Phoma lingam - a rapeseed parasite in Serbia

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Abstract

The rapeseed (*Brassica napus* L.) was grown in Serbia already in 1930s. However, organized production and a steady increase in the acreage under winter rapeseed have been registered in recent years. The main reason for the increase in rapeseed acreage is that rapeseed can be used for production of biodiesel. Although the current acreage is relatively small, disease symptoms caused by certain phytopathogenic fungi have already been observed in rapeseed fields. This paper describes the symptoms caused by the pathogenic fungus *Phoma lingam*. In addition to the description of symptoms, the paper contains descriptions of morphological characteristics of the fungus cultured on PDA and oat agar, results of pycnospore and pycnidium counts and the results of field trials for rapeseed resistance to the disease.

Key words: winter rapeseed, Serbia, *Phoma lingam*, symptoms, biodiesel, pycnospores, pycnidia, genotypes.

Introduction

Phoma lingam (Tode. Fr.) Desmaz causes symptoms on a large number of plant species from the family *Brassicacae* (Punithalingam and Holliday, 1972). The fungus is present for a long time in some European countries (Great Britain, Germany, Denmark, France, etc.), Canada, USA and Australia. Stem canker is the major rapeseed disease on the global scale (Gosende et al. 2003, Howlett et al. 2001). West et al. (cit. loc. Salam et al. 2003) report that stem canker is an economically important disease of rapeseed in the main production regions of Australia, Canada and Europe. Pedras et al. (1996) stated that annual crop losses due to *P. lingam* in Canada exceed 30 million dollars. In Great Britain, yield reductions with susceptible cultivars reach up to 50% in years when stem canker attack is intensive (Gladders and Musa, 1979).

Leptosphaeria maculans (Desm.) Ces. and de Not, the anamorf of *Phoma lingam* (Tode. Fr.) Desmaz, is capable of causing disease symptoms on rapeseed from cotyledon stage till maturity. The pathogen causes spots on cotyledons, leaves and pods, canker on stems and root crowns and lesions on upper stems (Gabrielson, 1983; Petrie, 1979; Paul and Rawlinson, 1992). During primary infection in the fall, the fungus releases ascospores from mature pseudothecia (Huang et al., 2003, Marcroft et al., 2003). Ascospore release lasts for 3-4 months, but it is most intensive in first two months (McGee, 1977, cit. Salam et al., 2003). In addition to ascospores, pycnospores too may cause disease symptoms (Gosende et al., 2003).

Depending on isolate and location of origin, on PDA, the fungus forms a white to greyish-white mycelium with round, brown pycnidia (Pound, 1947). Two types of pycnidia are formed: type 1 are brown scleroids with a narrowed down ostiole, and type 2 are round black pycnidia (Punithalingam and Holliday, 1972).

Material and Method

Isolation of the parasite. Samples of infected rapeseed plants have been collected in production fields around the Vojvodina Province. They were analyzed in the laboratory of phytopathology at Institute of Field and Vegetable Crops in Novi Sad. Isolation was done from plant parts (root crown, stem, leaf, pod) with clear symptoms of the disease. Pieces of infected tissue were dipped in a sublimate, rinsed with sterilized water and air dried. Isolation was done by applying small pieces of infected tissue on PDA medium.

Inoculation. Inoculation of young rapeseed plants was done at the stage of 2-4 leaves by spraying plants with a conidial suspension of *P. lingam*. The inoculated plants were kept in a plastic tunnel for 24 hours and then transferred to a greenhouse.

Mycological characteristics. Pure cultures served for the study of morphological and culture characteristics of the parasite as well as inoculation effectiveness.

To identify the species of the parasitic fungus, width and length were measured in 100 conidia and 100 pycnidia from 20-day cultures cultured on PDA at 25°C. Growth of *P. lingam* colonies was monitored on two media, PDA and oat agar. Condition, color and shapes of colonies were inspected visually.

Evaluation of disease incidence in the field. Rapeseed genotypes were evaluated for disease incidence in the second half of May 2005, on the scale 1-9:

1- resistant cultivar (genotype), no disease symptoms, disease incidence 0%
2- tolerant cultivar (genotype), disease incidence less than 2%
3- cultivar (genotype) prone to disease attack, disease incidence below 5%
4- cultivar (genotype) slightly sensitive, disease incidence below 10%.
5- cultivar (genotype) medium susceptible, disease incidence below 20%.
6- cultivar (genotype) susceptible, disease incidence below 40%.
7- cultivar (genotype) highly susceptible, disease incidence below 60%.
8- cultivar (genotype) very susceptible, disease incidence below 80%.

