First publ. in: Microbiology of the Phyllosphere / ed. N. J. Fokkema. Cambridge: Cambridge Univ. Pr., 1986, pp. 50-59

QUANTITATIVE SEROLOGICAL ESTIMATIONS OF FUNGAL COLONIZATION

K. Mendgen

Universität Konstanz, Fakultät für Biologie, D-7750 Konstanz, Fed. Rep. Germany

INTRODUCTION

A variety of methods has been used in the past to quantify pathogenic fungi present in or on host plants. Observations on degree of colonization range from verbal descriptions (e.g. very slight to abundant) and number of plants infected to more elaborate light-microscopical estimations of amounts of hyphae in sectioned tissue (Toth & Toth 1982). Phase-contrast lenses (Frankland 1974) and, in my experience, optics for differential interference contrast, help to recognize very thin and even empty hyphae. Specific fluorescent stains for hyphal walls make it easy to discriminate between fungal and plant structures (Patton & Johnson 1970; Rohringer *et al.* 1977).

In morphometric studies, the relative proportions of different fungal components or fungal structures are determined in tissue sections or on host surfaces. Instrumentation is now available that makes such an analysis easy (Moesta *et al.* 1983).

Direct methods also include isolations from freshly collected tissue or air-dried material followed by dilution plating on a selective medium (e.g. Davis *et al.* 1983). This technique needs specific adaptations for each fungus assayed. It may indicate the amount of propagules in an infected tissue, but not the amount of fungal biomass. The value of these assays is discussed in the proceedings of the previous meeting in this series (Blakeman 1981).

Ride & Drysdale (1972) recommended the chemical determination of the chitin content as a quantitative measure for fungal invasion. This method is rapid and simple and has been used for different fungi: *Botrytis cinerea* (Harding & Heale 1978), *Erysiphe cichoracearum* (Onogur & Schlösser 1976), *Fusarium* spp. (Zak 1976; Raghu Kumar & Subramanian 1977; Schönbeck *et al.* 1977; Dehne & Schönbeck 1979), *Puccinia* spp. (Lösel & Lewis 1974; Pearce & Strange 1977; Whipps & Lewis 1980), *Verticillium* spp. (Pegg 1978) and some mycorrhizal fungi (Becker & Gerdemann 1977; Hepper 1977; Haselwandter 1979). This method has its pitfalls since, in contrast to hyphae, spores often have much higher chitin contents and this results in an inaccurate estimate of fungal growth. Furthermore, the chitin content is not directly correlated with hyphal growth (Mayama *et al.* 1975; Kaminsky & Heath 1982), probably because of changes in the fungal wall composition during growth in the host (Mendgen *et al.* 1985).

Similary, ergosterol has been recommended as a measure of fungal growth (Seitz *et al.* 1979). This sterol is the predominant sterol component of most fungi and is either absent or a minor constituent in most higher plants. This method seems to be more sensitive than the chitin assay. However, like the chitin determination, the ergosterol assay cannot discriminate between different fungi although different fungi seem to have different ergosterol contents. Therefore, this assay is only a rough method for estimating fungal biomass in or on a solid substrate.

As an alternative to the methods mentioned above, the total amount of fungal material present in the infected tissue may be measured serologically (e.g. with the enzyme-linked immunosorbent assay, ELISA). In ELISA, the antibody binding reaction is combined with the adsorption of the antibodies to a solid plastic matrix; this complex selectively retains antibodies linked with an enzyme. Alternatively, the enzyme-linked complex may be replaced by a radioactive-labelled antibody (radioimmunosorbent assay, RISA), but this test is more complicated to use (Savage & Sall 1981; Richardson & Warnock 1983) and the isotopes may be difficult to handle under normal laboratory conditions.

