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Growth of *Venturia inaequalis* on apple tissue cultures

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Abstract

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Apple tissue cultures were colonized by *Venturia inaequalis* during the active growth period of the tissue. The fungus grew and sporulated to a similar extent on tissue cultures from susceptible apple varieties (Golden Delicious and Gravensteiner) and on tissues from varieties with the gene Vf for resistance against scab (Sir Prize, Liberty and Mac Free).

Introduction

In many host – parasite systems the expression of disease resistance in tissue cultures has already been investigated (Helgeson 1983). However, few attempts have been made to study host – pathogen interactions in the apple – *Venturia inaequalis* system. Saad (1965), in studies reviewed by Boone (1971), did not succeed to infect actively growing apple tissue in cultures with either spores or mycelial pellets of *V. inaequalis*. To our knowledge no further efforts were undertaken to verify the results obtained by Saad (1965).

The present paper reports the growth of *V. inaequalis* on non – senescent apple tissue cultures derived from the susceptible and from scab resistant apple varieties.

Materials and methods

Tissue cultures. – Branch sections from varieties of apple trees susceptible to the fungus *V. inaequalis* (Golden delicious and Gravensteiner) and from varieties carrying the gene Vf for resistance to apple scab (Liberty, Mac Free and Sir Prize) were cleaned in running tap water and sterilized by rinsing with absolute ethanol, immersing for 20 min in 2% (v/w) sodium hypochlorite and washing three times in sterile water. After removing the cortical tissue and cutting off the ends of the sections, the branch pieces were sliced into segments between 0.5 and 1 cm in length and planted on the solid nutrient medium. These procedures were done aseptically. The medium used for tissue culture induction, growth and maintenance was composed as described by Pech et al. (1975) with the following modifications: thiourea was omitted; 1% Bacto Agar Difco per 1 l medium was added; and the pH was adjusted to 5.6 with 1M NaOH. The medium was dispensed in 40 ml portions in Petri dishes (diameter 9 cm) and the dishes were sealed with Parafilm. Tissues

raised from the explants were the parental lines; the tissues used in experiments being the third subculture from them. Tissue cultures were incubated in total darkness at 24 °C and transferred to fresh media at intervals of 25–30 days.

Fungus. – Stock cultures of the *V. inaequalis* (Cke.) Wint. ETH-strain No. 3 (a monosporic isolate) were maintained at 20 °C on solid nutrient medium (malt, yeast, agar: 2%, 0.4%, 2% / 1l H_2O). Inoculum was obtained from 6 day old fungal cultures. Pieces of the mycelium were stripped off the plates aseptically. The mycelial strips (2 mm^3 pellets) were then floated in 2 ml of sterile water in a Petri plate. Spore suspension were obtained by rinsing the plate with 2 ml of sterile water and diluting this suspension to the desired spore concentration.

Inoculation procedure. – Tissues were inoculated asceptically with a 0.1 ml drop of spore suspension (10^5 spores/1 ml H_2O) or with a mycelial pellet. The inoculation was done immediately after transferring the tissue to fresh media. The inoculum was carefully placed on top of the tissue in such a way as to prevent contact with the medium. Inoculated tissues were incubated at 20 °C and transferred to fresh media at monthly intervals. The experiments were repeated 3 times using 12 tissue pieces per apple variety and experiment.

Results

Callus formation of all tested apple varieties started 7 days after culturing the explants. The tissue cultures were heterogenous in nature, with a variation in cell shape and size. White, compact calli from the varieties Mac Free (Fig. 1,1), Sir Prize or Gravensteiner, appeared to be slower growing than the fast expanding friable, yellow calli of Golden Delicious (Fig. 1,3) or Liberty. The growth of *V. inaequalis* was observed on all tested apple tissue cultures 5 to 6 days after inoculation with mycelial pellets. Sporulation started 4 to 5 days later. The mycelium expanded slowly (Figs. 1, 2 and 1, 4), eventually covering the entire tissue. Infected tissues continued to grow but at a reduced rate. The growth of the fungus on apple tissue cultures inoculated with mycelial pellets was independent of the morphological form of the tissues.

Examination of spore-suspension inoculated tissues did not reveal consistent results. In certain cases the growth of the fungus was already macroscopically observable 8 to 9 days after inoculation, whereas in other cases the visible development took place after 4 to 5 weeks of incubation. Increasing the concentration of spore inoculum from 10^5 to 3×10^5 or 5×10^5 (spores/ml H_2O) stimulated a slightly faster and more abundant growth of the fungus.

The soft, friable types of tissue from Liberty or Golden Delicious responded better to inoculation with spore-suspension than the hard, compact tissues from Gravensteiner, Sir Prize or Mac Free.

The growth of the pathogen on all tested apple tissue cultures, regardless of the method of inoculation, was not accompanied by any macroscopic changes in the tissues such as a change of colour or texture.

