

Effect of residue type and burial depth on survival of *Mycosphaerella pinodes* in Manitoba

J.X. Zhang, A.G. Xue, and W.G.D. Fernando

Abstract: Survival of *Mycosphaerella pinodes* in leaf and stem residues placed at 0, 5, and 10 cm below the soil surface was examined at Morden and Winnipeg, Manitoba, from October 1999 to October 2000. Survival was measured as production of spores from residue and pathogenicity of residue washings on the susceptible field pea cultivar 'AC Tamor'. Spore production on the residues decreased over the 12-month sample-retrieval period, regardless of residue type and burial depth. Spores were rarely produced from residues after 11 months at Morden and 9 months at Winnipeg. In general, more spores were produced on leaf residues than on stem residues. No or few lesions developed from residue washings after 9 months for leaf residue and 8 months for stem residue at both locations. Residues on the soil surface had greater spore production and subsequently produced greater disease severities on pea plants inoculated with residue washings than those of the buried residues. There was no statistical difference in spore production and disease severity produced by residue washings between residues buried 5 and 10 cm deep.

Key words: mycosphaerella blight, *Mycosphaerella pinodes*, field pea, *Pisum sativum*, survival.

Résumé : D'octobre 1999 à octobre 2000, la survie du *Mycosphaerella pinodes* dans des débris de feuilles ou de tiges enfouis à 0, 5 ou 10 cm de profondeur a été étudiée à Morden et à Winnipeg, Manitoba. La survie a été mesurée par la production de spores issues des débris et par le pouvoir pathogène de solutions de lavage de débris sur le cultivar sensible de pois protéagineux 'AC Tamor'. La production de spores sur les débris a diminué au cours de la période de 12 mois de récupération des échantillons, peu importe le type de débris et la profondeur d'enfouissement. Des spores se sont rarement formées sur les débris après 11 mois à Morden et 9 mois à Winnipeg. Généralement, il y a eu plus de spores produites sur les débris de feuilles que sur ceux de tiges. Aux deux endroits, avec les solutions de lavage, il y a eu peu ou pas du tout de lésions qui se sont développées après 9 mois pour les débris de feuilles et après 8 mois pour les débris de tiges. Par rapport aux débris enterrés, les débris en surface du sol ont produit plus de spores et, par la suite, une intensité de maladie plus grande sur des pois inoculés avec les solutions de lavage. Il n'y a pas eu de différence statistique entre les débris enfouis à 5 cm et ceux à 10 cm quant à la production de spores et l'intensité de maladie causée par les solutions de lavage.

Mots clés : ascochytose, *Mycosphaerella pinodes*, pois protéagineux, *Pisum sativum*, survie.

Introduction

Mycosphaerella blight, caused by *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr., is the most important disease of field pea (*Pisum sativum* L.) in western Canada (Warkentin et al. 2000; Xue and Warkentin 2001; Xue et al.

2003). This disease causes yield losses of 10% annually and more than 50% in field trials (Wallen 1974; Xue et al. 1997). Yield reduction from *M. pinodes* is the result of leaf and stem infection by pycnidiospores and ascospores arising from residues of previous pea crops, and secondary infection spreading from diseased plants at later stages of plant development (Bretag and Ramsey 2001; Xue et al. 1997). *Mycosphaerella pinodes* survives between seasons by means of thick-walled mycelia, chlamydospores, and sclerotia in host residue on the soil surface or buried in soil (Bretag and Ramsey 2001; Sheridan and Dickinson 1968). Survival of the fungus can vary from 4 months to more than 20 years (Carter and Moller 1961; Cruickshank 1952; Wallen et al. 1967), depending on environmental conditions. The impact of residue type and burial depth on pathogen survival was first examined by Wade (1951), who

Accepted 21 October 2004.

