THE EFFECT OF VARIOUS TREATMENTS OF CULTURE MEDIUM UPON THE GROWTH OF CERTAIN FUNGAL ANTAGONISTS

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Many soil fungi have been found to be antagonists of *Sclerotinia trifoliorum* ERIKSS., *S. sclerotiorum* (LIB.) BREF. and *Botrytis cinerea* PERS. (1, 2, 9, 10). Since external conditions have an influence on these antagonists (3, 4, 5) and thus indirectly affect their ability to suppress the occurrence of harmful parasitic fungi of cultivated plants, this study dealt with the effect of various treatments of culture medium upon the growth of such soil fungi, whose antagonism is based on their capacity to destroy the sclerotia of their hosts.

Materials and methods

The following fungal antagonists, which have been found to be antagonists of *S. trifoliorum*, *S. sclerotiorum* and *B. cinerea*, were used in these studies:

Acrostalagmus roseus BANIER, isolate I (cf. 10). Isolated from a sclerotium of *S. trifoliorum* in 1954.

A. roseus, isolate II. Isolated from a sclerotium of *S. trifoliorum* in 1963.

Coniothyrium minitans, CAMPB., isolate I. Isolated from foreign red clover seed in 1962.

C. minitans, isolate II. Isolated from a sclerotium of *S. trifoliorum* in 1962.

Fusarium avenaceum (FRIES) SACC. Isolated from carnation at the Department of Plant Pathology, Tikkurila, in 1955.

Gliocladium roseum (LINK) THOM. (cf. 7). Isolated from a sclerotium of *S. trifoliorum* in 1955.

Gliocladium sp. Isolated from a sclerotium of *S. trifoliorum* in 1955.

Mucor hiemalis WEHM. (cf. 7). Isolated from a sclerotium of *Claviceps purpurea* in 1959.

M. spinosus TIEGH. Isolated from Henneberg agar medium treated with pentachloronitrobenzene in 1962.

Rhizopus nigricans EHRENB. Isolated from dead clover in 1962.

Sporotrichum carnis BROOKS & HANSF. Isolated from a sclerotium of *S. trifoliorum* in 1962.

Trichoderma viride (TODE) HARTZ (cf. 7). Isolated from a sclerotium of *S. trifoliorum* in 1955.

Trichothecium roseum LINK. Isolated from mycelia of *S. trifoliorum* in potato stem in 1963.

The fungi were cultured on a basic medium consisting of Henneberg agar (10 g peptone, 2 g $NH_4H_2PO_4$, 2 g KNO_3, 0.5 g $MgSO_4$, 0.1 g $CaCl_2$, 100 g glucose, 20 g agar and 1000 ml distilled water). In the different trials the following additions were made to the basic medium:

Potassium was added at a rate of 200 g/m² in the form of 50 % potassium chloride (KCl) or as potassium sulphate (K_4SO_4).

In the tests with pentachloronitrobenzene (PCNB), Brassicol dust at rates of 50 and 100 g/m² was applied to the solidified surface of the agar in the Petri dishes.

The pH of the Henneberg agar — normally 6.7 — was made more acidic by adding 10 % hydrogen chloride (HCl) or more basic by adding 10 % potassium hydroxide (KOH).

In addition to the above treatments, the effect of oxygen deficiency on the growth of the fungi was studied by sealing the Petri dishes with modelling clay after inoculation of the fungi. The control dishes were left unsealed.

The fungi were transferred with an inoculating needle to the centre of the agar medium in the Petri dish, and the size of the colony was measured from time to time. In determining the effect of PCNB, the vigour of the fungal growth was estimated visually on a scale of 0—3. All the trials were carried out at room temperature in the laboratory. The control organism used was *S. trifoliorum* which had been isolated in the autumn of 1960 from a first-year clover ley at Viik.

Results

E f f e c t o f p o t a s s i u m. The addition of potassium — either as chloride or as sulphate — had no appreciable effect on the growth of the fungi (Fig. 1). The differences in colony size of the same isolate on the differently treated plates were either very small or completely lacking.

E f f e c t o f p e n t a c h l o r o n i t r o b e n z e n e (PCNB). The fungi in these tests were cultivated on Henneberg agar which had been treated with Brassicol dust, and their growth was compared with that of fungi on untreated plates. It was found that the different isolates responded differently to PCNB. All of them grew more poorly on the treated than on the control medium on which their growth

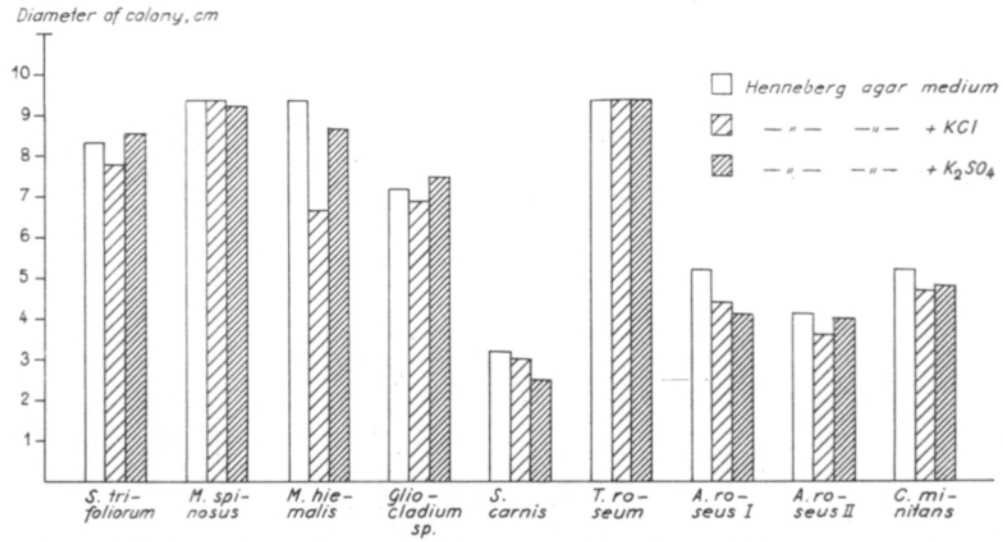

Fig. 1. The effect of potassium on the growth of some fungi. The trial period was 14 days.

