Verticillium lecanii a Natural Parasite on the Fig Rust *Ceratolium fici*

A. Zouba and A. J. Khan
Sultan Qaboos University, College of Agriculture
P.O. Box-32484, Al-Khod, Oman

Abstract

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Verticillium lecanii was isolated from uredia of the fig rust *Ceratolium fici*, collected from Al-Batinah area in the Sultanate of Oman. Pathogenicity was conducted *in vitro* on water agar and *in vivo* on detached fig leaves. *Verticillium* colonized urediospores internally resulting into vacuolated and disintegrated cytoplasm of urediospores. *V. lecanii* reduced significantly the number of uredia on detached leaf disks when it was applied together with rust urediospores. In the presence of *V. lecanii* uredia became black and covered by a web-like white mycelium. The number of viable urediospores taken from infected uredia was significantly reduced.

Key words: Bidogical control, *Vaticillium lecanii, Ceratolium fici*, Oman.

Introduction

Verticillium lecanii (Zimm) Viegas is known to parasitize several fungi and arthropods (3,7). Isolates from aphids and fungi are reported to be virulent on the bean rust *Uromyces appendicallus* (Pers.) Unger (1), the wheat rust *Puccinia graminis* (4) and the carnation rust Uromyces dianthi (Pers.) Niessel (6). In this work we report the fig rust *Ceratolium fici* as a natural host of *Verticillium lecanii.*

Materials and Methods

Fig leaves infected by the rust fungus *Ceratolium fici* were collected from orchards in Al'Batinah area. Infected leaves with rust sori covered by a white fungal growth were used for the isolation of *Verticillium lecanii*. The tip of a sterile dissecting needle was introduced into the uredia and streaked on potato-dextrose agar plates. Streaked plates were incubated at 25± C for seven days. Pure culture was obtained by single colony isolation.

The pathogenicity of *V. lecanii* to the urediospores of the fig rust was confirmed with two inoculation procedures, using urediospores obtained from artificially inoculated detached healthy leaves, treated with the fungicide benomyl to avoid *V. lecanii* conidia and 0.1 ml of the mixed suspension was spread onto each of six water agar plates. Plates were incubated in a moist chamber to maintain high relative humidity. Germination of the urediospores was observed after 24 hr and 48 hr of incubation using light microscopy. In a second experiment, the pathogenicity of *V. lecanii* was studied on detached fig leaf disks. The disks, 18 mm in diameter were cut from mature healthy leaves using a cork borer. Nine leaf disks were dipped for one minute either in an urediospore suspension containing approximately 3×10^3 spores/ml or in a suspension of urediospores (3×10^3 spore/ml) mixed with *V. lecanii* conidia (10^5 conidia/ml). Disks were transferred onto a moist filter paper in a sterile petri dish and incubated at room temperature. Disks dipped into sterile distilled water served as control. The number of uredial formation on each disk was recorded, 13,20 and 27 days after inoculation. All experiments were repeated twice. Colonization of the urediospores by *V. lecanii* was obseved by light and scanning electron microscopy.

The germination of urediospores from healthy and infected uredia was determined 2,6,11 and 18 days after the eruption of the uredial sori. Urediospores collected from several uredia were spread onto water agar plates and germination was recorded after 24 hr using a light microscope. From each plate at least 250 urediospores were counted. Urediospores having germ tube longer than spore width were considered as germinated.

Results

Isolation from infected uredia yieled a white Deuteromycetes fungus fitting the species concept of *Verticillium lecanii* (2) (Fig.1). Pathogenicity of *V. lecanii* was confirmed by two methods. Incubation of urediospores mixed with *V. lecanii* showed that after 24 hr the germ tube of the *V. lecanii* conidia grew towards the urediospore and became closely attached and coiled to it. After 48 hr urediospores were completely covered by the mycelium (Fig.2).

The number of uredial formation on the fig leaf disks was significantly reduced when disks were treated with rust urediospores mixed with conidial suspension of *V. lecanii* (Table 1). The number of uredia remained constant over three weeks post uredial eruption. Infected uredia became black and covered with a web-like, white mycelium, whereas healthy uredia remained yellow. The number of germinated urediospores, collected periodically after uredial eruption was, substantially lower in infected uredia (Table 2).