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reported that deep burial of diseased residue enhanced pathogen survival in soil in Tasmania. Cruickshank (1952) observed that the pathogen survived less than 4 months in the absence of a host in New Zealand, suggesting that the extent of survival was related to the persistence of host residues. Sheridan (1973) showed that mycelium was capable of growing saprophytically on pea root in competition with normal soil microflora for at least 18 months in Ireland. Davidson et al. (1999) in Australia demonstrated that pea stubble placed on the soil surface or buried 4 cm deep, remained highly infectious only during the first 4 months, and subsequently did not cause significant disease on seedling crowns after this time. Little is known about the survival of *M. pinodes* in western Canada. This information can be important for formulating disease management strategies, especially where there are no resistant cultivars available (Xue and Warkentin 2001). The objectives of this research were to determine how the duration of *M. pinodes* survival is affected by residue type and depth of burial (on or below the soil surface) in soil in Manitoba, Canada.

Materials and methods

Diseased leaves and stems that were naturally infected with *M. pinodes* were collected from experimental fields at the Morden Research Centre, Agriculture and Agri-Food Canada, Morden, Manitoba, in August 1999 and stored at 3 °C until used. Intact leaf and stem segments (3 cm long) were put into separate nylon mesh bags (10 cm × 10 cm), sealed, and tied to plastic stakes at 0, 5 and 10 cm below the soil surface. At each burial depth, there were three sealed bags each for leaves and stems attached to each stake. Forty-eight stakes, each separated by 30 cm, were placed in the field with clay loam soil at Morden and loam soil at the Research Farm, University of Manitoba, Winnipeg, Manitoba, on 18 October 1999.

Plant residue samples were retrieved at monthly intervals from 18 November 1999 to 18 October 2000. On each sampling date, four stakes from each field (Morden and Winnipeg) were randomly selected and treated as four replicates for treatments of residue type and burial depth. Retrieved bags were rinsed under running water to wash off adhering soil, dried with paper towels, and kept in a moist glass chamber for a week at room temperature, to induce production of fungal fruiting bodies and spores on the residues. The three bags of the same plant residue from each stake at the same burial depth were pooled together as one replicate, from which 0.5 g of leaf or stem residue was placed into a 50-mL sterile test tube, soaked in 10 mL of sterile distilled water containing 0.05% Tween® 20 (polyoxyethylene sorbitan monolaurate) (Fisher Scientific, Ottawa, Ontario) for 30 min, ground with a round-tipped glass rod for 5 min, and shaken on an orbital shaker at 210 rpm for 1 h to dislodge spores from the existing and newly developed fungal fruiting bodies. The resulting spore suspension (pycnidiospores and ascospores) was filtered through two layers of cheesecloth and the concentration determined with a hemacytometer.

The pathogenicity of the spores recovered from residue samples was evaluated on susceptible field pea cultivar 'AC

Tamor'. Plants were grown in 20-cm diameter plastic pots containing a mixture of soil, sand, and peat (2:2:1 by volume) in a growth room at 20 °C with a 14-h photoperiod at light intensity of 360 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Plants were thinned to four per pot after emergence and inoculated at the 4–6 node stage, approximately 3 weeks after emergence. Each plant was inoculated with 0.5 mL of the spore suspension from the residue washings using a DeVilbiss Model 15 atomizer (The DeVilbiss Co., Somerset, Pennsylvania, USA). Inoculated plants were allowed to dry for 30 min and then transferred to a humidity chamber in the growth room for 48 h. The humidity chamber was maintained at or near 100% relative humidity by the continuous operation of an ultrasonic humidifier. Plants were subsequently returned to the growth room for incubation. For each residue type and burial depth combination, four replicate pots were used. Pots were arranged in a randomized complete block design in both the humidity chamber and the growth room.

