Blackleg disease (*Leptosphaeria maculans*) on oilseed rape—evidence for it being a polycyclic disease in Australia and implications for disease management

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Abstract

Blackleg, caused by *Leptosphaeria maculans,* is a major disease of oilseed rape worldwide, including Australia. Generally, the pathogen cycle is considered to be monocyclic, with the windborne ascospores (sexual) released from infested stubbles being the primary source of inoculum for the disease. Outside of Australia, the role of pycnidiospores (asexual) has generally been assumed be minor in disease epidemics, primarily because under controlled conditions, high concentrations of pycnidiospores are needed for reliable infection compared with the reliable and severe infection produced even with relatively low concentrations of ascospores. Although pycnidiospores are produced from stubbles from the beginning of the season and continue to be produced in abundance throughout the cropping season, major ascospore showers in Western Australia predominantly occur in the first half of the cropping season. Various attempts over several decades have been made to define the role pycnidiospores in the epidemiology of blackleg disease in Western Australia. Early studies in Western Australia implicated an important role played by pycnidiospores, and that this role may be largely defined by weather conditions and timing of release of these spores. Recent studies in Western Australia have shown that pycnidiospores, even at relatively low concentrations, can reliably cause severe disease provided the same seedling, but on different cotyledons, is co-inoculated with ascospores. It is noteworthy that ascospores even at low levels approximating 5 per cotyledon could be sufficient to allow pycnidiospores to initiate severe disease, even when pycnidiospore levels on the other cotyledon also are as low as approximately 1 spore per cotyledon. This is a significant finding and at least partly explains the occurrence of severe epidemics at times in the oilseed rape cropping season when ascospore showers have been shown to be relatively low. It may also explain why field epidemics initiated by pycnidiospores alone develop relatively slowly. A particular area that requires further study is in relation to determining the relative roles of ascospore and pycnidiospore epidemic components in the production of crown cankers. Overall, the results of studies undertaken in Western Australia may explain why continuing severe blackleg disease cycles occur there throughout the cropping season even when ascospore fallout is low or constrained only to a brief period or phase of the cropping season. Taken together, the evidence suggests that disease epidemics, at least in Australia, can be polycyclic rather than monocyclic. These findings could have significant implications in the field management of the disease, for example, involving the need for extended periods of application or activity of fungicides and/or utilization of high levels of adult host resistance. Additionally, the findings from Western Australian studies over the past three decades could have more wide-ranging implications, including for other ascomycete necrotrophic pathogens (*e.g., Mycosphaerella pinodes* on *Pisum* spp.) that also produce both ascospores and pycnidiospores during the cropping cycle.

Key words: blackleg, canola, oilseed rape, *Leptosphaeria maculans*, ascospores, pycnidiospores, polycyclic, *Mycosphaerella pinodes*

Introduction

The ascomycete fungus, *Leptosphaeria maculans* (Desm.) Ces. et de Not. (anamorph: *Phoma lingam*), commonly known as the blackleg pathogen, has the ability to infect a number of cruciferous crops. Blackleg disease causes severe yield losses of oilseed rape (*Brassica napus* L.) world-wide (Barbetti, 1975*a*; Gugel and Petrie, 1992; West *et al*., 2001). The pathogen cycle is generally considered to be monocyclic (West *et al*., 2001), with the windborne ascospores (sexual) released from infested stubbles being the primary source of inoculum for the disease (Brunin and Lacoste, 1970; Bokor *et al*., 1975). Pycnidia, produced on infested stubble and on cotyledon, leaf and stem lesions on infected plants during the growing season, produce pycnidiospores (asexual) that can be dispersed via direct contact or rain splash (Hall, 1992). Although pycnidiospores are produced from stubbles from the beginning of the season and continue to be produced in abundance throughout the season, major ascospore showers predominantly occur in the first half of the growing season. Pycnidiospores are considered to be the secondary source of inoculum (Hammond *et al.,* 1985), and are usually required in higher concentrations than ascospores to ensure lesions are produced (Barbetti, 1976; Wood and Barbetti, 1977). Although attempts have been made to define the role of ascospores and pycnidiospores in the epidemiology of blackleg disease (*e.g*., Brunin and Lacoste, 1970; Barbetti, 1976, respectively), the role of pycnidiospores still remains unclear. In Australia, pycnidiospores have been implicated to play an important role (Barbetti, 1975*b*, 1976), and this may be directly related to weather conditions and timing of release of these spores. However, outside of Australia, the role of pycnidiospores has generally been assumed be minor in disease epidemics (West *et al*., 2001). One of the reasons for this is that, under controlled conditions, high concentrations of pycnidiospores are

needed for reliable infection compared with reliable and severe infection produced even with relatively low concentrations of ascospores (Wood and Barbetti, 1977). The role of pycnidiospores in disease epidemics clearly warrants investigation, especially in relation to epidemics which are not closely associated with heavy ascospore showers (Barbetti 1975*b*). This paper reports the results of recent studies by Hua Li *et al*. (2006b) that provided significant new insights into the role that can be played by pycnidiospores in circumstances where there is the presence of ascospores on a different cotyledon of the same plant. The implications of these new findings are discussed.