SEROLOGICAL ESTIMATIONS WITH EILISA

The ELISA method of Clark & Adams (1977) is used very often for virus detection in plants: non-labelled antibodies are adsorbed to the wells of microtiter plates. A test solution with the antigen is added, and then enzyme-labelled antibodies are added. The latter antibodies also bind to the antigen. Subsequently, an enzyme substrate is added and the colour is measured. Alkaline phosphatase and horse-radish peroxidase are mainly used as enzyme label. Assuming that the rate of colour formation is proportional to the amount of conjugate reacted, the assay will indicate the amount of antigen or antibody present. The value of this technique in plant pathology has been reviewed recently (Clark 1981; Gugerli 1983). In the present review, new aspects for the estimations of fungal structures are summarized. Table 1 lists the fungal species for the detection of which ELISA has been used.

SPECIFICITY OF THE ANTIGEN AND SENSITIVITY OF ELISA

In most cases whole cells are used as antigen. These may consist of unbroken thalli, e.g. of *Botrytis cinerea* (Savage & Sall 1981), obtained from cultures in the growth phase, or the complete fungal mycelium including spores (Casper & Mendgen 1979; Mendgen 1981; Johnson *et al.* 1982). Also an acetone precipitate of culture fluid, e.g. of *Phoma tracheiphila* (Nachmias *et al.* 1979), may be used. More specific antigens are enzymes from fungi, e.g. invertase from *Phytophthora megasperma* f. sp. *glycinea* (Moesta *et al.* 1983), $NADP^+$-glutamate dehydrogenase from *Sphaerostilbe repens* (Martin *et al.* 1983) or polygalacturonases from *Fusarium oxysporum* (Suresh *et al.* 1984). Scheffer & Elgersma (1981) used a phytotoxic glycopeptide from *Ophiostoma ulmi*, the causal organism of Dutch elm disease, as an antigen. Banowetz *et al.* (1984) used membranes and proteins, carbohydrates and complex polysaccharides from spore surfaces of *Tilletia controversa* and *T. caries*. In this

latter study, monoclonal antibodies were used. Although this new technique seems to become very promising because of its high specificity (Benhamou & Ouellette 1985), it was not possible to differentiate with monoclonal antibodies between *T. controversa* and *T. caries* (Banowetz *et al.* 1984).

When polyclonal antibodies against *Epichloë typhina* were used, only three (*Thelephora*, *Rhizoctonia* and *Claviceps*) of 14 different genera of plant-pathogenic fungi tested, reacted to any extent in the ELISA system. A high concentration (50 mg ml^{-1}) of sclerotia of *Claviceps* sp. gave an absorbance value of 0.043, roughly equivalent to that of mycelium of *E. typhina* at 100 ng ml^{-1} (Johnson *et al.* 1982). Obviously, the ELISA technique may also be useful in studying taxonomic relationships between fungi. Testing different Endogonaceae, Aldwell *et al.* (1985) were able to differentiate between these mycorrhizal fungi serologically. The data obtained corresponded well with the current classification based on morphological features and demonstrated the high specificity of ELISA.

Table 1. Fungi detected in plant tissues with ELISA.

Fungal species	Reference
Acremonium coenophialum	Welty *et al.* (1984)
Alternaria solani	Vargo & Baumer (1984)
Armillaria sp.	Lung-Escarmant & Dunez (1979)
Aspergillus sp.	Hommell *et al.* (1976)
Bipolaris sp.	Vargo & Baumer (1984)
Clitocybe sp.	Lung-Escarmant & Dunez (1979)
Endomycorrhizal fungi (*Gigaspora margarita*, *G. calospora*, *Glomus mosseae*, *G. clarum*, *Acaulospora laevis*, *Sclerocystis dussii*)	Aldwell *et al.* (1983, 1985)
Endophytic fungi	Funk *et al.* (1983); Johnson *et al.* (1983); Musgrave (1984)
Epichloë typhina	Funk *et al.* (1983); Halisky *et al.* (1983); Johnson *et al.* (1983, 1985); Musgrave (1984)
Gremmeniella abietina	Liese *et al.* (1982)
Phytophthora syringae	Kimishima *et al.* (1984)
Phoma tracheiphila	Nachmias *et al.* (1979)
P. exigua	Aguelon & Dunez (1984)
Plasmopara halstedii	Liese *et al.* (1982)
Verticillium albo-atrum	Leach & Swinburne (1984)
V. lecanii	Casper & Mendgen (1979); Mendgen (1981)