Symptoms of mycosphaerella blight on the inoculated plants were rated for disease severity 10 days after inoculation using a 0–5 scale modified from Nasir and Hoppe (1991) and Xue et al. (1998). Disease severity was estimated visually: 0, symptomless; 1, few small flecks on leaves; 2, <20% of leaf area covered by lesions, but no symptoms on stem; 3, numerous necrotic flecks and a few large lesions on leaves and stems, covering <50% of inoculated plant area; 4, large, coalescing lesions on leaves and stems, covering >50% of inoculated plant area, plant surviving; 5, large, coalescing lesions on leaves, girdling lesions on stems, plant withering and dying. Disease severity scores and a log transformation of spore concentration from the residue washing were statistically analyzed with SAS/STAT® (SAS Institute Inc., Cary, North Carolina), to determine effects of residue type, burial depth, and sample retrieving date. Where treatment effects were significant, treatment means were separated by the least significant difference (LSD) test at a probability level of $P \leq 0.05$.

Results and discussion

The effects of residue type, burial depth, sample retrieving date, and residue type × sample retrieving date interaction were significant ($P < 0.05$), while the residue type × burial depth interaction was not significant on spore production and disease severity at both Morden and Winnipeg (Table 1). The interactions of burial depth × sample retrieving date and residue type × burial depth × sample retrieving date were significant in disease severity, but not in spore production at either location.

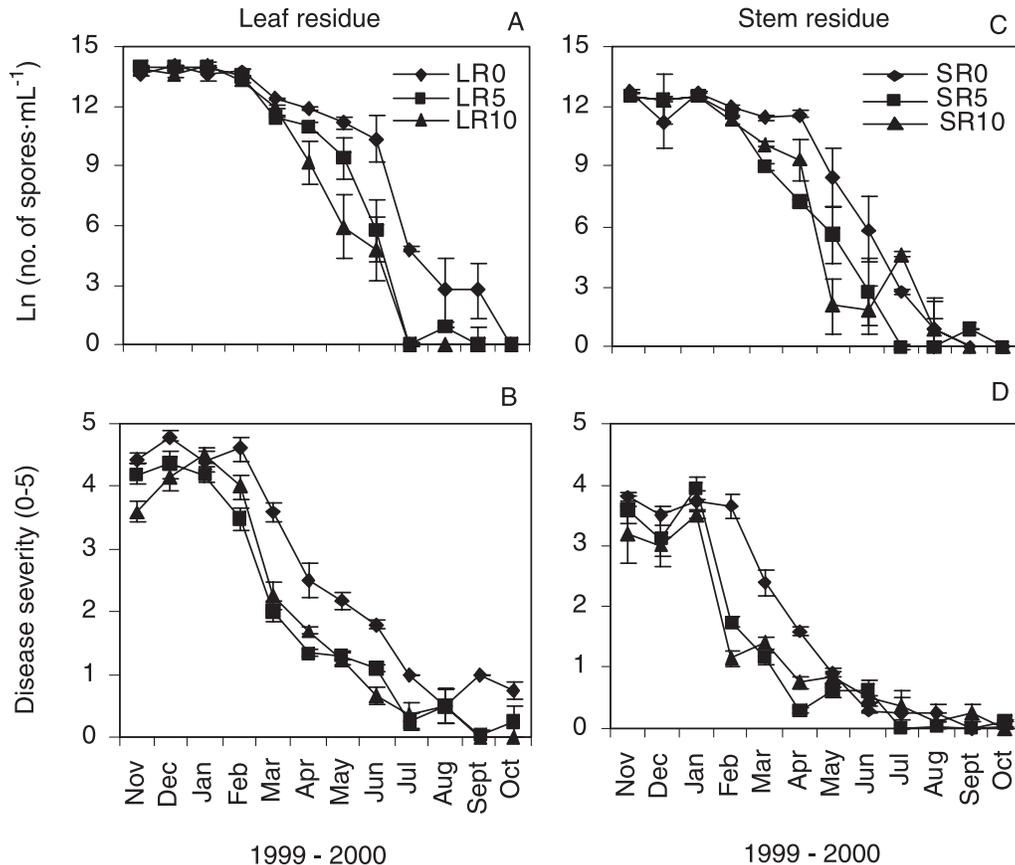
Spore production and corresponding disease severity of both leaf and stem residues decreased over the 12-month sample retrieving period regardless of burial depth at Morden and Winnipeg (Figs. 1 and 2). Spores were rarely produced from residues after 11 months at Morden and 9 months at Winnipeg. Leaf residues produced more spores and subsequently, washings from leaf residues caused more severe disease symptoms than stem residues. No or few lesions developed from residue washings after 9 months from leaf residue and 8 months from stem residue at both locations. Residues on the soil surface had greater spore produc-

