DEVELOPMENT OF AN IMMUNOLOGICAL APPROACH TO MONITOR AIRBORNE INOCULUM OF FUNGAL PATHOGENS OF OILSEED RAPE

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ABSTRACT

Monoclonal antibodies (mAbs) were raised to germinating spores of *Alternaria brassicae*, the cause of dark leaf and pod spot in oilseed rape. An ELISA procedure was developed to quantify the amount of specific antigen present in a spore sample. A rotating-arm spore trap was designed to collect airborne spores direct in the ELISA wells. The spores were germinated before being assayed. The trap was tested in a wind tunnel and in the field. The system has the potential for rapidly detecting airborne inoculum providing suitable mAbs are available.

INTRODUCTION

There is a need for more accurate and reliable disease forecasting methods for use in decision support systems. The estimation of disease potential is a necessary part of such forecasting systems. For air-dispersed fungal pathogens this potential may be estimated by monitoring the amount of inoculum present at critical stages in the development of the crop. Current methods of estimating exposure to airborne inoculum are labour intensive and time consuming and require skilled personnel. Immunological techniques have been used to identify fungal pathogens in the soil and plant tissue, and have the potential to provide an objective and straightforward method for identifying and quantifying air-dispersed fungal propagules. We describe a sampler which uses an enzyme-linked immunosorbent assay (ELISA) to detect and quantify airborne conidia of *Alternaria brassicae*, the cause of dark leaf and pod spot in oilseed rape.

EXPERIMENTAL

Monoclonal antibody production

A female Balb\c mouse (10 weeks old) was given 5 intraperitoneal injections of $0.8\text{-}2.5 \times 10^5$ germinating *A. brassicae* conidia suspended in 0.5 ml of 0.85 % saline. Two days after the final injection, the spleen cells were fused with SP-2 myeloma cells. Hybridoma supernatants were screened by ELISA, using Nunc PolySorp polystyrene ELISA plates coated with *Alternaria brassicae* conidia, which had been allowed to germinate overnight. A total of 96 antigen positive cell lines were cloned. The most promising 18 clones (8 clones of IgG_1 isotype and 10 clones of IgM isotype) were tested against a library of 20 fungal species (representing 13 fungal genera) most commonly found in air samples above oilseed rape crops. Most clones showed multiple reactivities,

but one designated IACR Abr 347G (IgG_1) showed no cross-reactivity with any of the fungi tested. The relationship between the ELISA signal (OD 405nm) and the number of spores/well using mAb IACR Abr 347G as a probe is shown in Fig.1.

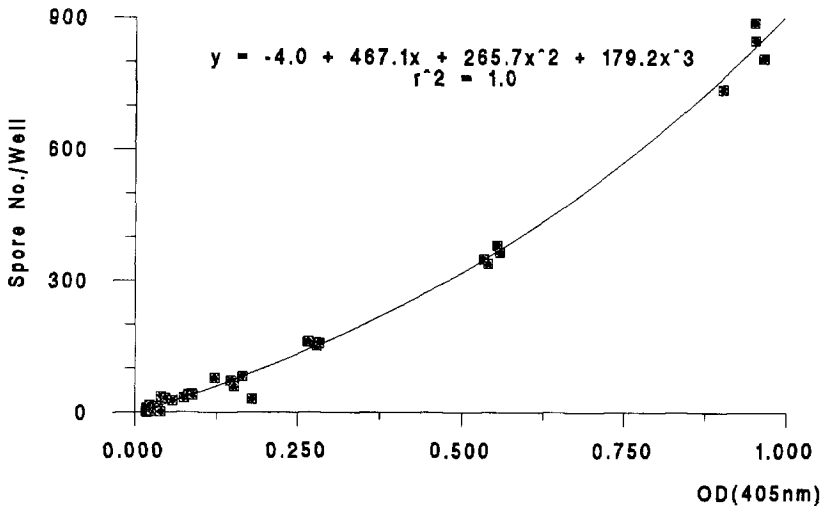

Fig.1 Correlation between the number of germinated spores per well and optical density (OD) at 405 nm after a substrate incubation time of 1h.

