Initial PCR results[a]	Reference
Jan 2010	18	2–3 kg (shipload)[b]	1 g seed	18 *Lm*	Wang *et al.*, 2010
Jan 2010	7	2–3 kg (storage silo)[c]	1 g debris	7 *Lm*	Wang *et al.*, 2010
Jan 2010	8	2 kg (meal)[d]	1 g meal	0 *Lm*	Wang *et al.*, 2010
Jan 2010	11	1–2 kg (debris)[b]	1 g debris[d]	11 *Lm*	Wang *et al.*, 2010
Jan 2011	15	2–3 kg (shipload)[b]	1 g seed	15 *Lm*	Wang *et al.*, 2011

[a]PCR primers used for detection of *L. maculans* (LMR1-DF/LMR1-DR) in seed, debris or meal samples are described by Taylor (1993).
[b]The 2–3 kg samples were sieved (sieve pore diameter 2·5 mm) to separate out the stem and pod debris, and the abnormal, wrinkled and discoloured seeds were separated from the normal seed (Wang *et al.*, 2010, 2011). Then 1 g of the abnormal seed and 1 g of oilseed rape stem and pod debris obtained after sieving were sampled for DNA extraction and PCR.
[c]A storage silo is a container for storage of oilseed rape seed on shore after unloading from the ship.
[d]Meal is the by-product after processing seeds to extract the oil.

available from GenBank (Yi *et al.*, 2010). Sequences of two *L. maculans* isolates from seeds or debris imported from Canada at Wuhan port showed 96% similarity to the sequence of *L. maculans* isolate v23.1.3 (CT4857890; Rouxel *et al.*, 2011; Wang *et al.*, 2011). Furthermore, inoculation onto cotyledons also confirmed identification of *L. maculans* isolates at both Shanghai and Wuhan (Tables S1 & S2).

Spread of *L. maculans* across Alberta, Canada (spring oilseed rape) and Poland (winter oilseed rape)

The logistic regression method correctly predicted 86% of crops infected by *L. maculans* in the Canadian data set, whereas only 33·5% of crops infected by *L. biglobosa* were correctly predicted (Table 5). Conversely, the neural network method and KNN method were good at predicting crops infected by *L. biglobosa* (74 and 83%, respectively) and satisfactory in predicting crops infected by *L. maculans* (56 and 57%, respectively). However, the classification tree was the best method in terms of correctly predicting both crops infected by *L. maculans* (64%) and crops infected by *L. biglobosa* (79%). The results were similar with the Polish data set. Consequently, the classification tree was selected for use with both data sets.

Node 0 had 894 observations for the Canadian data (probabilities of infection by *L. maculans* 0·375 or *L. biglobosa* 0·625), which were partitioned into six homogeneous groups (nodes 1–6) based on year (1984–1998). Two of the year categories (node 2, node 6) were further divided on the basis of ln (minimum distance) between the current crop and the nearest crop infected by *L. maculans* in the previous year. There were thus nine terminal nodes (nodes 1, 3, 4, 5, 7, 8, 9, 10, 11) for the Canadian data. For example (Fig. S1), if the year was >10 (i.e. after 1994) and the ln (minimum distance) was <3, there was a probability of 0·72 of being infected by *L. maculans*. For the Polish data, node 0 had 1169 observations with a probability of infection by *L. maculans* of 0·49 and the classification tree partitioned the data into three groups on the basis of year (Fig. S2). Two of the year categories (node 2, node 3) were further divided into six homogeneous groups based on ln (minimum distance) between the current crop and the nearest crop infected by *L. maculans* in the previous year. There were thus

Table 5 Evaluation of the sensitivity, specificity and overall classification accuracy of four models for describing spread of *Leptosphaeria maculans* into areas previously colonized by *Leptosphaeria biglobosa* that were growing spring oilseed rape (Canadian data set) or winter oilseed rape (Polish data set)

Model[a]	Sensitivity[b] (%)	Specificity[c] (%)	Classification accuracy[d] (%)
Canadian data set			
Logistic regression	86	34	65
Classification tree	64	79	73
Neural network	56	74	67
KNN	57	83	73
Polish data set			
Logistic regression	47	51	49
Classification tree	73	57	65
Neural network	27	97	62
KNN	75	60	67