Table 1. Mean squares from analysis of variance for spore production and disease severity of *Mycosphaerella pinodes* in field pea (*Pisum sativum*) as affected by residue type, burial depth, and sample retrieving date in Manitoba from 1999 to 2000.

Source of variation	df	Spore production		Disease severity	
		Morden	Winnipeg	Morden	Winnipeg
Residue type (RT)	1	141.8**	122.3**	40.1**	6.8**
Burial depth (BD)	2	58.7**	34.9*	10.7**	5.8**
Sample retrieving date (SRD)	11	724.3**	664.5**	56.3**	58.0**
RT × BD	2	6.1	9.9	0.6	0.3
RT × SRD	11	11.1*	11.2*	1.3**	7.5**
BD × SRD	22	9.4	4.1	0.7**	5.8**
RT × BD × SRD	22	3.4	4.3	0.4**	3.7**
Error	216	3.6	4.0	0.1	0.1

Note: *, statistical significance at $P < 0.05$; **, statistical significance at $P < 0.01$.

Fig. 1. Effect of residue type, burial depth, and sample retrieval date on spore production and pathogenicity of *Mycosphaerella pinodes* in field pea (*Pisum sativum*) at Morden, Manitoba, from 1999 to 2000. (A) Spore production from leaf residue on the soil surface (LR0), or buried 5 (LR5) or 10 cm (LR10) deep. (B) Disease severity caused by leaf residue washings. (C) Spore production from stem residue on the soil surface (SR0), or buried 5 (SR5) or 10 cm (SR10) deep. (D) Disease severity caused by stem residue washings. Vertical bars indicate LSD at $P = 0.05$.