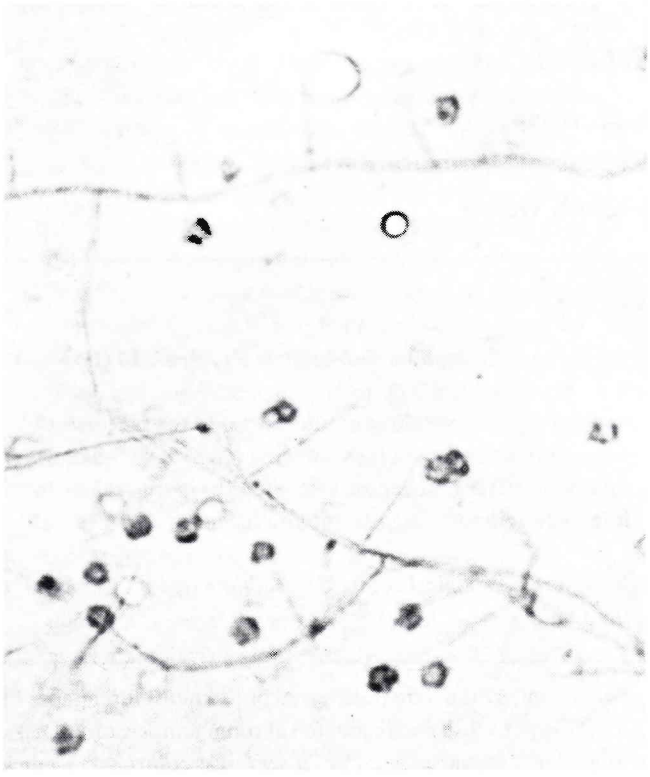

Figure 1. Light micrograph of conidia and conidiophores of *Verticillium lecanii* isolated from the fig rust fungus, *Ceratolium fici* (×400).

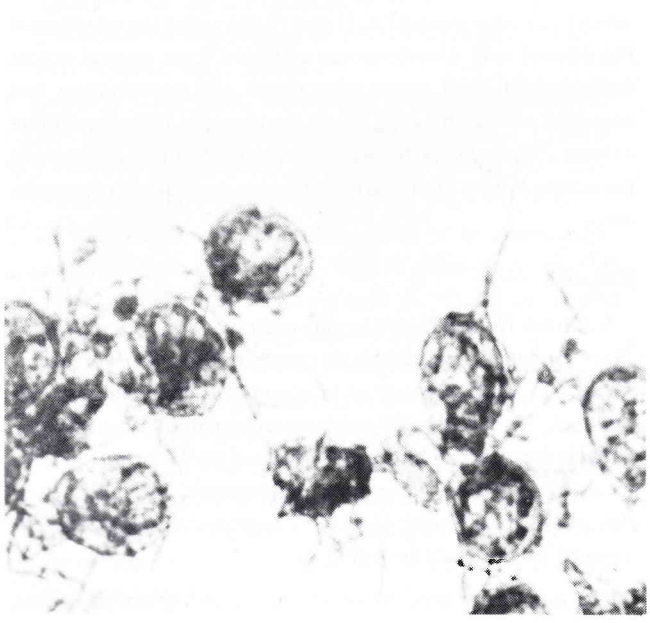

Figure 2. Urediospores of the fig rust fungus attacked by hyphae of *Verticillium lecanii*, 48 hr after mixing with *V. lecanii* and plating on water agar.

Light microscopic studies showed that urediospores from infected uredia became vacuolated and developed yellowish to brown discoloration and eventually becoming dark brown in color (Fig.3). Results obtained from scanning electron microscopy showed that *V. lecanii* mycelium became coiled and firmly attached to the urediospore surface and sometimes penetrating into the spors (Fig. 4 A & B).

Table 1. Effect of *Verticillium lecanii* on the uredial formation of the fig rust fungus using the leaf disk assay. Values are the mean ± SE of two experiments each with nine replicates. Treatment means with the same letter are not significantly different at 5 % level as determined by SAS/ANOVA using LSD and Tukey's Studentized Range (HSD) test.

Day	Treatment	Average No. Uredia leaf disk
13	Rust	155.23±3.2[a]
	Rust + *V. lecanii*	21.21 ±1.1[b]
21	Rust	155.66 ± 4.0[a]
	Rust + *V. lecanii*	23.76 ± 1.5[b]
27	Rust	158.00 ± 3.8[a]
	Rust + *V. lecanii*	25.11 ± 1.5[b]

Table 2. Effect of *Verticillium lecanii* on the germination of the urediospores of fig rust fungus. Values are the mean ± SE of three experiments each with three replicates. At least 250 urediospores were counted from each replicates. Treatment means with the same letter are not significantly different at 5 % level as determined by SAS/ANOVA using LSD and Tukey's Studentized Range (HSD) test.