[a]KNN is K-nearest neighbour, one of the statistical methods used to analyse and classify the data on spread of *L. maculans* in China.
[b]Sensitivity was estimated by comparing values predicted by the model against observed data for crops infected by *L. maculans* (value 1) to calculate the proportion of these crops that were correctly classified by the model.
[c]Specificity was estimated by comparing values predicted by the model against observed data for crops infected by *L. biglobosa* (value 0) to calculate the proportion of these crops that were correctly classified by the model.
[d]Classification accuracy indicated the overall proportion of the crops that were correctly classified as infected by *L. maculans* or by *L. biglobosa*.

seven terminal nodes (nodes 1, 4, 5, 6, 7, 8, 9) for Polish data. For instance, if year was >17 (after 2001) and ln (minimum distance) was 2–2·6, there was a probability of 0·69 of the crop being infected by *L. maculans*.

Ascospore dispersal as a potential mechanism for observed spread of *L. maculans* across Canada

The majority of *L. maculans* ascospores were deposited near the crop debris source (Fig. 5), as expected because the release of these spores occurred very close to the soil surface where gravitational settling is a dominant force. However, some ascospores were carried by wind to greater heights and transported over greater distances, depending on the weather conditions. At a distance of 20 km, maximum ascospore deposition on spring oilseed rape crops in a 1 ha cell was *c.* 10^5, $2{\cdot}5 \times 10^5$, 8×10^5 and $2{\cdot}5 \times 10^6$ spores for stability classes A, B, C and D, respectively. The resulting density of phoma leaf spot lesions (and ultimately of stem cankers) depended on many factors such as cropping density, infection conditions and cultivar resistance, that were not simulated. Nevertheless, the predicted number of ascospores deposited at distance from an infected source suggested that a single wind dispersal event per year could account for the observed rate of spread of *L. maculans* across Alberta, Canada (17 km per year).

Projection of the spread of *L. maculans* across China

For both the Canadian and Polish versions of the classification tree, the chosen node set for spreading the disease in a given year was selected at random. For nodes where distance was not relevant, it was assumed that only crops at grid points in neighbouring patches could be infected. Node sets were chosen with probability proportional to size. Selection of a node with a distance dependence resulted in all nodes at that point being selected, thus allowing spread to be related to ln (minimum distance). A crop at a grid point was considered as infected if its expected probability of infection by *L. maculans* was greater than the randomly generated probability of infection. Patches were discarded if their number of infected crops at grid points did not reach the required density, chosen as four to six out of 25 for northern China and eight out of 25 for central China. These values reflect the level of classification accuracy from fitting classification trees to the original data (73% for the Canadian set and 65% for the Polish data). The contour plots were constructed from tabulation over the patches corresponding to 1-degree increments over the regions and gave an average density (probability of infection) within a patch. Figures 6a and 6b show the results for north and central China, respectively, at 4-year intervals, starting with year 3. The simulated spread of *L. maculans* across north China or central China was estimated to occur at rates of 47 or 70 km per year, respectively.

Discussion

The survey data provide no evidence that *L. maculans* is already present on oilseed rape crops in China. The extensive surveys of pathogens associated with phoma stem canker on oilseed rape crops in China over the period 2005–2012 were done on winter crops in 11 provinces in central China and on spring crops in five provinces, mostly in north China. In all cases when the causal pathogen was isolated from upper stem lesions/basal cankers on these crops, it was identified as *L. biglobosa* by morphology in culture and/or species-specific PCR. Thus, this widespread survey supports previous evidence that only *L. biglobosa* is currently associated with phoma stem canker on Chinese oilseed rape crops (West *et al.*, 2000; Mendes-Pereira *et al.*, 2003; Fitt *et al.*, 2008; Hao *et al.*, 2012). Furthermore, Chinese farmers and extension workers are now being trained to recognize symptoms of the disease (http://sainclimatechange.org/details.asp) and a new book to help farmers and extension workers has been published in Chinese (Liu, 2009). Nevertheless, some Chinese oilseed rape cultivars are very susceptible to *L. maculans* (Fitt *et al.*, 2008; Li *et al.*, 2008); even those Chinese cultivars/lines that were not killed were much less resistant to *L. maculans* than European or Australian cultivars. Furthermore, the climatic and agronomic conditions in China are suitable for *L. maculans* (West *et al.*, 2002; Fitt *et al.*, 2006b,

