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**Quantitative Serological Estimation of a Hyperparasite:
Detection of *Verticillium lecanii*
in Yellow Rust Infected Wheat Leaves by ELISA**

By

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With one figure

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Detecting the presence and amount of a fungus in leaf tissue, especially when it is suspected that other fungi are present, is quite difficult. Culture methods can be unreliable because a few spores of a fast growing fungus may overgrow other fungi within a few days (e.g. MCKENZIE and HUDSON 1976). Methods which measure the chitin content of fungal walls cannot differentiate between fungi; in addition, recent evidence suggests that there is no direct correlation between the amount of fungal hyphae and the amount of chitin (MAYAMA et al. 1975).

Serological tests, because of their high specificity could be a possible solution for these problems. We therefore used the technique of enzyme-linked immunosorbent assay (ELISA), as modified for the detection and identification of plant viruses by CLARK and ADAMS (1977). Since ELISA enables quantitative measurement of antigen we used it to estimate *Verticillium lecanii* at different stages of infection in leaves of wheat heavily infected with yellow rust (*Puccinia striiformis*).

Material and Methods

To prepare an antiserum with maximum specificity *Verticillium lecanii* was cultured in 0.5% malt solution for 10 days, after which the mycelium was harvested, washed in water and freeze-dried. The freeze-dried powdered mycelium was resuspended in 5 volumes of water (w/v), emulsified with Freund's complete adjuvant (Difco), and injected intra-

muscularly into a rabbit. The rabbit was bled four weeks after the injection and the γ -globulin fraction of a portion of the antiserum prepared for ELISA according to CLARK and ADAMS (1977). In agar gel tests (Ouchterlony) the antiserum showed a specific titer of 1:16 to *Verticillium lecanii*, but the precipitation line was weak and diffuse.

For use in the ELISA test the coating and enzyme-labelled γ -globulin preparations were both diluted 1:500. ELISA values were obtained by measuring absorbance at 405 nm.

Wheat cv. Michigan Amber was raised in a growth chamber at 15°C with fluorescent light (2000 lux) 16 h/day. After 10 days, the plants were inoculated with yellow rust (*Puccinia striiformis* West., race 37 E 132). After 14 days, when the rust was sporulating the leaves were sprayed with a suspension of *Verticillium lecanii* spores, isolated from a rust pustule collected in the field. Samples of these leaves were taken 7 days later for the serological tests.

Incubating infected plants maintained in growth chambers at different humidities influenced the development of *V. lecanii* in the rust pustules. At 80% humidity, no *V. lecanii* was observed, however *Verticillium* hyphae could be detected by microscopical examination in a few pustules at 90% and at 95% in all rust pustules. To measure the amount of *Verticillium* hyphae in these leaves, 200 mg of leaf tissue was freeze-dried and subjected to the ELISA test.

Results and Discussion

In Figure 1 the condition of the leaf samples at time of harvest is shown and correlated to the results obtained in ELISA tests. Positive reactions were obtained with leaf samples containing *V. lecanii* (Figs. 1c and d) but not with the control uninfected wheat leaf or *Puccinia striiformis* infected wheat leaf

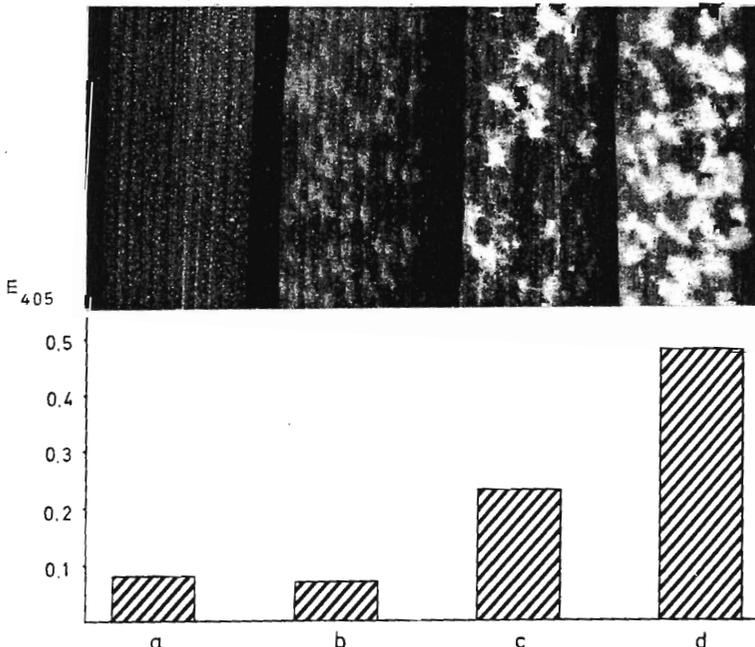


Fig. 1. ELISA extinction values for wheat leaf extracts: (a) uninfected wheat leaf; (b) yellow rust infected wheat leaf; (c) wheat leaf with yellow rust and *Verticillium lecanii* grown at 90% humidity and (d) grown at 95% humidity

samples. This shows that there is no immunological reaction between *P. striiformis* and the antiserum against *V. lecanii*.

The small amount of mycelium developed at 90 % humidity, was enough to obtain an extinction value of 0.23. In contrast at 95 % humidity the larger amount of mycelium induced an extinction value of 0.48. These data show that the ELISA technique is capable of quantitatively indicating the amount of *V. lecanii* in *P. striiformis* infected leaf tissue.

This is the first report of detection of fungal mycelium by ELISA. This technique may have a general usefulness for detecting fungal infections.

Summary

The ELISA technique enabled the quantitative serological determination of *Verticillium lecanii* in yellow rusted wheat leaves. Detection of *V. lecanii* was not affected by the presence of *Puccinia striiformis* in the same leaf.

Zusammenfassung

Quantitative serologische Bestimmung eines Hyperparasiten: Nachweis von *Verticillium lecanii* in gelbrost-infizierten Weizenblättern mit dem ELISA-Test

Mit dem ELISA-Test kann *Verticillium lecanii* in Gelbrost (*Puccinia striiformis*) befallenen Weizenblättern quantitativ bestimmt werden. Diese quantitative Bestimmung wird durch die Anwesenheit von *P. striiformis* nicht gestört.

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Literature

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