Development of a rapid technique for early detection of *Pyrenopeziza brassicae* (light leaf spot) on winter oilseed rape in the U.K.

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Correct diagnosis of crop disease is important in agricultural practice, allowing appropriate control measures to be applied before unnecessary losses are incurred. Traditionally, disease diagnosis has relied upon recognition of disease symptoms or identification of the causal organism morphologically after isolation from infected plants. Both methods have drawbacks. Similar symptoms caused by different pathogens may lead to misdiagnosis and use of inappropriate control measures. By the time that visual symptoms are seen, the disease is often at such an advanced stage that control measures have little effect on yield losses. Recently, use of molecular techniques has enabled the detection and diagnosis of plant pathogens to be done more rapidly and efficiently than was previously possible using the traditional methods. In particular, the fact that such techniques have the potential to detect extremely small amounts of the pathogen, for example a single fungal spore, enables crops at risk to be identified prior to the onset of disease symptoms.

Light leaf spot, caused by the pathogenic fungus *Pyrenopeziza brassicae*, is one of the most damaging diseases of winter oilseed rape in the UK. Despite expenditure of over £5 M on fungicide treatments, the disease, which is prevalent in northern and western parts of the UK, is responsible for economic losses estimated at >£30 M per annum (Fitt *et al.*, 1997). Although the disease can be effectively controlled by fungicide sprays, patterns of fungicide application do not relate well to the actual amounts of disease present, with many crops being sprayed unnecessarily or not being sprayed when required. In addition to the extra costs incurred, the unnecessary spraying of crops with little disease could encourage the development of fungicide resistance within the pathogen population. The discrepancy between disease levels and fungicide application may occur because light leaf spot epidemics differ in severity between seasons and between regions in the UK (Fitt *et al.*, 1996). In addition, although light leaf spot epidemics in the UK are often initiated by infections occurring in the autumn (which is the optimal time for fungicide treatment), disease symptoms are often not visible until the following February or March. Consequently, spray decisions are often taken without knowing if crops are affected by the disease. To avoid the possibility of unnecessary application of fungicides, and to improve the timing of fungicide applications when necessary, work has commenced on the development of a scheme for forecasting the severity of light leaf spot epidemics (Fitt *et al.*, 1996). At present, the scheme is based upon the factors that contribute to the variability of the epidemics within the UK such as previous disease levels, geographic location, weather data and cultivar resistance. An additional important component of the scheme is the early detection of infection in the autumn when symptoms are not visible. To achieve this, work is being done at the University of Cambridge and IACR-Rothamsted to develop a detection method based upon a molecular technique known as the polymerase chain reaction (PCR; Mullis *et al.*, 1986).

PCR has made important contributions to disease diagnosis in both animals and plants. The technique is based upon the ability of an enzyme (DNA polymerase) to amplify large quantities of a specific DNA sequence, which can be visualised using standard laboratory procedures, from a small amount of starting material. The most important part of the procedure is the design of PCR primers, short DNA sequences which bind specifically to the template DNA (for example, from a plant pathogen), providing a site from which the DNA polymerase can synthesise new copies of the target DNA sequence. Thus, identification of a DNA sequence which is specific to a plant pathogen can provide the information required to design PCR primers which will facilitate the amplification of DNA specific to that pathogen. Hence the technique can be used as a method to determine the presence of a pathogen in plant material. Because the procedure involves the exponential amplification of any target DNA present, the technique has the potential to determine even the presence of single fungal spores. In addition, the speed with which the procedure can be performed means that a large number of plant samples can be analysed within a single working day.

Work has started on the development of a PCR based diagnostic tool for the specific identification of *P. brassicae* and hence diagnosis of light leaf spot. To be of use as a diagnostic tool, such a technique must fulfil two criteria. Firstly, the PCR primers must be designed to amplify DNA from the target organism alone and not from any other pathogens or organisms present within or on the plant material which is being tested. Secondly, the technique should be sufficiently sensitive to detect extremely small amounts of the pathogen within the plant material. Results obtained thus far have demonstrated that PCR primers, designed from a DNA sequence involved in sexual development of *P. brassicae*, amplify DNA extracted from pure cultures of *P. brassicae* and not from other fungi pathogenic to oilseed rape [e.g. *Leptosphaeria maculans* (phoma leaf spot, stem canker), *Alternaria brassicae* (dark pod spot) and *Sclerotinia sclerotiorum* (sclerotinia stem rot)]. Although further testing is required, it appears that the first criterion has been fulfilled. Work is now being done to determine whether the technique is sensitive enough to identify the fungus in infected plant material and determine the earliest stage in the infection process at which the fungus can be detected. When this technique has been fully developed, it will be used as part of the forecasting scheme to identify winter oilseed rape crops at risk from light leaf spot and improve the efficiency of fungicide applications.

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