

## Kurze Mitteilungen / Short Communications

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### Detection of *Verticillium lecanii* in Pustules of Bean Rust (*Uromyces phaseoli*) by Immunofluorescence

By

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*With 2 figures*

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*Verticillium lecanii* has been found growing in pustules of the uredial stage of a number of rust fungi (GAMS 1971, SCHROEDER and HASSEBRAUK 1957, CASPER and MENDGEN 1979, SPENCER 1980), and in some insects (HALL 1980). SCHROEDER and HASSEBRAUK (1957) observed that this fungus is able to penetrate the urediospores of *Puccinia striiformis*. It remains to be shown, however, whether *Verticillium* is restricted to the spores of a pustule or whether it also penetrates into the fungal mycelium or into the leaf tissue. To answer this question, an antiserum against *V. lecanii* was prepared to label *Verticillium* hyphae by immunofluorescence. This would allow differentiation between *Verticillium*, rust-hyphae and plant tissue since such an antiserum does not react with the rust or the plant tissue (CASPER and MENDGEN 1979).

#### Methods

The antiserum against *Verticillium lecanii* (Zimm.) Viégas was prepared as described earlier (CASPER and MENDGEN 1979). Bean plants (*Phaseolus vulgaris*, cv. Favorit) were grown in a growth chamber at 21 °C with fluorescent light (6000 lux) 16 h/day. Primary

leaves were inoculated with urediospores of bean rust (*Uromyces phaseoli* Pers. var. *typica* Arth.) under high humidity conditions. When pustules appeared, the leaves were sprayed with a spore suspension of *V. lecanii* and the plants were kept under a plastic cover to keep high humidity. Ten days later, the pustules were completely covered by the mycelium of *Verticillium*. Samples of such pustules were fixed with 2% formaldehyde and embedded in paraffin. After sectioning and removal of the paraffin, sections were washed in 0.01 M phosphate buffer, pH 7.2 with 0.15 M NaCl and incubated with antiserum diluted 1:50 with the same buffer. After washing with buffer, the sections were incubated in fluoresceine-conjugated anti-rabbit-IG from sheep (Miles) diluted with buffer 1:20. Photographs were taken using a Leitz fluorescence microscope equipped with filter blocks A, D and I 2.

### Results and Discussion

Figure 1 shows a cross section through a bean rust pustule in a fluorescence microscope after broad band excitation (340—380 nm). The same result was obtained with an excitation between 355 and 425 nm. The walls of hyphae and of host cells can clearly seen. Urediospores show more or less pronounced fluorescence. Figure 2 shows the same section, but this time after selective excitation for the fluoresceine-conjugated antibodies (450—490 nm). The labelled *Verticillium* hyphae can be recognized by a bright yellow green fluorescence. *Verticillium* hyphae were restricted to the urediospore area in the pustule, and grew within and on the surface of the urediospores. They were

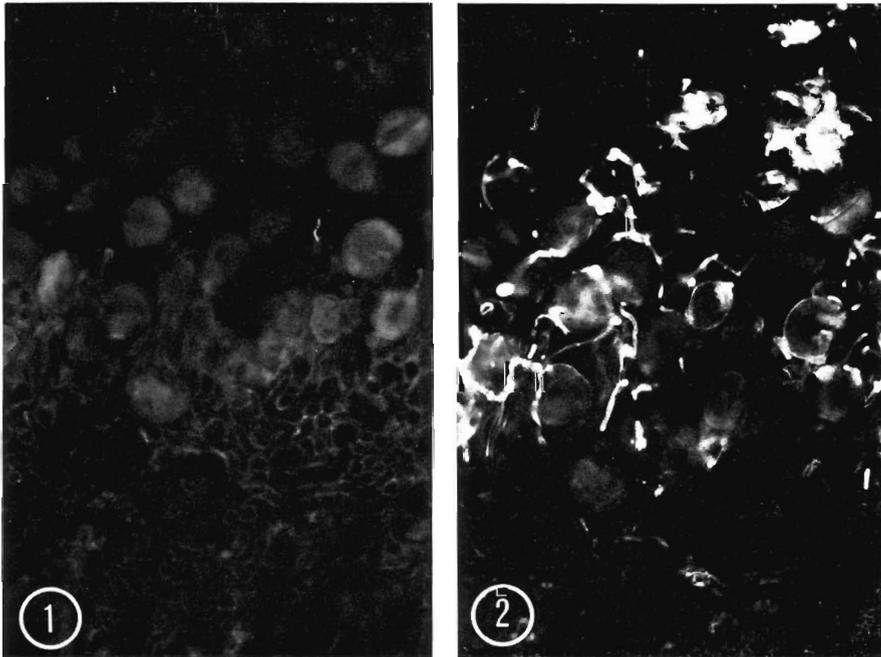


Fig. 1. Cross section through a bean rust pustule, infected with *Verticillium lecanii*, after broad band (340—380 nm) excitation ( $\times 550$ )

Fig. 2. The same section after selective excitation (450—490 nm) for fluoresceine-conjugated antibodies to demonstrate *Verticillium lecanii* ( $\times 550$ )

not observed in the leaf cells or in the intercellular spaces, which were completely filled with rust hyphae (sporogenous tissue). This result indicates that *Verticillium lecanii* does not infect bean leaves, at least as long the leaf tissue is not degenerated. Therefore, the hyperparasite may be useful in biological control. Furthermore, the results show that it is possible to differentiate with immunofluorescence the hyphae of different fungi in or on a leaf.

### Summary

*Verticillium lecanii*, originally isolated from uredial pustules of wheat stripe rust (*Puccinia striiformis*) was used to inoculate the pustules of bean rust (*Uromyces phaseoli*). An antiserum prepared against *V. lecanii*, reacted specifically with the *V. lecanii* hyphae growing in bean rust pustules. The hyperparasite was restricted to the spore layer in the pustules, where it penetrated into the urediospores, and did not penetrate into sporogenous tissue of the fungus nor the host leaf tissue.

### Zusammenfassung

#### Nachweis von *Verticillium lecanii* in Bohnenrostpusteln (*Uromyces phaseoli*) durch Immunfluoreszenz

Ein Antiserum gegen ein *Verticillium lecanii*-Isolat, das aus Gelbrost-pusteln (*Puccinia striiformis*) isoliert worden war, reagierte auch mit *Verticillium lecanii*-Hyphen in Bohnenrostpusteln (*Uromyces phaseoli*). Das Wachstum der Hyphen von *Verticillium lecanii* beschränkte sich auf den Sporenbereich der Pustel. Die Hyphen des Hyperparasiten wurden weder in den Pilzhypen noch im Pflanzengewebe angetroffen.

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### Literature

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