IMMUNOLOGY

Serological Differentiation of *Pythium aphanidermatum* from *Phytophthora parasitica* var. *nicotianae* and *Ph. parasitica*

THIS report describes the use of serological techniques to distinguish two morphologically similar pathogenic fungi in soil. *Pythium aphanidermatum* (Edson) Fitzp. causes damping-off in many plants, including the tobacco (*Nicotiana tabacum* L.) and tomato (*Lycopersicon esculentum* Mill.) plants¹. Recently Dukes *et al.* reported that this fungus also causes dark brown to black stem cankers on tomato seedlings grown in fields in South Georgia². *Phytophthora parasitica* (Dast.) var. *nicotianae* (B. de Haan) Tucker [= *Phytophthora nicotianae* var. *nicotianae*³] causes black shank of tobacco in this same area of South Georgia⁴, and has been reported to cause damping-off of tobacco seedlings, as well as a disease of eggplant, tomato and other plants⁵. It is difficult to differentiate quickly between these two indigenous soil-borne fungi in the vegetative state, whether in culture or in host tissues. To identify these pathogens in host tissue when reproductive structures are lacking, it is necessary to isolate and grow them in culture on media suitable for production of asexual bodies. Some isolates of both species may produce reproductive structures very slowly or not at all^{6,7}. Proper identification of these species thus may require considerable time and effort, especially by workers not familiar with this group of fungi.

It was therefore decided to apply serological techniques towards developing a rapid method of distinguishing these species. In addition, it was decided to compare serological properties of *Ph. parasitica* [= *Ph. nicotianae* var. *parasitica*³] with those of *Ph. parasitica* var. *nicotianae*, because the only apparent difference between these two fungi is the ability of the latter to cause black shank of tobacco⁸. Recently Haasis reported that isolates of *Phytophthora* from several hosts other than tobacco caused a disease on a susceptible tobacco variety⁹. Whether these isolates caused typical black shank symptoms, however, is not known.

Previous serological work with pythiaceae fungi includes that of Burrell *et al.*¹⁰, who found that species-specific sera were efficient for the identification of *Ph. cactorum* (Leb. & Cohn) Schroet., *Ph. cinnamomi* Rands and *Ph. erythroseptica* Pethyb. Antiserum of *Ph. cactorum* cross-absorbed with *Ph. erythroseptica* did not react with antigens of several other fungi, including *Ph. parasitica* and three *Pythium* species.

To prepare the antisera, the three fungi were grown for 1 week at 28° C on a rotary shaker in potato glucose broth which consisted of the filtered juice from 200 g of fresh boiled potatoes, 20 g glucose, and distilled water to make up to 1,000 ml. Mycelia were then separated on a fine mesh nylon screen, washed twice with physiological saline, homogenized for 2 min in a blender and separated by centrifugation. They were resuspended in physiological saline, disintegrated in an ice bath for 10 min with a 'Branson S-75 Sonifier' at 6 amp, preserved with 1:10,000 'Thimersol', and kept at 4° C during the inoculation series. At first the preparations were injected intravenously into rabbits; however, 100 per cent mortality resulted with *Ph. parasitica* var. *nicotianae* inoculations. A modified injection schedule was therefore used as follows for each preparation: intramuscular injections of 1 ml. fungus suspension, plus 1 ml. 4 per cent sodium alginate adjuvant on the first and tenth day; intraperitoneal injections of 1, 2, 3, 4 and 5 ml. on the first, fourth, seventh, tenth and eighteenth days, respectively; and exsanguination on the twenty-eighth day. The antisera were frozen without preservatives.

Fungal materials for absorption studies were similarly prepared as used for the injection series except that no preservative was included. Antisera were absorbed by

mixing, intermittently, 2 ml. of absorbent with 2 ml. of antisera for 2 h at 37° C, and then clarifying antisera by centrifugation. Control antisera were treated similarly except that physiological saline was used instead of absorbent. Resulting preparations of antisera were tested in immuno agar diffusion plates against suspensions of disintegrated mycelia; reactant wells were 10 mm apart. Also, attempts were made to detect *Py. aphanidermatum* mycelium serologically in tomato stem lesions by homogenizing diseased tissue from several plants in physiological saline, filtering out coarse debris with a cheese cloth, concentrating the material by centrifugation, disintegrating the material by ultrasonification and testing the material against *Py. aphanidermatum* antiserum in immuno agar diffusion plates.

The results showed that *Py. aphanidermatum* antiserum was serologically specific; it was therefore unnecessary to absorb it. Undiluted *Ph. parasitica* var. *nicotianae* antiserum produced a faint band against *Py. aphanidermatum*, which was not evident after the antiserum was diluted 1:1 with physiological saline and incubated as an absorption control. The antiserum caused two sharp bands against each *Phytophthora* variety, but absorption with either variety removed all detectable antibodies. *Ph. parasitica* antiserum was serologically distinct from *Py. aphanidermatum* but was otherwise similar to *Ph. parasitica* var. *nicotianae* antiserum.

No precipitin lines formed when *Pythium*-infected tomato stems were tested against *Pythium* antiserum.

It is concluded that *Py. aphanidermatum* can be readily distinguished serologically from either *Ph. parasitica* var. *nicotianae* or *Ph. parasitica*, and that the two varieties of *Ph. parasitica* are serologically very similar. It was not possible to detect *Py. aphanidermatum* in infected tomato stems using immuno agar diffusion methods; however, suspected fungi could be isolated and then identified serologically. Also, the specificity of *Py. aphanidermatum* antiserum suggests that immunofluorescent techniques could effectively detect the species in mounts of infected tissue.

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Development of Immunological Memory during the Primary Immune Response

THE existence of immunological memory has been classically recognized by the appearance of a "secondary" type of antibody response to antigenic challenge of a previously immunized animal. Whereas the kinetics of the early phases of antibody production have been intensively investigated¹, little is known about the development of immunological memory during the early stages of