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A selective medium for Botrytis cinerea to be used in a spore-trap

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Abstract

A selective medium has been developed for the use in spore-traps to study the dispersion of *B. cinerea* on gerbera grown in glasshouses.

Additional keywords: dispersal, gerbera.

To study the dispersion of air-borne fungi in glasshouses different types of spore-traps were used (Frinking et al., 1987; Hirst, 1959; Jarvis, 1962). These traps were based on air-suction or on impaction on sticky surfaces by natural airflows. In the case presented here fungal spores in glasshouse air were trapped using Petri dishes containing an agar-based culture medium to study the dispersion of the air-borne spores of *B. cinerea* on gerbera grown in glasshouses.

In glasshouse air, spores of many different fungi are present. When an universal medium was used, for example Potato Dextrose Agar (PDA), it was very difficult to recognize colonies of specific fungi such as *Botrytis cinerea*. Within 24 hours of incubation fast growing fungi such as *Mucor* spp. covered the whole surface of the universal medium, suppressing other fungi. For this reason it is necessary to use a selective medium in the spore-trapping device.

A selective medium for *B. cinerea* was described by Kritzman and Netzer (1978). They developed their medium for the isolation and identification of *Botrytis* spp. from soil and onion seed. The medium contains tannic acid as a substrate for polyphenoloxidase (PPO) activity. PPO activity of growing mycelium of a culture of *Botrytis allii* was found to convert the tannin in the medium into a dark-brown pigment. Other fungi were found to have PPO too, but were inhibited by PCNB (pentachloronitrobenzene), Maneb and $CuSO_4$. Germination of conidia, hyphal elongation and mycelium growth of *B. allii* and *B. cinerea* were not prevented on the selective medium. Brown pigmented colonies could be identified 48 hours after incubation at 24 °C (Kritzman and Netzer, 1978). This selective medium is likely to be very useful for the study of the development of *B. cinerea*.

The following selective medium for *B. cinerea* is an adjustment of the selective medium used by Kritzman and Netzer. The basal medium, which was almost the same as that used by Kritzman and Netzer, consists of the following components (g/l distilled water): $NaNO_3$, 1.0; K_2HPO_4, 1.2; $MgSO_4.7H_2O$, 0.2; KCl, 0.15; glucose, 20.0 and agar, 25.0. The medium was sterilized for 20 minutes at 121 °C. After cooling til 65 °C the follow-

ing ingeredients were added (g/l distilled water): Terrachlor (PCNB, pentachlorobenzene 75% WP), 15×10^{-3}; Maneb (manganese ethylene bisdithiocarbamate), 1×10^{-2}; chloramphenicol (antibiotic), 5×10^{-2}; $CuSO_4$, 2.2; Rubigan (fenarimol), 0.1 ml/l and tannic acid, 5.0. The pH of the supplemented basal medium (SBM) was adjusted to 4.5 with 5.0 N NaOH. Rubigan was added into the selective medium to suppress airborne spores of *Penicillium* spp.

At pH 4.5 colonies of *B. cinerea* formed mycelium, conidiophores and conidia. At pH 6.5, used by Kritzman and Netzer (1978) *B. cinerea* was not able to form conidia.

Germination and mycelial growth of *B. cinerea* was not prevented on this selective medium. It is therefore reliable and useful for monitoring *B. cinerea*-spores in a glasshouse environment.

The SBM was put into plastic Petri dishes (∅ 9 cm), 20-25 ml per Petri dish. These Petri dishes were mounted in spore-traps. The traps were custom-made. Each trap was built around a wooden block. To each of the four sides of the block the bottomhalf of a Petri dish containing the selective medium was attached (Fig. 1).

The traps were placed in the greenhouse for eight hours. After incubation for seven days at 20 °C and under a lightperiod of 24 hours per day dark brown spots were counted as colony forming units of *B. cinerea* (Fig. 2). Other fungi were inhibited by PCNB, Maneb, $CuSO_4$ and Rubigan. *Penicillium* spp. and *Trichoderma* spp. were still able to grow on the selective medium, but very slowly. They did not convert the medium to a dark-brown colour. With this selective medium it is possible to count more than 200 plates, with 10 to 15 colony forming units of *B. cinerea*, per hour.

After fourteen days the dark brown *Botrytis*-colonies started to produce conidia. Conidia from these colonies tested on Gerbera-petals all formed small lesions, typical for infection of *B. cinerea* (Salinas et al., 1989).