9- cultivar (genotype) exceptionally susceptible, disease incidence over 80%.

The evaluation method was issued by Ministry of Agriculture of the Republic of Serbia.

Disease index, i.e., disease attack intensity was calculated according to Bansal et al. (2002), using the formula: (disease severity×disease scale category)/(total number of plants).

Results

Symptoms. In Serbia, weak symptoms of black leg occur on rapeseed rosette in September and October. Pale to pale orange spots, round to irregular-shaped, occur on rosette leaves. Many fields are completely symptom-free. Next year, the earliest appearance of symptoms, on bottom leaves, is late March and early April. Later on, the spots enlarge, their central part turns grey and a dark ring appears at their edge. Two to three days after the central part of the spots turns grey, individual, round, shiny black pycnidia start to be formed. Later on the tissue in the spots cracks and drops out during rainy days. Spot size ranges from several millimeters to 2 cm in diameter. Here it should be mentioned that the non-infected part of the leaf remains green for a long time. Such leaves stay on the plant long, in some cases till maturity.

The pale grey spots on the root crown have a dark border. The tissue inside the spots cracks and lesions are formed. A longitudinal cross section shows that the parasite penetrates the crown to the pith, causing necrosis of inner tissues and the pith itself. Pycnidia develop on the mycelium at the surface of the root, but they are absent inside the root. The plants with infected root crown wilt and dry, failing to bring yield.

In addition to the above symptoms, the pathogen also attacks rapeseed stems and pods. Elongated, greyish-white to milk-white spots with a dark border occur mostly on the upper part of the stem. The spots typically appear before flowering or at the beginning of pod forming. The spots occurring on the pods are similar to those on the stem, but somewhat shorter. In both cases pycnidia develop inside the spots. They occur individually and are round, black and partly embedded in plant tissue, which are diagnostic signs for rapid determination of the parasite. Under humid conditions, pycnidia release pycnospores in the form of a pale pink gelatinous mass. *Leptosphaeria maculans*, the perfect stage of the fungus, has not been registered yet in Serbia.

Pathogenicity of isolates. Of the five isolates used for inoculation, four caused leaf spots after 10 days and one after 13 days. Reisolations from the infected tissues produced the identical fungus.

Morphological and culture characteristics of the parasite. On PDA medium, the mycelium develops slowly, forming round to wedge-shaped colonies. The aerial mycelium is sparse, loose, whitish to grey. The substrate mycelium was grey-whitish. All isolates release dark brown pigment into the medium. A somewhat better growth was registered in the isolate that caused the occurrence of disease symptoms 13 days after inoculation. After 7 days, the fungus formed round, dark brown to black pycnidia whose size was 185-384μ×220-510 μ. The pycnidia occurred individually at first, forming stromata later on. Pycnospores were single-celled, hyaline, short cylindrical, mostly strait but some slightly bent, with oil drops on both end, their size ranging around 1.25-2.36 μ×2.60-5.70 μ.

On oat-agar medium, the colonies were round-shaped. After 15 days, their diameter was 9 cm, i.e., they filled the entire petri dish. The aerial mycelium was white at the beginning, somewhat more compact than that developing on PDA, turning light brown later on. Pigment color in this medium is somewhat lighter. Pycnidia occur individually and are typically round and light brown. Pycnospores correspond in shape, color and size to those growing on PDA.

Assessment of genotypes

Table 1 shows disease indices for different parets of rapeseed plant expressed in percents. It should be mentioned here that this paper review a part of the breeding material used at Institute of Field and Vegetable Crops in Novi Sad.

The results presented in Table 1 exhibit the intermediacy of the tested genotypes. Based on disease intensity percentages, the genotype R – 47 could be considered as susceptible while the genotypes R – 52 and R – 53 exhibited high resistance to *P. lingam*. The other genotypes, depending on plant organ observed, showed high or low resistance to black leg. All genotypes in Table 1 were free from symptoms on the stem.

Discussion

The basic reason for a low occurrence of spots on rosette leaves in September and October was most probably the absence of the teleomorph stage of the fungus, which is not the case in Great Britain (Hammond et al., 1985) or Australia (Selam et al., 2003). Sporadic occurrence of spots in the fall may be the results of seed contamination or contamination with pycnospores from the surrounding flora. Both ascospores and pycnospores can infect rosette leaves of rapeseed (Gosende et al., 2003). The increase in rapeseed acreage in Serbia may increase the infection pressure, like it happened in Australia (Marcroft et al., 2003), which may result in a higher percentage of infected plants in the field. The symptoms we observed on root crowns, leaves, stems and pods were similar to those reported by Gabrielson (1983), Petrie (1979) and Paul and Rawlinson (1992), but not to those reported by Gaetan (2005). The appearance and size of pycnidia and pycnospores were not essentially different from the data of Punithalingham and Holliday (1972) and Gabrielson (1983).

