PEANUT SCIENCE

VOLUME 17 JANUARY JUNE, 1990 NUMBER 1

Parasitism of Rust, Early and Late Leafspot Pathogens of Peanut by *Verticillium Lecanii*

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ABSTRACT

Verticillium lecanii was found parasitizing rust early and late leafspot pathogens of peanut in the glasshouse at ICRISAT Center and in farmers fields in the Indian States of Andhra Pradesh Karnataka and Tamil Nadu In inoculation experiments there was a significant reduction in the extent of rust and late leafspot development on peanut leaves inoculated with *V lecanii* Receptivity and percentage leaf area damage of rust and late leafspot were reduced when inoculated with *V lecanii* The potential use of *V lecanii* in biological control of rust and leafspot diseases of peanut is discussed

Key Words groundnut foliar diseases biological control

The isolates of rust (*Puccinia arachidis* Speg) early leafspot (*Cercospora arachidicola* Hori) and late leafspot (*Phaeoisariopsis personata* (Berk & Curt) v Arx syn *Cercosporidium personatum* (Berk & Curt) Deighton) of peanut (*Arachis hypogaea* L) maintained on potted plants of a rust and leafspots susceptible cultivar TMV 2 in the glasshouse at the International Crops Research Institute for the Semi Arid Tropics (ICRISAT) near Hyderabad India were found parasitized by a fungus The rust and leafspot lesions were covered by a whitish mycelial growth which gave them a downy appearance (Figs 1 and 2) The fungus was observed on both surfaces of the lesions but was more evident on sporulating areas of lesions i e lower surfaces of rust and late leafspot lesions and on the upper surfaces of early leafspot lesions Growth of the fungus on the lesions was abundant when temperatures ranged from 20 25 C and relative humidity was in excess of 80% The fungus invaded almost all rust and leafspot lesions on glasshouse grown plants The fungus was also found on rust and leafspot lesions of field-grown plants at ICRISAT Center and in farmers fields in Andhra Pradesh Karnataka and Tamil Nadu States in India

Submitted as Journal Article No 955 by the International Crops Research Institute for the Semi Arid Tropics (ICRISAT)

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This paper describes the isolation of the fungus and testing of its pathogenicity on rust early and late leafspot pathogens in the laboratory Its possible use in biological control of rust and late leafspot diseases is discussed

Materials and Methods

Microscopic examination

Peanut leaves showing rust early and late leafspot lesions invaded by the fungus in the glasshouse were brought to the laboratory Surfaces of rust and leafspot lesions on which the fungus occurred were gently scraped with a sterile scalpel Uredinospores of the rust and the conidia of leafspot pathogens and the fungus invading the lesions were stained with methylene blue for examination under a light microscope Lesions were also processed through the critical point dry method (9) using liquid carbon dioxide coated with gold and examined under a scanning electron microscope (Model JSM 35 C JEOL Ltd Akishima Tokyo Japan)

Isolation and pathogenicity tests

The fungus present on the rust early and late leafspot lesions was isolated in pure culture by scraping lesion surfaces with a sterile scalpel and plating urediniospores of the rust and conidia of the leafspot pathogens on potato dextrose agar (PDA) Plates were incubated in a growth chamber at 25 C with a 12 hr photoperiod The fungus was isolated and multiplied on PDA at 25 C and a conidial suspension (*ca* 10^6 conidia/ml) was prepared

Fig. 1 Uredinia of peanut rust colonized by the hyperparasite *Verticillium lecanii*

Fig. 2. Late leafspot lesions of peanut colonized by the hyperparasite *Verticillium lecanii*.

in sterile distilled water from a 10-day-old culture.

Inocula of the rust, early and late leafspot pathogens were multiplied on detached leaves of TMV 2. Conidia were collected with a cyclone spore collector (ERI Instrument Shop, Iowa State University, Ames, Iowa 50011) and suspensions (*ca* 50,000 conidia/mL) were made in sterile distilled water containing 0.2 mL/1,000 mL of water of the wetting agent, Tween 80 (polyoxyethylene sorbitan monooleate).