The results mentioned above show that it may be possible to differentiate between two fungi in or on a leaf. For example, the hyperparasite, *Verticillium lecanii*, could easily be distinguished in pustules of various rust fungi (Casper & Mendgen 1979; Mendgen 1981). In that system, there were no cross-reactions between extracts of the plants, the rust fungus and the hyperparasite. The specificity of the antibody could easily be checked with a microscope equipped for epifluorescence: fluorescein isothiocyanate-labelled antibodies against *V. lecanii* were restricted to this fungus in the cross-sections through a leaf of *Phaseolus vulgaris* infected with *Uromyces appendiculatus* and *V. lecanii* (Mendgen & Casper 1980). Moreover, the specificity of the antiserum may be enhanced by pretreatment with antigens (proteins) from similar fungi and discarding the precipitate formed (Frankland *et al.* 1981; Leach & Swinburne 1984).

The sensitivity of ELISA was at least 10^3 to 10^5 times greater than that of the double diffusion test (Nachmias *et al.* 1979). *E. typhina* was detected in concentrations down to 100 ng ml^{-1} (Johnson *et al.* 1982). This value is similar to that possible with RISA for the detection of

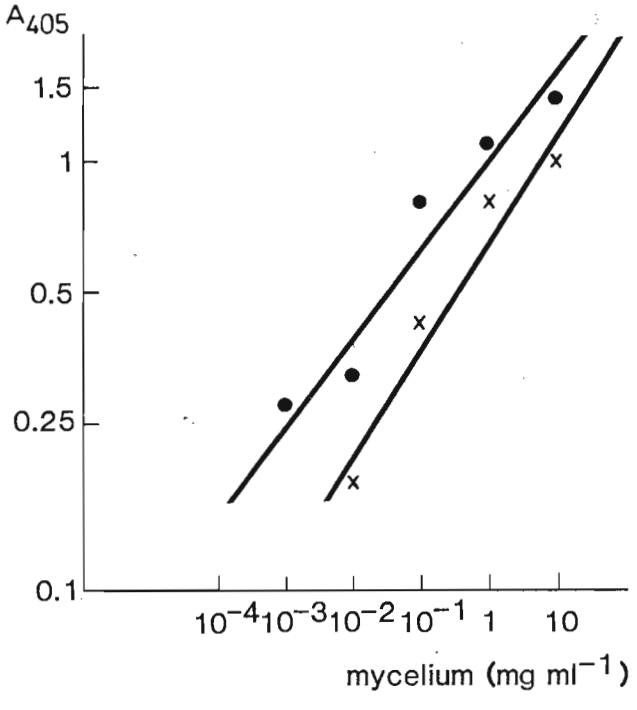

Fig. 1. Enzyme-linked immunosorbent assay (ELISA) for a dilution series of mycelium of two *E. typhina* isolates. Absorbance at 405 nm after incubation of *E. typhina* isolated from tall fescue (•) and bentgrass (x). The absorbance of the control buffer (PBS) was 0.007. Drawn after data from Johnson *et al.* (1982).

B. cinerea (Savage & Sall 1981). Johnson *et al.* (1983) detected antigens in samples consisting of only one endophyte-infected seed extracted together with 19 endophyte-free seeds.

