# New Molecular Tools for Diagnosis of Light leaf spot on Winter Oilseed Rape

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Variation in the severity of epidemics of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape between seasons and between regions within the UK may have contributed in the past to the inappropriate application of fungicides (Fitt *et al.*, 1996). To more accurately determine whether or not a crop should be sprayed to control the disease, work done in collaboration between IACR-Rothamsted and the Department of Plant Sciences at Cambridge University has produced two useful PCR-based diagnostic tools for this important pathogen of winter oilseed rape. One of the tools was specific to *P. brassicae* (Foster *et al.*, 1999) and was used to identify infection prior to the development of disease symptoms. The second tool was also specific to *P. brassicae* and was able to differentiate the two mating types of the fungus. Such tools will be of great benefit in the management of light leaf spot and, when used in conjunction with a forecasting scheme (Fitt *et al.*, 1996), will facilitate the diagnosis of the disease at the time when spray decisions need to be made.

The *P. brassicae* general diagnostic PCR primers, Pb1 and Pb2 (Foster *et al.*, 1999) were shown to be specific to *P. brassicae* and capable of amplifying the specific marker from all isolates tested and from infected plant material. This PCR-based diagnostic tool will be invaluable in the correct diagnosis of light leaf spot. Light leaf spot can be difficult to identify on crop plants (Fitt *et al.*, 1998a). The majority of the symptoms, such as chlorosis and necrosis of infected areas, are also characteristic of infection by a number of other fungal pathogens of oilseed rape, and the white pustules (conidiomata), which contain the conidia and are the only reliable characteristic symptoms of this disease, are often not visible after periods of rain.

The PCR-based diagnostic technique has many advantages over current, more traditional methods of diagnosis which involve the isolation of the fungus from infected leaves, or the incubation of leaves in polyethylene bags to promote the production of the characteristic conidiomata (Fitt *et al.*, 1998a). The PCR-based technique is faster; analysis can be performed in a few hours, as opposed to incubation of leaf samples for a number of days. The PCR-based technique is also more sensitive; using a nested PCR approach, the *P. brassicae* diagnostic assay was sufficiently sensitive to detect the presence of the fungus in pre-symptomatic oilseed rape tissue which did not show signs of infection even after incubation for five days. Hence, this technique is potentially of great importance for the identification of infected plants early in the autumn when decisions regarding the application of fungicides to treat the disease need to be made.

In an ideal situation, the diagnostic tool would be best employed to monitor individual crops at regular intervals throughout the season to determine the presence or absence of disease. However, this would entail a large amount of work and expense to process the large number of plant samples necessary to provide an accurate indication of the incidence of light leaf spot on several different occasions. Furthermore, the assay currently requires a substantial amount of sample processing to extract DNA of suitable quality for use in the assay. Even if the sample processing could be reduced by the use of a cruder method of DNA extraction, the PCR assay itself has to be carried out in a laboratory by staff with the necessary expertise. Consequently, the

technique is limited in that it cannot be used by non-experts such as farmers and would require plant samples to be sent to a laboratory for testing. This would increase both the cost of the assay and the speed with which diagnosis could be given.

Although it may not be feasible for the *P. brassicae* specific diagnostic tools to be employed for the large-scale monitoring of light leaf spot within oilseed rape crops, the techniques could be useful for other purposes. The nested PCR approach was sufficiently sensitive to be capable of detecting as few as 10 *P. brassicae* genomes, the equivalent of 10 *P. brassicae* conidia or ascospores. This level of sensitivity could prove to be sufficient to use the diagnostic tool to identify the presence of light leaf spot inoculum prior to infection. Mathematical modelling is currently being used to describe the progress of light leaf spot epidemics on winter oilseed rape (Papastamati *et al.*, 1999) and it is hoped that this information will eventually be combined with data from the light leaf spot forecasting system to identify periods during which the environmental conditions are conducive to infection. One of the most important conclusions from the modelling work is that the timing of the appearance of inoculum, in the form of either air-borne ascospores or splash-dispersed conidia, must be known for the model to accurately predict the onset of disease.

Traditionally, the detection of air-borne spore inoculum has been achieved using spore traps which rely on the impaction of air-borne spores onto a solid surface. The surfaces upon which the spores are impacted are then examined under a microscope to determine which fungal species are represented in the air sampled. This technique has obvious drawbacks in that substantial expertise is required to reliably identify the different spores present, especially when species with morphologically similar spores may be present at the same time. Recently, the technique of spore trapping has been combined with antibody-based diagnostic assays to simplify the process of identifying trapped spores (Kennedy *et al.*, 1999; Schmechel *et al.*, 1994; 1996). It has been recognised for some time that the PCR technique also offers potential for simplifying and increasing the specificity and sensitivity of detection of air-borne fungal spores (McCartney *et al.*, 1997). Preliminary work has shown that it is possible to use the *P. brassicae* specific PCR assay to detect *P. brassicae* ascospores from DNA extracted from large populations of fungal spores trapped using a Burkard spore trap (C. Calderon & H. A. McCartney, IACR-Rothamsted, pers. comm.). Further work is currently being done to assess the possibility of using spore trapping in combination with the *P. brassicae* diagnostic PCR assays to predict the onset of light leaf spot epidemics.

Mating type specific PCR primers, PbM-1-3 and PbM-2, were also developed in this work. These can be used to reliably differentiate between the two mating types of *P. brassicae* and simultaneously function as diagnostic primers for *P. brassicae*. This is the first report of the development of a diagnostic tool able to combine these two functions. This assay will be of use in the rapid identification of the mating type of isolates of *P. brassicae* and could be used to perform a large-scale survey of mating types directly from lesions on infected leaves. This information is useful for the management of light leaf spot epidemics as it is currently thought that the ascospores of *P. brassicae* are likely to be responsible for initiating light leaf spot epidemics in the autumn (Gilles & Fitt, 1999). The ability to determine the presence of both mating types within a crop may therefore provide us with information that could be used to predict the availability of ascospores, which could initiate epidemics in the following season.

In conclusion, this study has resulted in the development of two useful PCR-based diagnostic tools for *P. brassicae*, which will undoubtedly be of use in studying the epidemiology of *P. brassicae*. However, these tools are currently too unwieldy to be of general use to non-experts. Although improvements could be made to the assay, particularly with respect to the extent of sample processing necessary, the technique will still be inherently lab-bound. However, steps

have recently been taken towards the development of an immuno-diagnostic tool for *P. brassicae* using a targeted molecular approach and such a tool may eventually be converted into a 'farmer-friendly' diagnostic kit.

For further information about these diagnostic tools, please e-mail simon.foster@bbsrc.ac.uk

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