Day	Treatment	% Germination of Urediospores
2	Healthy Uredia	84.5 ± 0.4[a]
	Infected Uredia	48.0 ±1.6[b]
6	Healthy Uredia	47.0 ±0.5[a]
	Infected Uredia	25.2±0.1[b]
11	Healthy Uredia	35.5 ± 1.0[a]
	Infected Uredia	12.6 ± 0.3[b]
18	Healthy Uredia	38.16 ±0.5[a]
	Infected Uredia	3.42±0.1[b]

Discussion

The suscepibility of the fig rust to *Verticillium lecanii* was verified *in vitro* and *in vivo*. The hyperparasite is capable of attacking urediospores internally. It was also shown to reduce the number of uredia and the viability of urediospores. This study reveals the potential of *V. lecanii* as a biological agent against the fig rust, which is a destructive disease in the Sultanate of Oman. A high relative humidity usually over 85 % and moderate temperatures 15° to 25° C are required to insure successful infection by *V. lecanii* (3). Alhough these conditions are not commonly met in the Sultanate, this isolate of *V. lecanii* has established itself on the fig rust and spreaded all over the Batinah area even during the hotest period of the year. Further studies on the host range and environmental requirements of this isolate are being undertaken.

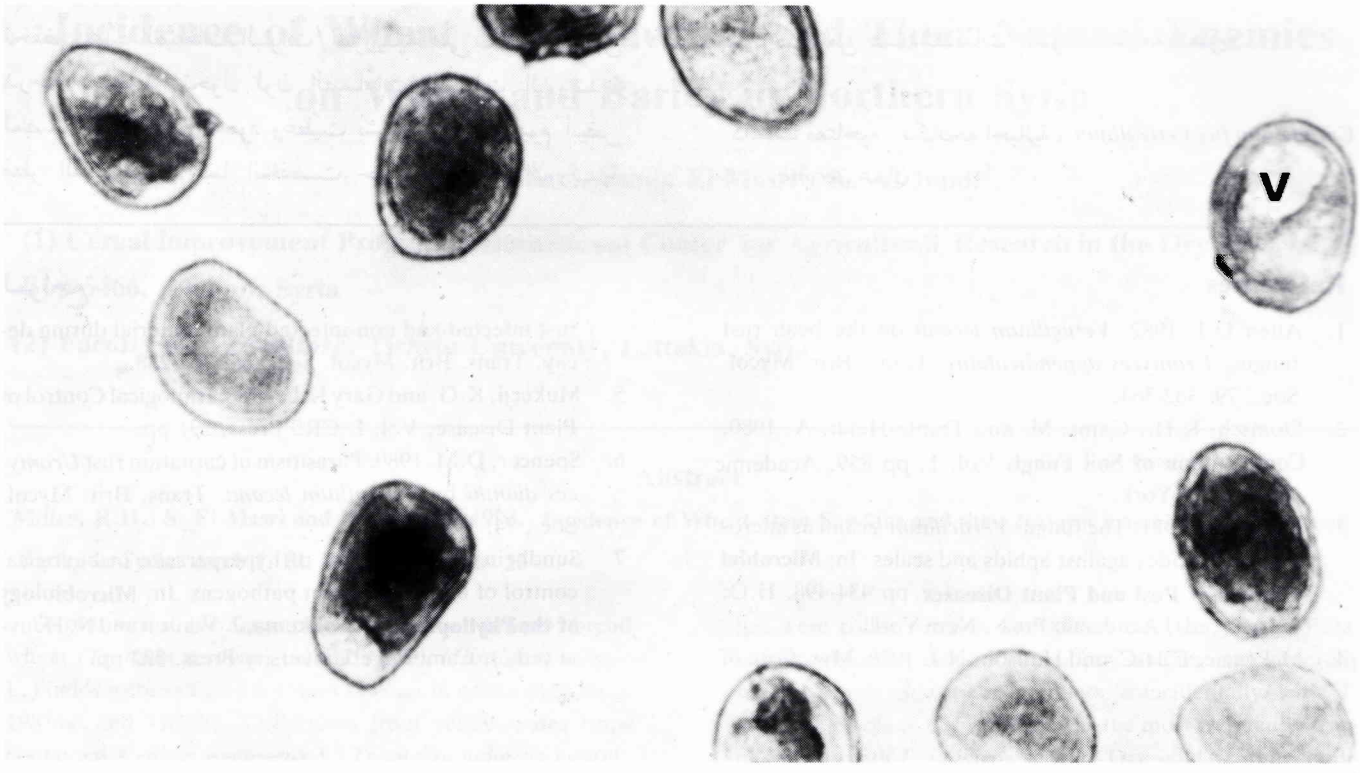

Figure 3. Fig rust urediospores from artificially infected uredia showing vacuolated (V) and disintegrated dark cytoplasm. (x 1000).