ELISA protocol

1. Collect spores in Nunc-Immuno-Modules, PolySorp F8 strips using the spore trap described below.
2. Remove strips from the spore trap and incubate impacted spores overnight (15h-16h) in distilled water supplemented with D-glucose (0.5%).
3. Wash the strips the following morning in washing buffer (phosphate-buffered saline with 0.05 % Tween-20, PBST) three times , 3 min each.
4. Apply acetic acid as a 60 % aqueous solution for 10 min at room temperature. This inhibits substrate relevant endogenous enzyme activity (Schmechel *et al*, 1994).
5. Wash as in step 3.
6. Incubate in blocker solution (PBS + 1% Nido milk powder) for 30 min at 37 °C.
7. Wash as in step 3.
8. Apply specific mAb culture supernatant diluted by half in PBST for 1h at 37 °C.
9. Wash as in step 3.
10. Apply secondary antibody at 1/1000 dilution in PBST for 30 min at 37 °C.
11. Wash as in step 3.
12. Apply substrate (*p*-Nitrophenyl phosphate tablets) for 1h at room temperature.
13. Read optical density at 405 nm.

Rotating-arm spore trap

Current immuno-methods rely on the extraction of antigens from the sample. This usually involves sample processing which may reduce accuracy and sensitivity in quantifying the antigen. A spore trap was designed to collect airborne spores in wells suitable for direct ELISA processing, thereby avoiding any sample manipulation before analysis. The spore trap consists of two ELISA strips (Nunc-Immuno-Modules) attached to arms (Fig 2). As

the arms rotate airborne particles are collected in the wells. The arms and strips were designed so that most of the trapped spores impacted on the base of the well. The strips are attached to the supporting arms by rubber bands for ease of removal. The arms are rotated at approximately 2800rpm by a 12V dc-motor, equivalent to sampling the air at about 350 l min^{-1}. The trap was tested in a wind tunnel at Rothamsted and the sampling efficiencies were between 1.7 ($1ms^{-1}$ wind speed) and 0.75 ($3ms^{-1}$) compared to a Burkard trap. For field operation the trap is operated in a cage covered in fine mesh to prevent the collection of small insects which can interfere with the signal. The device is highly mobile, inexpensive and simple to operate.

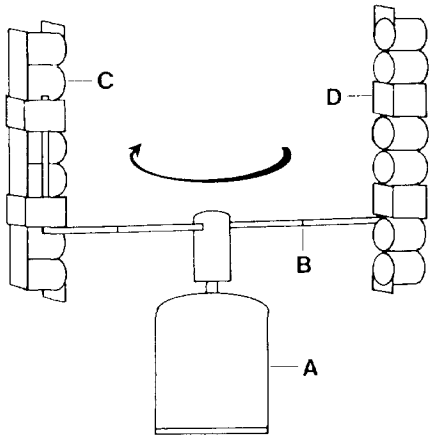

Fig. 2. Schematic diagram of the rotating-arm spore trap. Direction of rotation is indicated by arrow. A:- 12 V dc-electric motor; B:- support arms; C:- ELISA strip; D:- rubber bands to hold strips to support arms.

Discussion and Conclusions

These experiments demonstrate the potential for using monoclonal antibodies to detect and quantify airborne fungal inoculum, provided that antibodies with the appropriate specificity are available. The monoclonal antibody used was raised against germinated *A. brassicae* conidia and was highly specific and showed no cross-reactions to other fungal spores likely to be present in the air in an oilseed rape field. Because the monoclonal antibody reacted to germinated conidia only viable conidia were detected. The avoidance of minimum sample manipulation before ELISA analysis enhanced comparability and reproducibility of the results. The development of relatively inexpensive spore traps employing antibodies to detect and quantify airborne inoculum will allow early infection and disease spread to be more easily detected. However, for practical use in decision support systems further work is needed to simplify the antigen detection system.

REFERENCES

Schmechel, D., McCartney, H.A. and Halsey, K. (1994). The development of immunological techniques for the detection and evaluation of fungal disease inoculum in oilseed rape crops. In *Modern Assays for Plant Pathogenic Fungi Identification, Detection and Quantification*. Eds. A. Schots, F.M. Dewey and R.P. Oliver. pp. 247-253. Oxford: CAB International, UK.