Mature, undamaged leaves of TMV 2 were collected from 40-day-old potted plants raised in the glasshouse, washed in running tap water, and arranged with their petioles buried in a layer of sterilized river sand in plastic seed trays (56 cm long x 25 cm wide x 5 cm deep). The sand was moistened with Hoagland's nutrient solution. Trays were covered with clear plastic sheets and incubated for 24 hr in plant growth chambers (Percival Refrigeration and Mfg. Co., Boone, Iowa 50036) adjusted to 25 C with a 12 hr photoperiod. Trays were removed from growth chambers and spore suspensions of rust, early and late leafspot pathogens were sprayed over the leaves. Ten leaves were inoculated with each pathogen. The trays were again covered with plastic sheets and returned to the growth chambers.

Ten days after inoculation, when rust and leafspot lesions appeared, the trays were removed from plant growth chambers and the leaves were sprayed with a spore suspension of the test fungus. Trays were again covered with plastic sheets and returned to the plant growth chambers.

Biological control of rust and late leafspot diseases

The effects of the fungus isolated from rust and late leafspot lesions on rust and late leafspot development on peanuts were studied. Inocula of the rust and late leafspot pathogens and the test fungus were prepared as described above. Leaves of TMV 2 were obtained from glasshouse-grown plants and arranged in plastic trays as described above. There were five replications, and each replicate treatment had two leaves. The inoculation treatments were as follows:

1. Leaves inoculated with the test fungus only (check).
2. Leaves inoculated with either rust or late leafspot pathogen only (check).
3. Leaves inoculated with a mixture (50:50, v/v) of the test fungus and the rust or late leafspot pathogen.
4. Leaves inoculated with the test fungus two days before inoculation of rust and late leafspot pathogens.

Disease development was assessed at 20 and 30 days after inoculation (DAI). Parameters evaluated were:

Receptivity: At 20 DAI, the total number of lesions on each leaf was counted. Leaf areas were measured with a leaf area meter (Hayashi Denkoh Co. Ltd., Tokyo, Japan). Receptivity was expressed as number of lesions/cm^2 leaf area.

Percentage leaf area damaged: At 20 and 30 DAI, the percentage area of each leaf damaged by rust and late leafspot, which included yellowing and necrosis, was estimated by comparison with schematic diagrams depicting leaves with known percentages of their areas affected. In the case of late leafspot, the defoliation was also measured. The loss of each leaf was considered as 100% leaf area damage and included in calculating the percentage leaf area damaged.

Percentage data were subjected to angular transformation and an analysis of variance was carried out.

Results and Discussion

Microscopic examination of urediniospores of the rust and conidia of the early and late leafspot pathogens revealed the presence of a fungus extensively colonizing them (Fig. 3). In a majority of the cases, the fungus established contact directly through the spore wall and, in a few cases, through germspores. Mycelia were present inside the spores of rust and leafspot pathogens, but sporulation was not evident. Lysing of the spores was commonly observed (Fig. 4). Bursting of spores due to extensive growth of the fungus internally was also observed occasionly (Fig. 5).

Lesions of rust, early and late leafspots inoculated with the test fungus showed typical downy fungal growth within a week after inoculation. Reisolation of the fungus from the infected lesions yielded cultures identical to the parent cultures and were identified as *Verticillium lecanii* (Zimmerm.) Viegas by Dr. B. L. Brady, CAB International Mycological Institute, Kew, Surrey, UK.

There was a significant reduction in receptivity of rust and late leafspot when inoculated with *V. lecanii* either as a mixture with the pathogen or as a preinoculation treatment. Percentage leaf area damaged from rust was also significantly reduced when inoculated with *V. lecanii*, especially in the preinoculation treatment. Inoculation of *V. lecanii* as a mixture with the late leafspot pathogen was not effective in reducing the percentage leaf area damage; however, the preinoculation treatment with *V. lecanii* reduced the percentage leaf area damage (Table 1). *V. lecanii* was not pathogenic to peanut. These results indicate the potential for use of *V. lecanii* to control peanut rust and late leafspot diseases.

