ABSTRACT OF HABILITATION THESIS

Polish Academy of Sciences, Institute of Plant Genetics, Poznań, Poland

EPIDEMIOLOGY AND DAMAGE CAUSED BY STEM CANKER OF OILSEED RAPE IN POLAND

M. Jędryczka

Key words: *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, avirulence genes, minisatellite markers, disease resistance

Stem canker (blackleg) is a damaging disease of oilseed rape worldwide and it is also one of the most important diseases of the crop in Poland. The disease was reported for the first time in Poland in 1952 when stem canker was discovered on vegetable crops. Stem canker did not cause serious damage of oilseed rape until the early 1980s. Symptoms of the disease are caused by two pathogens, of which *Leptosphaeria maculans* (Desm.) Ces. et de Not. is regarded aggressive and *L. biglobosa* sp. nov. Shoemaker and Brun is described as nonaggressive or weakly aggressive. The aim of the work was to study the epidemiology, spread and damage caused by the disease and to evaluate morphological and genetic variation within the pathogen population in Poland.

In the course of twenty vegetative seasons (1984–2004) the spread and severity of the disease was observed in all regions of intensive oilseed rape production in Poland. In total, the work consisted of 1760 field visits to evaluate plant infection under natural conditions in 13 of 16 administrative regions of the country. Before 1990, half of the fields assessed were free from stem canker and the disease incidence was insignificant. However, since 1990 (except for 2003 when disease symptoms were observed on 14% of fields) the percent of rapeseed fields with the stem canker varied from 75 to 100%. The overall pattern of disease incidence varied across the country. Generally, the highest level of infection was observed in the west of Poland and the lowest in the east. This coincided with changes in the ratio of *L. maculans* to *L. biglobosa*, which indicated that the former species was predominant in the west of Poland and the latter prevailed in the east. Both species were isolated from disease symptoms on leaves and from lower and upper stems. The ratio *L. maculans* to *L. biglobosa* on leaves was ca 3:1. Before 2000, stems were mainly infected with *L. biglobosa* (97% of isolations from upper and 67% from stem bases). *Leptosphaeria maculans* was always co-isolated with *L. biglobosa*. From 2000 onwards, the incidence of *L. maculans* increased significantly, with the species being confined to the lower stem (60%) rather than the upper stem (26%). The results support the hypothesis that *L. maculans* is an invasive species in Poland. However, *L. biglobosa* dominated and was isolated alone from 75% of upper stems and 40% of stem bases.

In the studied period more than 10 thousand isolations were done. In general, half of the isolates belonged to *L. maculans* and half to *L. biglobosa*, but great disparities were observed in different seasons. In autumn nearly three quarters of isolates belonged to *L. maculans* (67.5%), whereas in summer 74.3% of isolates were *L. biglobosa*.

Due to the increasing spread and severity of *L. maculans* in Poland, studies were performed to investigate possible variation in the pathogen population. A population of 92 isolates originating from 2000–2001 was monomorphic with respect to *AvrLm7* (avirulent genotype) and *avrLm2, avrLm3* and *avrLm9* (virulent genotypes). Isolates were polymorphic to *AvrLm1* (58.7% of isolates) and *AvrLm4* (3.3%). The presence of *avrLm1* isolates was also studied using PCR with a *Vir1* marker. The result of molecular study agreed with the interaction phenotype (90.2%). The experiment demonstrated the existence of four *L. maculans* races, with the prevalence of Av1-7 (57.6%) and Av7 (39.1%) and negligible levels of Av4-7 (2.2%) and Av1-4-7 (1.1%). The population of *L. maculans* was also studied in respect to minisatellite markers *MinLm1-MinLm4*. There were 6, 9, 3 and 2 alleles of these sequences, with considerable variation of their frequencies.

Two hundred isolates, half *L. maculans* and half *L. biglobosa*, were characterised by colony morphology, including phenotypic features such as colour and abundance of aerial mycelium, number of pycnidia and colony shape and size. Experiments were done *in vitro* on PDA and V8 media at 10, 15 and 20°C. Significant differences were found between and within both groups of isolates. In general, isolates of *L. biglobosa* were growing about 2.5 times faster than *L. maculans*, and the difference between growth rate increased with the decrease of temperature. In contrast, sporulation of *L. maculans* at low temperatures was more abundant than that of *L. biglobosa*.

Growth rates *in vitro* on agar media were compared with data on fungal growth in plant tissues. Based on results of *in vivo* experiments, mathematical simulations were done using *Leptosphaeria* sp. pseudothecia maturation or ascospore concentrations in air samples and weather data from six consecutive growing periods (1998/99 to 2003/04). The progress of oilseed rape colonization differed between the two species, with plant colonization dominated by *L. biglobosa*. Statistical analysis supported the hypothesis that a percentage of leaves, infected in the autumn, drop off before the pathogen reaches the stem base. Due to the slower growth rate of *L. maculans* in comparison to *L. biglobosa,* this process is more likely to decrease cankers at stem base rather than upper stem symptoms caused by *L. biglobosa*. It explains lower incidence of *L. maculans* on plant stems in east Poland, where winters are more harsh.

In 1998/99 and 1999/2000, plants were tagged and assessed for disease symptoms several times during each season. Seed yield from individual plants was recorded and compared with the level of disease symptoms at harvest. In the experiment stem canker symptoms at harvest were caused solely by *L. biglobosa*. It was found out that severe symptoms of *L. biglobosa* are damaging to oilseed rape in respect to seed yield.

A large number of cultivars (44) and breeding lines (30) of winter oilseed rape were studied for resistance to *L. biglobosa* under controlled environment and under field conditions over five subsequent seasons (1991/92–1995/96). The results of field and glasshouse tests were statistically correlated. The experiment demonstrated that all cultivars tested were susceptible to *L. biglobosa* infection, but the degree of infection differed between plant genotypes. The cultivars 'Bolko', 'Bor', 'Prestol', 'Symbol', 'Libravo', 'Liradette' and 'Lirajet' were less damaged; they can be used in resistance breeding programmes.

*

The habilitation colloquium took place and the degree of *doctor habilitatus* was confirmed by the Council of the Faculty of Agriculture, The August Cieszkowski Agricultural University in Poznań, on March 9, 2007.

Author's address:
Doc. Dr. hab. Małgorzata Jędryczka, Polish Academy of Sciences, Institute of Plant Genetics, ul. Strzeszyńska 34, 60-479 Poznań, Poland, e-mail: mjed@igr.poznan.pl