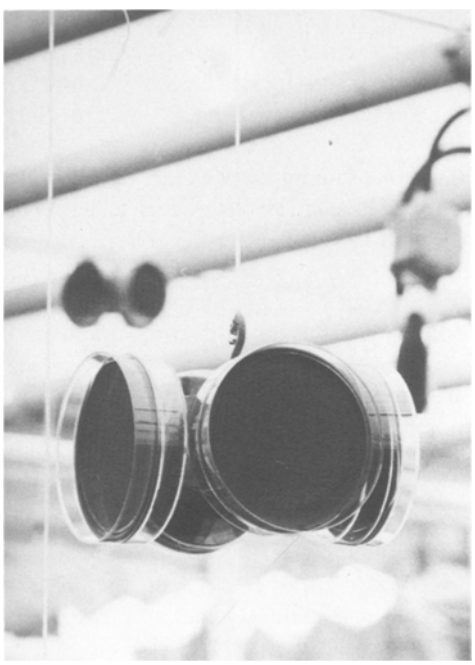

Fig. 1. Spore-trap device.

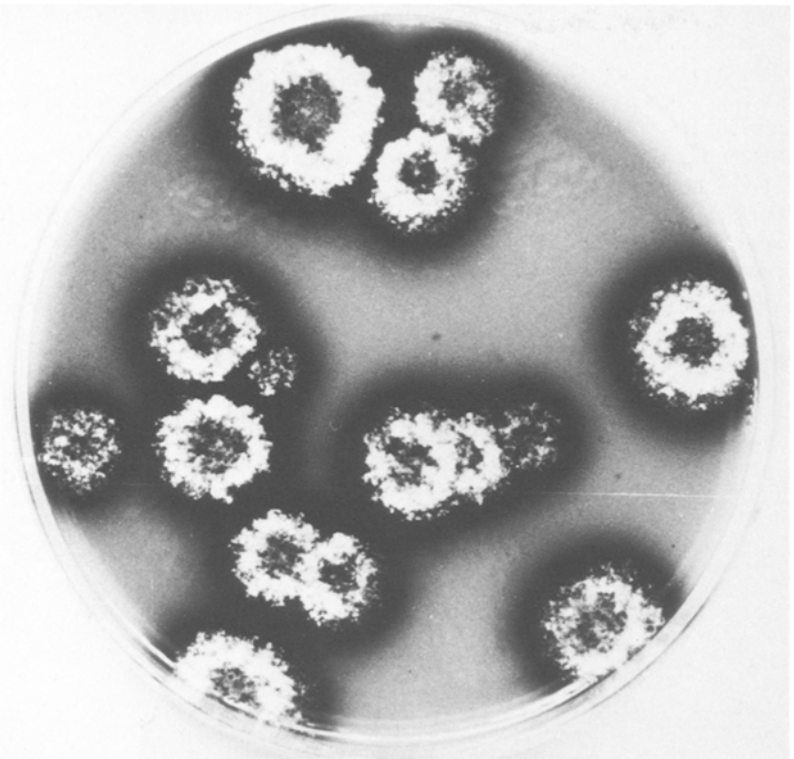

Fig. 2. Colonies of *Botrytis cinerea*, turning the colour of the selective medium into dark-brown.

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Samenvatting

Een selectief medium voor Botrytis cinerea, als onderdeel van een sporevanger

Een selectief medium voor *B. cinerea* is ontwikkeld voor het gebruik in sporevangers. Deze sporevangers werden gebruikt bij het bestuderen van de ontwikkeling van *B. cinerea* in gerbera geteeld onder glas.

References

Frinking, H.D., Gorissen, A. & Verheul, M.J., 1987. Dissemination of mildew spores in a glasshouse: Pattern or chaos? International Journal of Biometeorology 31: 147-156.

Hirst, J.M., 1959. Spore liberation and dispersal. Chapter XLVII in: Holton, C.S., Fisher, G.W., Fulton, R.W., Hart, H. and McCallan, S.E.A. (Eds), Plant pathology: Problems and progress, 1908-1958, Madison, The University of Wisconsin Press, 1959, 588 pp.

Jarvis, W.R., 1962. The dispersal of spores of *Botrytis cinerea* Fr. in a raspberry plantation. Plant Disease Reporter 56: 126-130.

Kritzman, G. & Netzer, D., 1978. A selective medium for isolation and identification of *Botrytis* spp. from soil and onion seed. Phytoparasitica 6: 3-7.

Salinas, J., Glandorf, D.C.M., Picavet, F.D. & Verhoeff, K., 1989. Effects of temperature, relative humidity and age of conidia on the incidence of spotting on gerbera flowers caused by *Botrytis cinerea*. Netherlands Journal of Plant Pathology 95: 51-64.