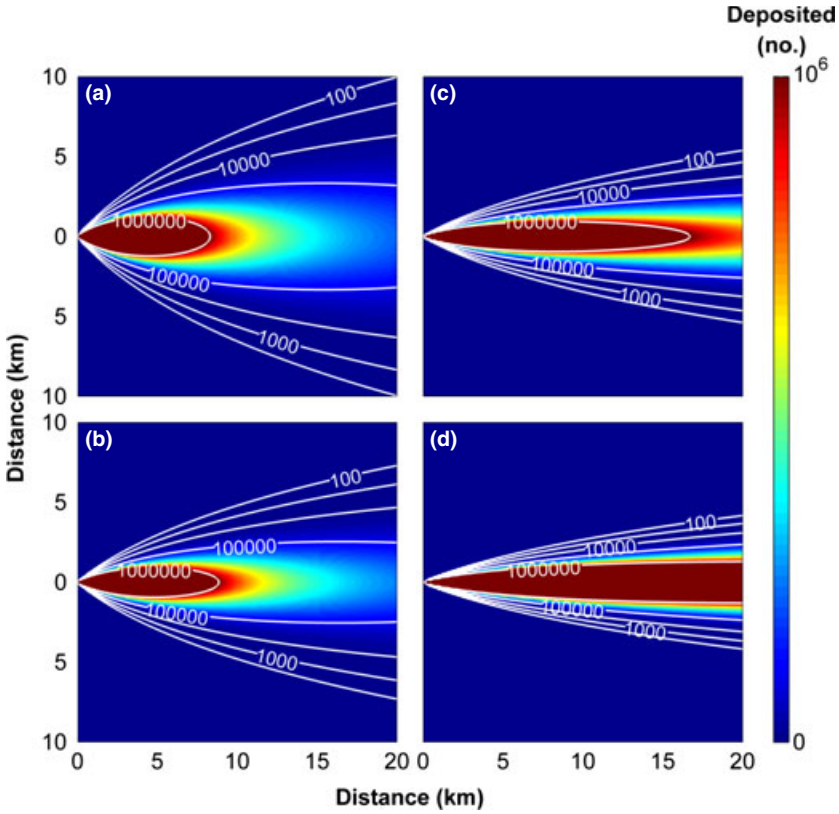

Figure 5 Variation in windborne transport of *Leptosphaeria maculans* ascospores over distance, as predicted by an atmospheric dispersion model. Panels (a) to (d) show ascospore deposition patterns for Pasquill–Gifford stability classes A (extremely unstable), B (moderately unstable), C (slightly unstable) and D (neutral atmospheric conditions), respectively. The colour bar shows the range of ascospore deposition values. White contour lines connecting points of equal deposition are shown in a series of logarithmic steps.