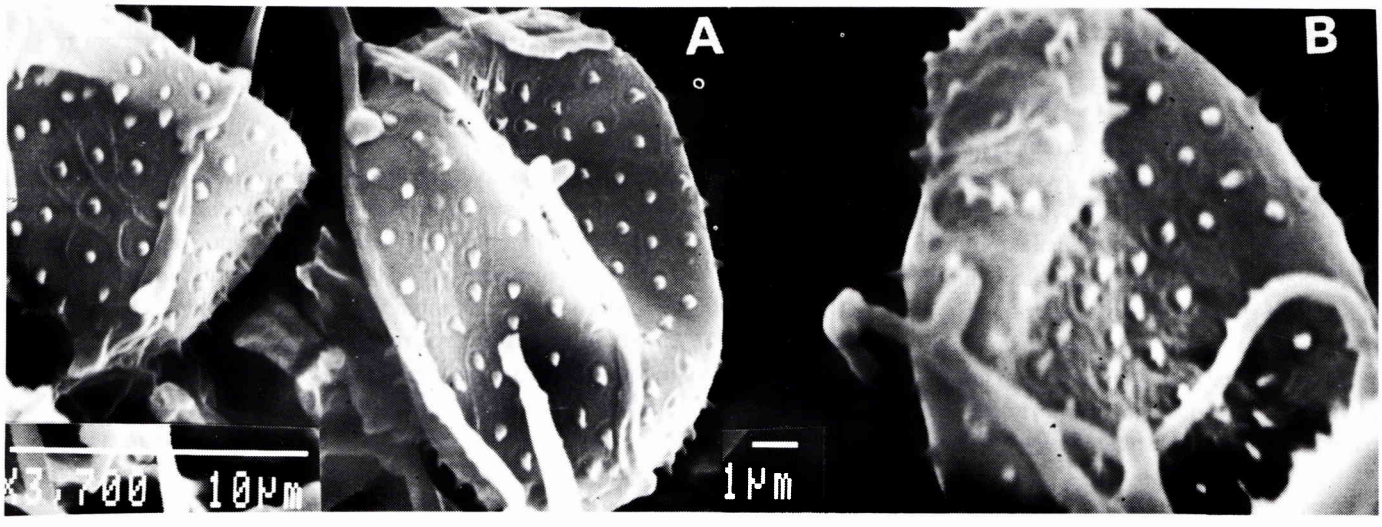

Figure 4. Scanning micrographs demonstrating fig rust urediospores colonized by *Verticillium lecanii*. (A) Hyphae of *V. lecanii* firmly attached to the urediospores. (B) *V. lecanii* hyphae penetrating the cell wall.

الملخص

زوبة علي وخان ج. 1992. فطر *Verticillium lecanii* كمتطفل طبيعي على فطر صدأ التين. مجلة وقاية النبات العربية 10 (1): 31 - 34

تم في منطقة الباطنة بسلطنة عمان عزل الفطر *Verticillum lecanii* متطفلاً على الأبواغ اليوريدية لفطر صدأ التين *Cerato-lium fici*. وقد درست القدرة الإمراضية لهذا الفطر مختبرياً على مستنبت ماء الآجار وطبيعياً على أقراص من اوراق تين مفصولة. وتمكن فطر الفرتسيليوم من غزو الأبواغ اليوريدية لفطر صدأ التين داخلياً وأحدث فجوات في السيتوبلازم؛ وعندما أعديت أقراص من ورق التين بلقاح معدي مكون من الأبواغ الكونيدية لفطر فرتسليوم والأبواغ اليوريدية لفطر الصدأ

انخفض عدد الضامّات البثرات/اليوريدية على تلك الأقراص بدرجة معنوية، وتحول لون الضامّات/البثرات اليوريدية المصابة إلى اللون الأسود وغُطيّت بشبكة من ميسيليوم أبيض لفطر الفرتسليوم. كما انخفضت حيوية الأبواغ اليوريدية المأخوذة من هذه الضامّات السوداء بدرجة معنوية.

كلمات مفتاحية: مكافحة احيائية، *Certotelium fici,Verticillium lecanii*، عمان.

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