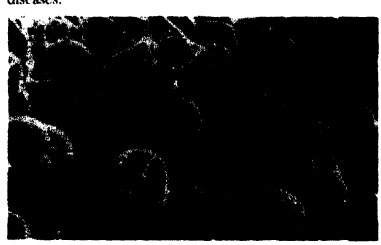

Fig. 3. Hyphae of *Verticillium lecanii* parasitizing urediniospores of peanut rust.

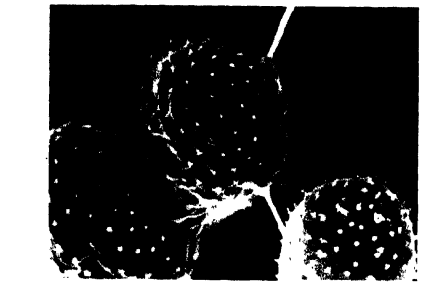

Fig. 4. Lysing of urediniospores of peanut rust due to invasion by *Verticillium lecanii*.

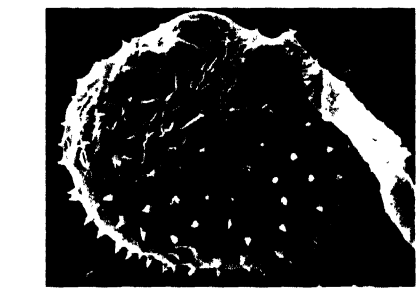

Fig. 5. Bursting of urediniospores of peanut rust due to invasion by *Verticillium lecanii*.

Hyperparasites such as *Penicillium islandicum* Sopp., *Eudarluca caricis* (Fr.) O. Ericks, *Acremonium persicinum* (Nicot.) W. Gams, *Darluca filum* (Biv. Bern ex Fr.) Cost., *Tuberculina costaricana* Syed., and *Hyalodendron* sp. have been reported to parasitize the peanut rust pathogen (8, 12); however, no serious attempts have been made to use any of these organisms in biological control of peanut rust. *Dicyma pulvinata* (Berk. & Curt.) v. Arx (=*Hansfordia pulvinata* (Berk. & Curt.) Hughes) has been observed to parasitize the leaf spot pathogens of peanut (3, 10, 13). It was found to be effective in controlling late leafspot both under field and greenhouse conditions (6, 7, 14).

Verticillium lecanii is a polyphagous fungus and has been reported as parasitic on insects and on a number of fungal pathogens (1, 5, 11). *V. lecanii* has also been reported parasitic on peanut rust in India (12) and Burkina Faso (15), and on leafspot pathogens in India (4). *V. lecanii* has a high potential use in biological control of rust, early and late leafspot diseases of peanut since it can parasitise all three pathogens which normally occur together and may cause severe damage to peanut crops wherever peanut is grown. Preliminary studies conducted with *V. lecanii* in India have shown considerable reduction in late leafspot severity (2). However, further studies are required to assess the possible use of *V. lecanii* in biological control of rust and leafspot diseases of peanut at the field level.

Table 1. Effect of inoculation with Verticillium lecanii on receptivity and leaf area damage by rust and late leafspot pathogens on peanut.

	(lesions/cm²)	Leaf area damaged (%)		Receptivity r (lesions/cm²)	Leaf area damaged (%)	
		20 DAI[1]	30 DAI		20 DAI	30 DAI
Pathogen alone[2]	12.6	21	30	8.7	48	90
Pathogen + *V. lecanii* (mixture)[3]	7.3	9	22	4.5	43	91
Preinoculation with *V. lecanii*[4]	5.3	7	10	3.3	30	78
LSD (5%)	4.13	7.1	8.5	1.83	14.1	14.2
CV (%)	33.7	23.0	22.5	25.9	25.0	13.9

1. Days after inoculation
2. Leaves inoculated with either rust or late leafspot pathogen
3. Leaves inoculated with a mixture (50 : 50, v/v) of *V. lecanii* and rust or late leafspot pathogen.
4. Leaves inoculated with *V. lecanii* two days before inoculation of rust or late leafspot pathogens.

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