Because of a small number of studied isolates there were no differences in virulence, although Pedras et al. (1996) and Mitrović (1997) reported the presence of virulent and avirulent strains.

Disease indices in the field

Genotype	Root crown	Stem	Leaf	Pod
R – 46	1.48	1	1	1
R – 47	3.64	1	1	1.43
R – 48	2.69	1	1	1
R – 49	1.73	1	1	1
R – 50	2.21	1	1	1
R – 51	1.48	1	1	1
R – 52	1	1	1	1
R – 53	1	1	1	1
R – 54	1.24	1	1.93	1
R – 55	1.24	1	1.65	1
R – 56	2.21	1	2.84	1

References

Bansal, K. V., Blenis, P., Sringam, G. R., Thiagarujah, M. R. and J. P. Tewari, 2002. Screening of *Brassica napus* against black leg caused by *Leptosphaeria maculans*: effects at inoculum concentration, subculturing of the pathogen and time of disease screening. Can. J. Plant Pathol. 24: 323-326.

Gabrielson, R. L., 1983. Blackleg disease of crucifers caused by *Leptosphaeria maculans* (*Phoma lingam*) and its control. Seed Science and Technology 11: 749-780.

Gaetan, A. S. 2005. First outbreak of blackleg caused by *Phoma lingam* in commercial canola fields in Argentina. Plant Disease 89: 435.

Gladers, P. and T. M., Musa, 1979. The development of *Leptospheria maculans* in winter oilseed rape and its implications for disease control. Pests and Diseases: 129-136.

Gosende, S., A. Penaud, N. J. Aubertat, O. Schnieder and X. Pinochet, 2003. Evolution of soil surface oilseed rape stubbles and their ability to produce spores of *Leptosphaeria maculans*: Preliminary results. 11th International Rapeseed Congress, Proceeding 4, AP 11. 14: 1166-1168 Denmark.

Hammond, K. E., B. G., Lewis and T. M. Musa, 1985. A systemic pathway in the infection of oilseed rape plants by *Leptosphaeria maculans*. Plant Pathology 34:557-565.

Howlett, B. J., A. Idnurm and M. S. Pedras, 2001. *Leptospheria maculans*, the causal agent of blackleg disease of *Brassicas*. Fungal Genet. Biol. 33: 1-14.

Huang, Y., B. Fitt, Y. Steed and A. Hall, 2003. Maturation of *Leptosphaeria maculans* pseudothecia in relation to forecasting phoma leaf spots (stem canker) on oilseed rape. 11th International Rapeseed Congress, Proceeding 4, AP 11. 9: 1153-1156 Denmark.

Marcroft, S., S. Sprague, P. Salisburg and B. Howlett, 2003. Survival and dissemination of *Leptospheria maculans* in southern Australia. 11th International Rapeseed Congress, Proceeding 4, AP 11. 11: 1157-1159 Denmark.

Mitrović, P. (1997). Paraziti kupusa. Magistarska teza, Poljoprivredni fakultet, Novi Sad.

Solam, U. M., K. R. Khangura, J. Diggle and J. M. Barbetti, 2003. Blackleg Sporacle: An aid to understanding and managing blackleg in canola (*Brassica napus*). 11th International Rapeseed Congress, Proceeding 4, AP 11. 3: 1141-1143 Denmark.

Paul, V. and C. J. Rowlinson, 1992. Diseases and pests of rape. Verlag Theodore Mann, Gelsenkirchen- Buer, Germany.

Pedras, C. S. M., L. J. Taylor, M. V. Morales, 1996. The blackleg fungus of rapeseed: How many species. Acta Hort. (ISHS) 407: 441-446.

Petrie, G. A., 1979. Blackleg of rape. Canadian Agriculture, 24: 22-25.

Pound, S. G. 1947. Variability in *Phoma lingam*. Journal of Agricultural Research, Vol. 75, No 4, Washington, D. C.

Punithalingam, E., P. Holliday, 1972. *Phoma lingam*. CMI Descriptions of pathogenic fungi and bacteria. No 331.