Discussion

V. inaequalis established itself on the apple tissue cultures during the period of active growth of the tissue and not only as the tissue became senescent (Saad 1965), (Fig. 1, 5). Apparently no inhibitory substances were produced in the tissue preventing the fungus from growing, contrary to what Saad (1965) hypothesized and Boone (1971) stated in his review. The Vf gene for disease resistance in intact plants was not

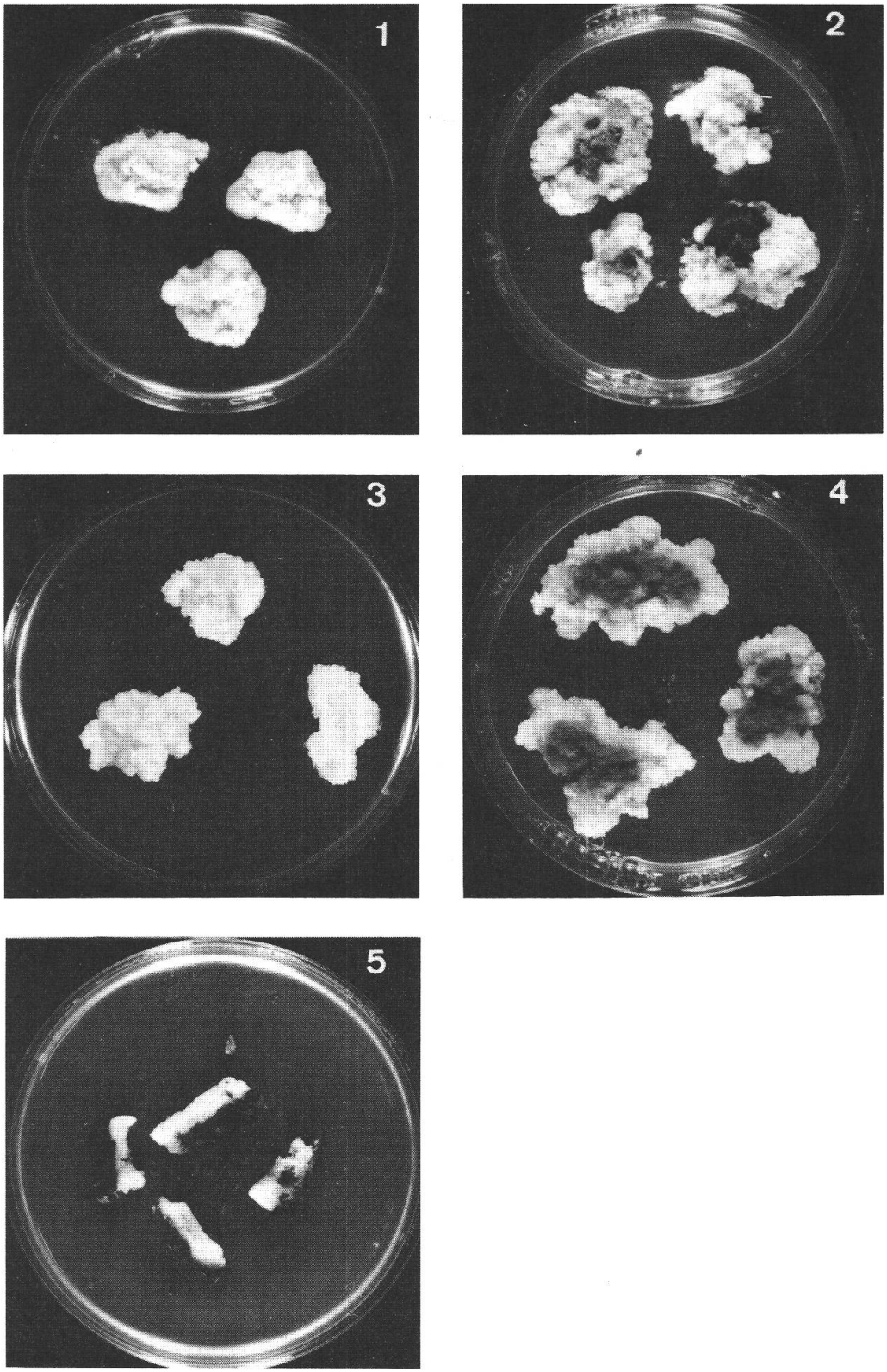

Fig. 1. Apple tissue cultures raised from the resistant variety Mac Free (1–2) and from the susceptible variety Golden Delicious (3–5). – 1 and 3, healthy tissues. 2 and 4, infected tissues one month after inoculation with mycelial pellets of *Venturia inaequalis*. 5, sections through tissue pieces totally colonized by *V. inaequalis* revealing healthy, living tissue beneath the mycelium.

expressed in the apple tissue cultures when tissues were cultivated on the medium supplied with the specific concentration of hormones employed in this study. Thus the possibility of examining the expression of resistance in apple varieties with the gene Vf by varying the hormonal regime remains to be investigated.

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Zusammenfassung

Wachstum von *Venturia inaequalis* auf Apfel-Gewebekulturen. Aktiv wachsende Kalli von Apfel wurden von *Venturia inaequalis* besiedelt. Der Pilz wuchs und sporulierte auf Kalli von den für Schorf anfälligen Apfelsorten (Golden Delicious und Gravensteiner) gleich gut wie auf den Kalli von Apfelsorten mit dem Resistenzgen gegen Schorf Vf (Sir Prize, Liberty und Mac Free).

References

Boone D. M. 1971. Genetics of *Venturia inaequalis.* Ann. Rev. Phytopathol. 9: 297–318.

Helgeson J. P. 1983. Studies of host-pathogen interactions in vitro. In Helgeson, J. P., B. J. Deverall (Eds.), Use of tissue culture and protoplasts in plant pathology, 9–38.

Pech J. C., Latche A., Austervy M. et Fallot J. 1975. Croissance in vitro de tissus et de suspension cellulaires de pomme. Bull. Soc. Bot. Fr. 122: 183–194.

Saad A. T. 1965. The culture of apple callus tissue and its use in studies on pathogenicity of *Venturia inaequalis.* Ph. D. thesis, Univ. Wisconsin, Madison, U.S.A.