QUANTITATIVE ESTIMATIONS

It may be possible to correlate spectrophotometric ELISA readings with the total amount of fungal material in infected host plants. An example of such a correlation is shown for a dilution series of mycelium of two *E. typhina* isolates (Fig. 1). Quantitative measurements have also been made with *V. lecanii* in pustules of stripe rust (*Puccinia striiformis*) under different growth conditions (Fig. 2). A good correlation was obtained between the amount of the hyperparasitic fungus growing in the rust pustules and the ELISA values (Casper & Mendgen 1979; Mendgen 1981).

There are some principal restrictions in using the ELISA technique for the quantitative evaluation of fungal biomass or fungal growth. Comparable to the problems with quantitative chitin or ergosterol measurements, the antigen chosen for the detection of a fungus within plant tissue may not be evenly distributed within all fungal structures. As a consequence, the increase or decrease of that antigen, rather than the growth of the fungus will be measured. Changes in protein patterns during the development of a fungal pathogen have been reported (Huang & Staples

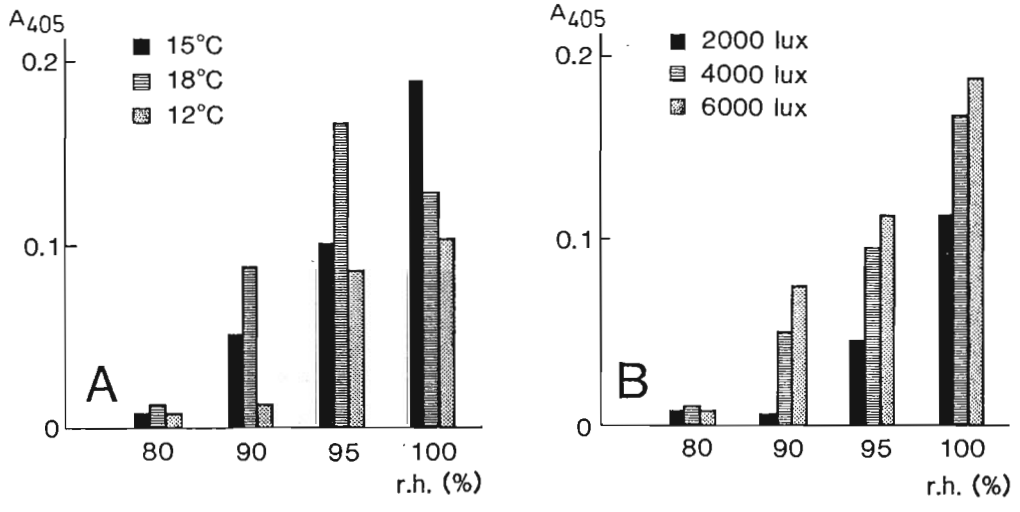

Fig. 2. The amount of *V. lecanii* in rust pustules on wheat, estimated by ELISA and expressed as absorbance at 405 nm (adapted from Mendgen 1981). A. Influence of relative humidity and temperature on the development of *V. lecanii* grown under 4000 lux. A_{405} values of the control (rusted leaves without *V. lecanii*) were <0.01. B. Influence of relative humidity and light on the development of *V. lecanii* grown at 15°C. A_{405} values of the control were <0.01.