was rated as »3» on the scale. In comparison with that the fungi grew on the PCNB-treated medium as seen below:

S. trifoliorum	0	*Gliocladium* sp.	2
A. roseus I	1	*M. hiemalis*	1
A. roseus II	2	*M. spinosus*	2
C. minitans I	1	*R. nigricans*	0
C. minitans II	1	*S. carnis*	0

The species *S. trifoliorum*, *R. nigricans* and *S. carnis* were most sensitive to PCNB since their development ceased completely. In contrast, such parasites of *S. trifoliorum* as *A. roseus*, *Gliocladium* sp. and *M. spinosus* were considerably more resistant to the chemical.

The tolerance of *A. roseus* isolate II to PCNB was further studied by culturing it for 4 weeks on Henneberg agar to which Brassicol dust had been added at rates of 50 and 100 g/m². According to the results shown below, the fungus grew relatively well at both consentrations.

Rate of PCNB	Diameter of colony, cm
0	9.4
50 g/m²	7.0
100 »	5.9

E f f e c t o f pH. It is generally known that fungi can exist over a wide range of pH values (6, 8). In the present tests, *S. trifoliorum*, *A. roseus* I, *Gliocladium* sp., *T. roseum* and *T. viride* were cultivated on Henneberg agar whose pH varied by 1-unit intervals from 2 to 9. It was found that all the fungi grew when the pH was between 4 and 9. The pH optimum of *S. trifoliorum* was 4—6 while *Gliocladium* sp. and *A. roseus* favoured a more alkaline substrate. *T. viride* was the only fungus which grew at pH 2. In this highly acidic medium, however, the appearance of the colony

differed from normal: it was slimy and pale-coloured, and no conidia were formed. At pH 8—9 the normal green colour of this fungus changed to yellow; abundant formation of conidia was not inhibited.

Effect of oxygen deficiency. A deficiency in the oxygen supply slowed the development of *S. trifoliorum*, *A. roseus* I and II, *M. hiemalis* and *T. viride*. Toward the end of the 4-week trial period their growth stopped completely. In such dishes no conidia were formed, nor did *S. trifoliorum* produce sclerotia. However, even four months after the end of the trial, all the fungi in the sealed dishes were still viable.

Conclusions

The addition of potassium chloride and potassium sulphate at a rate of 200 g/m² to the culture medium had no effect on the growth of *Sclerotinia trifoliorum*, *Acrostalagmus roseus* I and II, *Coniothyrium minitans*, *Gliocladium* sp., *Mucor hiemalis*, *M. spinosus*, *Sporotrichum carnis* and *Trichothecium roseum*.

Treatment of the culture medium with PCNB suppressed to some extent the development of *A. roseus* I and II, *Gliocladium* sp., *C. minitans* I and II, *Mucor hiemalis* and *M. spinosus*. The fungal species *S. trifoliorum*, *Rhizopus nigricans* and *Sporotrichum carnis* did not grow at all on PCNB-treated medium.

S. trifoliorum, *A. roseus* I, *Gliocladium* sp., *T. viride* and *T. roseum* were able to grow in the pH range of 4—9. When the pH of the medium was 2, only *T. viride* grew.

Oxygen deficiency suppressed the growth of *S. trifoliorum*, and the growth and the conidia formation of *A. roseus* I and II, *M. hiemalis* and *T. viride*.

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SELOSTUS:

KASVUALUSTAN ERILAISTEN KÄSITTELYJEN VAIKUTUKSESTA ERÄIDEN TUHOSIENIEN ANTAGONISTIEN KASVUUN

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Tutkittaessa kasvualustan erilaisten käsittelyjen vaikutusta eräiden maasienien kasvuun, todettiin, että kaliumkloridin (KCl) ja kaliumsulfaatin (K_2SO_4) 200 g/m² vastaava lisäys ravintoalustaan ei vaikuttanut *Sclerotinia trifoliorum*in, *Acrostalagmus roseus* I:n ja II:n, *Coniothyrium minitans*in, *Gliocladium* sp:n, *Mucor hiemalis*en, *M. spinosus*en, *Sporotrichum carnis*en ja *Trichothecium roseum*in kasvuun.

Ravintoalustan käsittely PCNB:llä heikensi jonkin verran *A. roseus* I:n ja II:n, *Gliocladium* sp:n, *C. minitans* I:n ja II:n, *Mucor hiemalis*en sekä *M. spinosus*en kasvua. Sensijaan *S. trifoliorum*, *Rhizopus nigricans* ja *Sporotrichum carnis* eivät kasvaneet olleenkaan PCNB:llä käsitellyllä ravintoalustalla.

Sienien *S. trifoliorum*, *A. roseus* I, *Gliocladium* sp., *T. viride* ja *T. roseum* todettiin menestyvän pH-rajoissa 4—9. Ravintoalustan pH-arvon ollessa 2 kasvoi vain *T. viride*.

Hapenpuute hidasti sienien *S. trifoliorum*, *A. roseus* I ja II, *M. hiemalis* ja *T. viride* kasvua ja kuromanmuodostusta.