Short communication

Infection of Pythium sylvaticum in vitro with tobacco mosaic virus

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The discovery that viruses occur in fungi is rather recent (Hollings, 1962). Seven examples of virus transmission by fungi have been described, all involving Oomycete fungi which infect plant roots in the soil (Hollings and Stone, 1969).

It has been possible to incite infection of a *Pythium* culture *in vitro* by tobacco mosaic virus (TMV) (Brants, 1969), but it was impossible to identify the *Pythium* culture used because it is probably heterothallic. Therefore experiments were started with an identified fungus: *Pythium sylvaticum* Campbell & Hendrix. A male and a female strain of this fungus were obtained from Mrs A. J. van der Plaats-Niterink of the Centraal Bureau voor Schimmelcultures at Baarn.

Little is known about the pathogenicity of this fungus. Gams and Domsch (1969) found that *P. sylvaticum* can infect seedlings of wheat, pea and rape. Blok (1970) tested seedlings of various commercial plants, including wheat, flax, pea, radish, lettuce, carrot and cucumber. Clear differences in pathogenicity between the male and female strain of *P. sylvaticum* were not noted.

The purpose of the experiments described here was to determine whether it is possible to introduce TMV into *P. sylvaticum*. Liquid cultures of *P. sylvaticum* were inoculated with TMV as described by Brants (1969). Virus-containing mycelia were subcultured in virus-free media and tested regularly for virus content for more than 2 months. After the mycelia had been washed with antiserum against TMV, virus could still be demonstrated in these mycelia though the concentration was low.

On solid media the fungus growth is retarded by virus infection. The growth of a virus-containing culture in liquid medium also differed markedly from a virusfree culture of the same age (Fig. 1).

As yet, it is not certain whether the virus occurs within the mycelium or attached to the outside. Maintenance of infectivity after washing the mycelium with antiserum favours the view that the virus particles occur inside the mycelium.

We do not know whether the virus can multiply in the mycelium. Even $2\frac{1}{2}$ years after inoculation a *Pythium* culture on solid medium contained TMV, indicating that the virus is rather persistent in the fungus. The striking differences between virus-free and virus-containing cultures strongly suggest that the latter becomes diseased.

When hyphal tips from a virus-containing liquid culture were used as inoculum for

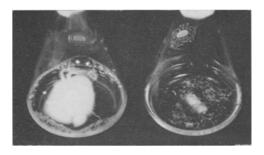


Fig. 1. Liquid shaken cultures of *P. sylvaticum* inoculated with TMV (right) and without virus (left), both of the same age.

Fig. 1. Vloeistofcultures van P. sylvaticum geinoculeerd met TMV (rechts) en zonder virus (links), beide van dezelfde leeftijd.

serial subculturing, eventually a virus-free culture was obtained. It is possible that rapidly growing hyphal tips cannot be reached by virus because virus transportation from the older parts of the mycelium towards the tips is too slow. The virus-free culture looked completely normal and no retardation of growth was observed. Whether the virus-containing mycelium can introduce virus into a plant is a question currently under study.

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Samenvatting

Infectie van Pythium sylvaticum in vitro met tabaksmozaïekvirus

Cultures van *P. sylvaticum* werden *in vitro* geïnoculeerd met tabaksmozaïekvirus (TMV). Virushoudende mycelia werden verder gekweekt in virusvrije media gedurende meer dan twee maanden en regelmatig getoetst op aanwezigheid van virus. Ook nadat de mycelia gewassen waren met antiserum tegen TMV kon virus er in worden aangetoond.

De virusconcentratie was laag. De groei van een virushoudende *Pythium*-cultuur verschilde duidelijk met die van een even oude, gezonde cultuur (Fig. 1). Een *Pythium*-cultuur, $2\frac{1}{2}$ jaar geleden geïnoculeerd met virus en verder gekweekt op een vaste voedingsbodem, bleek nog virus te bevatten. Of een virushoudend mycelium het virus op een plant kan overbrengen en zo als vector kan dienen kon nog niet worden aangetoond.

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