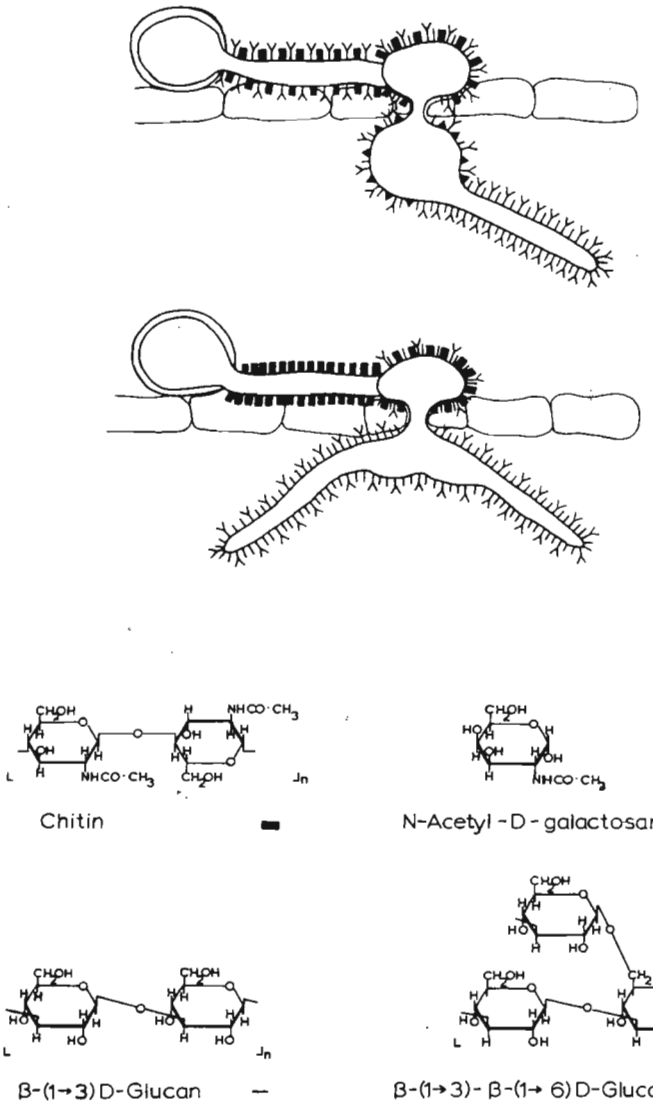

Fig. 3. A, B. Surface carbohydrate pattern on outer and inner infection structures of two rust fungi: *Uromyces appendiculatus* (A) and *Puccinia coronata* (B). C. Structures of the carbohydrates involved (Mendgen 1984).

1982; Kim *et al.* 1982). Also the wall carbohydrates (Bartnicki-Garcia 1968) and especially the surface carbohydrates (Fig. 3) of the wall change drastically during the course of infection of a rust fungus (Mendgen *et al.* 1985). Such carbohydrates (e.g. polyglucans or polygalactans) may be effective antigenic determinants. To avoid some of these problems, antibodies against a mixture of antigens derived from all parasitic structures of a fungus are recommended for quantitative studies of fungal development. This technique would overcome inaccuracies of other methods in which only one component of a fungus is measured, which may change with age, growth rate and environmental conditions.

ACKNOWLEDGEMENTS

I thank Dr R.E. Gold, Konstanz, for reading the manuscript and the Deutsche Forschungsgemeinschaft for supporting the research.

REFERENCES

Aguelon, M. & Dunez, J. (1984). Immunoenzymatic techniques for the detection of *Phoma exigua* in infected potato tissues. Annals of Applied Biology, *105*, 463-9.

Aldwell, F.E.B., Hall, I.R. & Smith, J.M.B. (1983). Enzyme-linked immunosorbent assay (ELISA) to identify endomycorrhizal fungi. Soil Biology & Biochemistry, *15*, 377-8.

Aldwell, F.E.B., Hall, I.R. & Smith, J.M.B. (1985). Enzyme-linked immunosorbent assay as an aid to taxonomy of the Endogonaceae. Transactions of the British Mycological Society, *84*, 399-402.

Banowetz, G.M., Trione, E.J. & Krygier, B.B. (1984). Immunological comparisons of teliospores of two wheat bunt fungi, *Tilletia* species, using monoclonal antibodies and antisera. Mycologia, *76*, 51-62.

Bartnicki-Garcia, S. (1968). Cell wall chemistry, morphogenesis and taxonomy of fungi. Annual Review of Microbiology, *22*, 87-108.

Becker, W.N. & Gerdemann, J.W. (1977). Colorimetric quantification of vesicular-arbuscular mycorrhizal infection in onion. New Phytologist, *78*, 289-95.