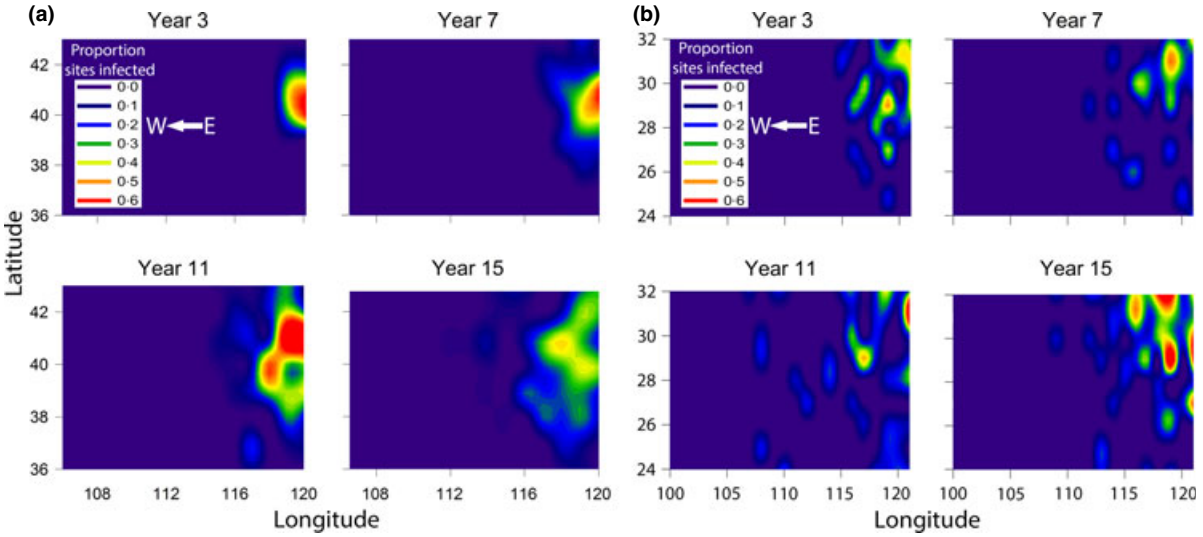

Figure 6 Projection of westwards spread of *Leptosphaeria maculans* across China by 3, 7, 11 or 15 years after its introduction through a port. (a) Potential westward spread of *L. maculans* across the spring oilseed rape growing area in north China, over a 16-year period, with the epidemic assumed to start from Qinhuangdao. Results presented indicate the proportions of the 25 crops at grid points within a patch that are infected by *L. maculans* at 4-year intervals. Each patch is taken as 1 degree of latitude by 1 degree of longitude. The spring oilseed rape growing area of north China is assumed to be 36–43°N and 106–120°E. (b) Potential westward spread of *L. maculans* across the winter oilseed rape growing area in central China along the Yangtze River valley, over a 16-year period, with the epidemic assumed to start from Shanghai. Results presented indicate the proportion of crops at grid points within a patch that are infected by *L. maculans* at 4-year intervals. Each patch is taken as 1 degree of latitude by 1 degree of longitude. The winter oilseed rape growing area in central China is assumed to be 24–32°N and 100–121°E.

2008). Therefore continued vigilance is recommended to ensure that if *L. maculans* does spread onto Chinese oilseed rape crops, it is recognized quickly. The effectiveness of detection of *L. maculans* could be improved if species-specific PCR were to be used directly on all diseased stems as it is with imported seed samples.

The pre-harvest surveys suggest that phoma stem canker is widespread on both winter oilseed rape crops in central China (in May) and spring oilseed rape in north China (in August). However, there was no evidence that the disease is currently causing economic losses on spring oilseed rape in north China and severe epidemics were rarely observed on winter oilseed rape in central China. In both regions, the symptoms were mainly the less damaging upper stem lesions associated with *L. biglobosa*. On the unsprayed Chinese winter oilseed rape crops, the epidemics were less severe than on UK crops (88·3% crops, 30% stems affected with damaging stem base canker from 2001 to 2010; http://www.cropmonitor.co.uk/wosr/wosr-intro.cfm; all crops sprayed against *L. maculans*) or Polish crops (100% crops, 5% stems affected; Jedryczka, 2007; *c*. 60% crops sprayed). On the unsprayed Chinese spring oilseed rape crops, the epidemics were less severe than on unsprayed spring oilseed rape in Canada (from 2010 to 2012, basal cankers on 72·4% crops with 11·7% of stems affected; upper stem lesions on 53·2% crops with 5·4% of stems affected; R. M. Lange, unpublished data). Whilst it was not possible to estimate yield losses for China in the way that fungicide experiment data were used to estimate yield losses for the UK (Butterworth *et al*., 2010), it seems most unlikely that losses were sufficient to justify the expense of fungicide application. However, it is important that steps continue to be taken to minimize spread of *L. biglobosa* in China. The Chinese practices of alternating winter oilseed rape with paddy rice in central China and removal of stem debris for use as fuel (Fitt *et al*., 2008) both contribute to this objective.