Materials And Methods

In three separate experiments Hua Li *et al.* (2006b) determined the potential of pycnidiospores to cause severe disease in the presence of ascospores. Across the three experiments, they evaluated the time of co-inoculation of pycnidiospores with ascospores, the resistance level of cultivars, and the concentration of pycnidiospores needed to produce reliable infection. To avoid additive effects, they inoculated the two spore types onto separate cotyledons of each *B. napus* seedling. Their first experiment utilized the historical cultivar Oro, a cultivar that had been utilized commercially in Australia in the 1970's when the oilseed rape industry collapsed following severe blackleg epidemics, was used. Their second experiment utilised the commercial cultivar Surpass 400, containing single dominant gene-based resistance derived from *B. rapa* ssp. *sylvestris*. In their third experiment, two cultivars were used, *viz*. cv. Westar, a highly susceptible Canadian cultivar; and cv. Dunkeld, an Australian commercial cultivar expressing polygenic resistance at seedling (including cotyledon) and/or adult plant stages depending upon the environment (Hua Li *et al*., 2006a).

Results

In their first experiment, Hua Li *et al.* (2006) found that there were >3 times more cotyledons with lesions from pycnidiospores in the presence of ascospores compared with absence of ascospores. In their second experiment, mean lesion size from pycnidiospore infection was >1.5 time greater in the presence of ascospores than in the absence of ascospores. In their third experiment, while pycnidiospores on their own, even at high concentrations, were generally unreliable in producing significant disease symptoms, pycnidiospores caused significantly more disease, even at very low concentrations, but only on the proviso that ascospores were present on the same plant.

Discussion

The studies by Hua Li *et al.* (2006b) clearly demonstrated for the first time that pycnidiospores, even at low concentrations, could reliably cause severe blackleg disease, provided the same seedling had different cotyledons co-inoculated with ascospores. This finding offers the first explanation of why severe epidemics can occur at times in the oilseed rape cropping season in Western Australia even when ascospore showers have been shown to be relatively low (*e.g*., Barbetti, 1975*b*), and as illustrated diagrammatically in Figure 1. It also offers an explanation as to why field epidemics in Western Australia that are initiated solely by pycnidiospores develop relatively slowly in comparison (Barbetti, 1976).

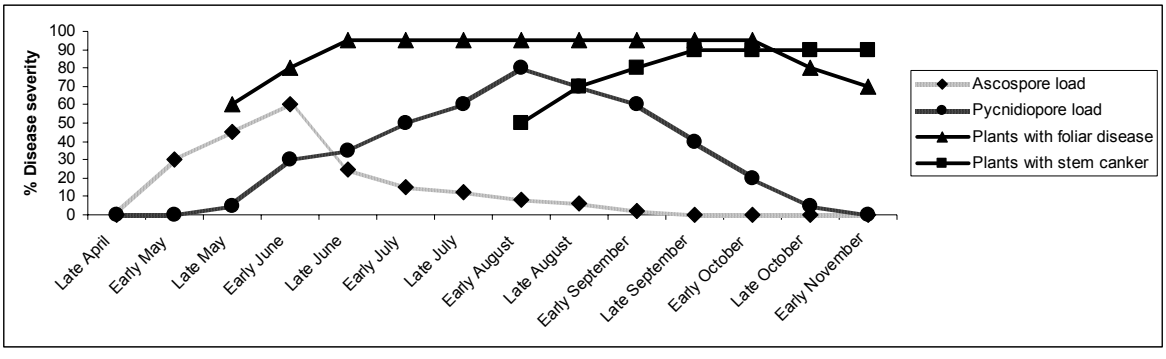

Figure 1. Diagrammatic representation of the hypothetical potentials for ascospore- and pycnidiospore-initiated disease epidemics in Western Australia. The hypothetical disease severity levels (0-100%) in relation to spore type and spore load.

Outside of Western Australia, pycnidiospores are generally considered to be, at best, of limited importance in the disease epidemiology. This current consideration outside of Western Australia that the involvement of pycnidiospores is non-significant in field epidemics appears to be based upon two facts. Firstly, that, in the absence of ascospores, large doses of pycnidiospore inoculum are required for the development of reliably severe disease symptoms (*e.g*., Wood and Barbetti, 1977) and, secondly, because severe epidemics are frequently associated with periods of release of ascospores (*e.g*., Brunin and Lacoste, 1970; Barbetti, 1975*a*; Salam *et al*., 2003), especially during the seedling phase when the host is particularly susceptible (Barbetti 1975*a*; Hua Li *et al*., 2004*a,b*, 2005). This may be due to the fact that field observations give no indication of the polycyclic nature of the epidemic, if that was in fact what was occurring. This is further complicated by the fact that the latency of the pathogen in early infection processes makes it difficult to pinpoint the actual time of infection. It is noteworthy in the studies by Hua Li *et al*. (2006b) that even at pycnidiospore concentrations as low as 100 pycnidiospores per mL (which in their studies related to approximately 1 spore per cotyledon inoculation site) the severity of the disease was markedly increased in the presence of ascospores elsewhere on the same seedling. Not only is this the first report of this