Benhamou, N. & Ouellette, G.B. (1985). Les anticorps monoclonaux: une technologie de pointe en phytopathologie. Phytoprotection, *66*, 5-15.

Blakeman, J.P. ed. (1981). Microbial Ecology of the Phylloplane. London: Academic Press.

Casper, R. & Mendgen, K. (1979). Quantitative serological estimation of a hyperparasite: detection of *Verticillium lecanii* in yellow rust infected wheat leaves by ELISA. Phytopathologische Zeitschrift, *94*, 89-91.

Clark, M.F. (1981). Immunosorbent assays in plant pathology. Annual Review of Phytopathology, *19*, 83-106.

Clark, M.F. & Adams, A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology, *34*, 475-83.

Davis, J.R., Pavek, J.J. & Corsini, D.L. (1983). A sensitive method for quantifying *Verticillium dahliae* colonization in plant tissue and evaluating resistance among potato genotypes. Phytopathology, *73*, 1009-14.

Dehne, H.-W. & Schönbeck, F. (1979). Untersuchungen zum Einfluss der endotrophen Mycorrhiza auf Pflanzenkrankheiten. I. Ausbreitung von *Fusarium oxysporum* f. sp. *lycopersici* in Tomaten. Phytopathologische Zeitschrift, *95*, 105-10.

Frankland, J.C. (1974). Importance of phase-contrast microscopy for estimation of

total fungal biomass by the agar-film technique. Soil Biology & Biochemistry, *6*, 409-10.

Frankland, J.C., Bailey, A.D., Gray, T.R.G. & Holland, A.A. (1981). Development of an immunological technique for estimating mycelial biomass of *Mycena galopus* in leaf litter. Soil Biology & Biochemistry, *13*, 87-92.

Funk, C.R., Halisky, P.M., Johnson, M.C., Siegel, M.R. Stewart, A.V., Ahmad, S., Hurley, R.H & Harvey, I.C. (1983). An endophytic fungus and resistance to sod webworms: association in *Lolium perenne* L. Bio/Technoloy, *1*, 189-91.

Gugerli, P. (1983). Use of enzyme immunoessay in phytopathology. *In* Immunoenzymatic Techniques, eds S. Avrameas, B.V.P. Druet, R. Masseyeff & G. Feldmann, pp. 369-84. Amsterdam: Elsevier Science Publishers.

Halisky, P.M., Funk, C.R. & Vincelli, P.C. (1983). A fungal endophyte in seeds of turf-type perennial ryegrass. Phytopathology, *73*, 1343 (Abstr.).

Harding, V. & Heale, J.B. (1978). Post-formed inhibitors in carrot root tissue treated with heat-killed and live conidia of *Botrytis cinerea*. Annals of Applied Biology, *89*, 348-51.

Haselwandter, K. (1979). Mycorrhizal status of ericaceous plants in alpine and subalpine areas. New Phytologist, *83*, 427-31.

Hepper, C.M. (1977). A colorimetric method for estimating vesicular-arbuscular mycorrhizal infection in roots. Soil Biology & Biochemistry, *9*, 15-8.

Hommell, M., Truong, T.K. & Bidwell, D.E. (1976). Technique immunoenzymatique (ELISA) appliquée au diagnostic sérologique des candidoses et aspergilloses humaines. Nouvelle Presse Médicale, *5*, 2789-91.

Huang, B.-F. & Staples, R.C. (1982). Synthesis of proteins during differentiation of the bean rust fungus. Experimental Mycology, *6*, 7-14.

Johnson, M.C., Anderson, R.L., Kryscio, R.J. & Siegel, M.R. (1983). Sampling procedures for determining endophyte content in tall fescue *Festuca arundinacea* seed lots by ELISA. Phytopathology, *73*, 1406-9.

Johnson, M.C., Pirone, T.P., Siegel, M.R. & Varney, D.R. (1982). Detection of *Epichloë typhina* in tall fescue by means of enzyme-linked immunosorbent assay. Phytopathology, *72*, 647-50.