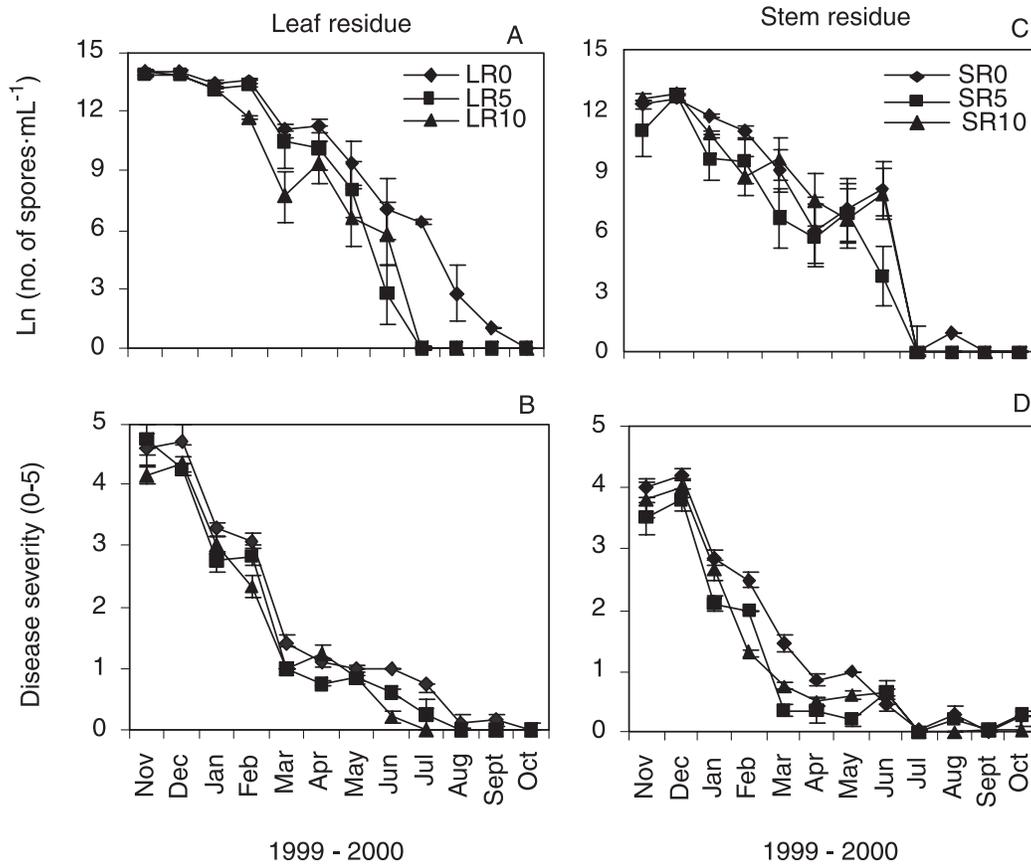


tion and their residue washings resulted in greater disease severities than those of the buried residues. There was no statistical difference in spore production and disease severity caused by residue washings between residues buried 5 and 10 cm deep.

This study demonstrated that survival of *M. pinodes* as measured by its ability to produce spores decreased over a 12-month sample-retrieval period regardless of residue type,

burial depth, and location. Although measurable levels of spore production and disease severity were detected during the first 8–9 months after residue burial, severe disease development (severity ≥ 3) resulting from residue washings was observed only for the first 4 months after burial at Morden and for the first 2 months at Winnipeg. These results were generally in agreement with Davidson et al. (1999), who reported that residues placed on or below the

Fig. 2. Effect of residue type, burial depth, and sample retrieval date on spore production and pathogenicity of *Mycosphaerella pinodes* in field pea (*Pisum sativum*) at Winnipeg, Manitoba, from 1999–2000. (A) Spore production from leaf residue on the soil surface (LR0), or buried 5 (LR5) or 10 cm (LR10) deep. (B) Disease severity caused by leaf residue washings. (C) Spore production from stem residue on the soil surface (SR0), or buried 5 (SR5) or 10 cm (SR10) deep. (D) Disease severity caused by stem residue washings. Vertical bars indicate LSD at $P = 0.05$.



soil surface remained highly infectious during the first 4 months, but did not cause a significant level of disease thereafter. However, length of survival observed in the present study was much shorter than those reported by Wallen et al. (1967), who isolated the pathogen from soil that had not been sown with peas for 20 years, and Sheridan (1973), who detected infectious mycelia of *M. pinodes* in host residues on the soil surface or those buried 15 or 25 cm deep for up to 18 months. The difference in the extent of pathogen survival between the two locations in the present study and from the other studies is likely the result of varying environmental conditions, which would affect the rates of residue decomposition. Cruickshank (1952) reported that the survival period of *M. pinodes* is shortened in the absence of a host, suggesting that survival period is related to the decomposition of host residues.

It appeared that *M. pinodes* survived better in residue on the soil surface as compared with residue buried in soil. Surface residue was drier and less decomposed than buried residue when collected during winter and spring months and produced more viable spores resulting in more severe disease development than buried residue (Figs. 1 and 2). In western Canada, spores produced from surface residue are the major source of inoculum for mycosphaerella blight in field pea (Xue et al. 1996). Some cultural practices such as

plowing surface residue below the soil surface and cleaning pea residue from the field could be effective in reducing initial inoculum and managing mycosphaerella blight for the next crop. Further research is needed to confirm the effect of moisture and temperature on residue decomposition and the survival of *M. pinodes* in soil in Canada.

Acknowledgements

This research was funded by the Manitoba Pulse Growers Association and the Agri-Food Research and Development Initiative. We thank Dr. T.C. Paulitz for valuable suggestions on data analysis and advice on the manuscript.

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