The greatest risk associated with import into China of oilseed rape seed from Canada, Australia and the Ukraine comes from the stem and pod debris ('dockage') associated with the seed. Such debris, from which *L. maculans* was isolated in both Shanghai and Wuhan, is known to be a potent source of ascospore inoculum (Fitt *et al*., 2006a) and infected stems were considered responsible for spreading the pathogen into Canada (McGee & Petrie, 1978). By contrast, the incidence of *L. maculans* in the seed imported into China from Canada, the Ukraine or Australia was generally smaller than the incidence of *L. maculans* in Canada in 1990/1991 (1·5–3·6%; Chigogora & Hall, 1995). Furthermore, there is little evidence that *L. maculans* is efficiently transmitted from infected seed to cause disease on seedlings in crops. There is no risk from import into China from Canada of oil or meal, obtained after the oil extraction process, which requires high temperatures that kill *L. maculans*. The current strategy of importing oilseed rape seed through Chinese ports in regions where little oilseed rape is grown (Fig. 3), testing for presence of *L. maculans* by the quarantine bureau and crushing the seed at the port of entry into China will further decrease the risk from infected seed. If the oilseed rape seed were to be cleaned in the exporting countries to minimize the presence of stem and pod debris, it would further decrease the risk of spread of *L. maculans* into China.

The predicted rates of spread of *L. maculans* across north (47 km per year) and central China (70 km per year), although greater than the observed rate of spread across Canada (17 km per year; Fitt *et al*., 2008), were compatible with spread by airborne ascospores (Fig. 5). A similar rate of spread (30–120 km per year) has been observed for *Hymenoscyphus pseudoalbidus*, cause of ash dieback, which has been spreading by means of airborne ascospores across Europe into areas previously colonized by the less damaging, widespread *Hymenoscyphus albidus* (Timmermann *et al*., 2011; Nik Cunniffe, Cambridge University, Cambridge, UK, personal communication). The strategies used in Canada to decrease the rate of spread of *L. maculans,* namely destruction of infected crops and restrictions on movement of seed or crop debris from affected to unaffected areas, could be applied in China if *L. maculans* were to be found on some oilseed rape crops there.

Strategies to decrease the risk from *L. maculans* to oilseed rape and other brassica crops in China should be a priority for Chinese government and industry. There is a need to breed new oilseed rape cultivars with effective resistance against *L. maculans* that are adapted to yield well in China. Breeding resistance against *L. maculans* should be easier, now that the first resistance gene operating against *L. maculans* has been cloned (Larkan *et al*., 2013). Nevertheless, because breeding new sources of resistance into crop cultivars is a long-term strategy that can take 10–15 years, it is important to start it as soon as possible and combine it with some short-term strategies to prevent entry of *L. maculans* into China and spread within China. Short-term strategies such as disease surveys, tests on imported seed, training of extension workers and farmers to recognize symptoms of the disease all require good collaboration between policy makers, researchers, extension workers and farmers. A combination of short-term and long-term strategies can be used to safeguard production of oilseed rape in China.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. Identification of *Leptosphaeria maculans* (*Lm*) or *L. biglobosa* (*Lb*) isolates obtained from seed or debris from shiploads of oilseed rape seed cargoes imported into China (Shanghai port) from Australia, Canada or the Ukraine by pathogen isolation and PCR, inoculation tests or sequencing.

Table S2. Identification of *Leptosphaeria maculans* (*Lm*) or *L. biglobosa* (*Lb*) isolates obtained from seed or debris from shiploads of oilseed rape seed cargoes imported into China (Wuhan port) from Canada by pathogen isolation and PCR.

Figure S1. Classification tree results for the Canadian (Alberta) survey data on the westward spread of *Leptosphaeria maculans* (phoma stem canker) across an area of spring oilseed rape previously colonized by *L. biglobosa*.

Figure S2. Classification tree results for the Polish survey data on the westward spread of *Leptosphaeria maculans* (phoma stem canker) across an area of winter oilseed rape previously colonized by *L. biglobosa*.