Johnson, M.C., Siegel, M.R. & Schmidt, B.A. (1985). Serological reactivities of endophytic fungi from tall fescue and perennial ryegrass and of *Epichloë typhina*. Plant Disease, *69*, 200-2.

Kaminskyj, S.G.W. & Heath, M.C. (1982). An evaluation of the nitrous acid - 3-methyl-2-benzothiazolinone hydrazone hydrochloride - ferric chloride assay for chitin in rust fungi and rust-infected tissue. Canadian Journal of Botany, *60*, 2575-80.

Kim, W.K., Howes, N.K. & Rohringer, R. (1982). Detergent-soluble polypeptides in germinated uredospores and differentiated uredosporelings of wheat stem rust. Canadian Journal of Plant Pathology, *4*, 328-33.

Kimishima, E., Nishio, T., Takayama, M. & Nagao, N. (1984). Studies on serological detection and identification methods for species of *Phytophthora*. 3. Detection of *Phytophthora syringae* in plant tissues by means of enzyme-linked immunosorbent assay. Research Bulletin of the Plant Protection Service, *20*, 1-6.

Leach, J.E. & Swinburne, T.R. (1984). An indirect ELISA for quantitative estimation of *Verticillium albo-atrum* in hops. Phytopathology, *74*, 845 (Abstr.).

Liese, A.L., Gotlieb, A.R. & Sakston, W.E. (1982). Utilization of the ELISA technique for the diagnosis of two fungal diseases: Scleroderris canker of conifers and downy mildew of sunflower. Phytopathology, *72*, 263 (Abstr.).

Lõsel, D.M. & Lewis, D.H. (1974). Lipid metabolism in leaves of *Tussilago farfara* during infection by *Puccinia poarum*. New Phytologist, *73*, 1157-69.

Lung-Escarmant, B. & Dunez, J. (1979). Differentiation of *Armillaria* and *Clitocybe* species by the use of the immunoenzymatic ELISA procedure. Annales de Phytopathologie, *11*, 515-8.

Martin, F., Botton, B. & Msatef, Y. (1983). Enzyme-linked immunosorbent assay of

fungal $NADP^+$-glutamate dehydrogenase. Plant Physiology, *72*, 398-401.

Mayama, S., Rehfeld, D.W. & Daly, J.M. (1975). A comparison of the development of *Puccinia graminis tritici* in resistant and susceptible wheat based on glucosamine content. Physiological Plant Pathology, *7*, 243-57.

Mendgen, K. (1981). Growth of *Verticillium lecanii* in pustules of stripe rust (*Puccinia striiformis*). Phytopathologische Zeitschrift, *102*, 301-9.

Mendgen, K. (1984). Wirtsfindung, Wirtserkennung biotropher Pilze und Abwehrreaktionen der Wirtspflanze. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem, *223*, 6-16.

Mendgen, K. & Casper, R. (1980). Detection of *Verticillium lecanii* in pustules of bean rust (*Uromyces phaseoli*) by immunofluorescence. Phytopathologische Zeitschrift, *99*, 362-4.

Mendgen, K., Lange, M. & Bretschneider, K. (1985). Quantitative estimation of the surface carbohydrates on the infection structures of rust fungi with enzymes and lectins. Archives of Microbiology, *140*, 307-11.

Moesta, P., Grisebach, H. & Ziegler, E. (1983). Immunohistochemical detection of *Phytophthora megasperma* f. sp. *glycinea* in situ. European Journal of Cell Biology, *31*, 167-9.

Musgrave, D.R. (1984). Detection of an endophytic fungus of *Lolium perenne* using enzyme-linked immunosorbent assay (ELISA). New Zealand Journal of Agricultural Research, *27*, 283-8.

Nachmias, A., Bar-Joseph, M., Solel, Z. & Barash, I. (1979). Diagnosis of mal secco disease in lemon by enzyme-linked immunosorbent assay. Phytopathology, *69*, 559-61.

Onogur, E. & Schlösser, E. (1976). Quantitative Bestimmung der Pilzmasse echter Mehltaupilze in infiziertem Gewebe. Phytopathologische Zeitschrift, *87*, 91-3.

Patton, R.F. & Johnson, D.W. (1970). Mode of penetration of needles of eastern white pine by *Cronartium ribicola*. Phytopathology, *60*, 977-82.

Pearce, R.B. & Strange, R.N. (1977). Glycinebetaine and choline in wheat in relation to growth of stem rust. Physiological Plant Pathology, *11*, 143-8.

Pegg, G.F. (1978). Effect of host substrate on germination and growth of *Verticillium albo-atrum* and *V. dahliae* conidia and mycelia. Transactions of the British Mycological Society, *71*, 483-9.

Raghu Kumar, C. & Subramanian, D. (1977). Studies on *Fusarium* wilt of cotton. I. Host colonization. Phytopathologische Zeitschrift, *90*, 223-35.

Richardson, M.D. & Warnock, D.W. (1983). Enzyme-linked immunosorbent assay and its application to the serological diagnosis of fungal infection. Sabouraudia, *21*, 1-14.

Ride, J.P. & Drysdale, R.B. (1972). A rapid method for the chemical estimation of filamentous fungi in plant tissue. Physiological Plant Pathology, *2*, 7-15.

Rohringer, R., Kim, W.K., Samborski, D.J. & Howes, N.K. (1977). Calcofluor: an optical brightener for fluorescence microscopy of fungal plant parasites in leaves. Phytopathology, *67*, 808-10.

Savage, S.D. & Sall, M.A. (1981). Radioimmunosorbent assay for *Botrytis cinerea*. Phytopathology, *71*, 411-5.

Scheffer, R.J. & Elgersma, D.M. (1981). Detection of a phytotoxic glycopeptide produced by *Ophiostoma ulmi* in elm by enzyme-linked immunospecific assay (ELISA). Physiological Plant Pathology, *18*, 27-32.

Schönbeck, F., Dehne, H.-W. & Zimmermann, J. (1977). Untersuchungen über den Einfluss von Diallat auf den Befall von Weizen mit *Fusarium culmorum* und *F. avenaceum*. Phytopathologische Zeitschrift, *90*, 77-86.

Seitz, L.M., Sauer, D.B., Burroughs, R., Mohr, H.E. & Hubbard, J.D. (1979). Ergosterol as a measure of fungal growth. Phytopathology, *69*, 1202-3.

Suresh, G., Balasubramanian, R. & Kalyanasundaram, R. (1984). Enzyme-linked immunosorbent assay of polygalacturonases in cotton plants infected by *Fusarium oxysporum* f. sp. *vasinfectum*. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, *91*, 122-30.

Toth, R. & Toth, D. (1982). Quantifying vesicular-arbuscular mycorrhizae using a morphometric technique. Mycologia, *74*, 182-7.

Vargo, R.H. & Baumer, J.S. (1984). Soaking as a method of preparing samples for an enzyme-linked immunosorbent assay (ELISA) for *Bipolaris sorokiniana*. Phytopathology, *74*, 884 (Abstr.).

Welty, R.E., Milbrath, G.M., Faulkenberry, D., Azevedo, M.D. & Meek, L. (1984). Detecting *Acremonium coenophialum* in seeds of tall fescue. Phytopathology, *74*, 1142 (Abstr.).

Whipps, J.M. & Lewis, D.H. (1980). Methodology of a chitin assay. Transactions of the British Mycological Society, *74*, 416-8.

Zak, J.C. (1976). Pathogenicity of a gibberellin-producing and a non-producing strain of *Fusarium moniliforme* in oats as determined by a colorimetric assay for N-acetyl glucosamine. Mycologia, *68*, 151-8.