



CERTAIN PARASITES OF FUNGAL SCLEROTIA

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Received October 2, 1964

Cultures have been made on a total of 51 different microbe isolates which have proved to be antagonistic to certain pathogenic fungi of cultivated plants. Among these isolates were some fungal species parasitizing the sclerotia of *Sclerotinia trifoliorum* ERIKSS., *S. borealis* BUB. & VLEUGEL, *S. sclerotiorum* (LIB.) BREF., *Claviceps purpurea* TUL. and *Botrytis cinerea* PERS., as well as some bacteria having an antibiotic effect on *S. trifoliorum* (5, 6, 8, 9, 10, 11, 12).

Materials and methods

The sclerotia of *S. trifoliorum* and *S. sclerotiorum* were cultivated in the laboratory at a temperature of about 10°C on Henneberg agar (10g peptone, 2g NH₄H₂PO₄, 2g KNO₃, 0.5g MgSO₄, 0.1g CaCl₂, 100g glucose, 20g agar, 1000ml distilled water). The sclerotia of *Botrytis cinerea* grew best when cultivated at 15°C on low-sugar Czapek's agar (3.0g NaNO₃, 1.0g K₂HPO₄, 0.5g MgSO₄ · 7H₂O, 0.5g KCl, 0.01g FeSO₄ · 7H₂O, 30g sucrose, 15g agar, 1000ml distilled water).

The sclerotia of *S. trifoliorum* and *S. sclerotiorum* were transferred outdoors in November 1961 and 1962. They were placed on the surface of soil in wooden boxes (15 × 25 cm) which had been buried in the ground and filled with earth. Every month during the winter and spring, up to the month of May, one box was brought inside and the sclerotia examined. In general, no fungal mycelium nor bacterial slime, was seen on the sclerotia. By scraping their surface with an inoculation needle a sample was taken to be cultivated on Czapek's agar medium in Petri dishes. The microorganisms which grew from these samples were isolated, and their parasitic or antibiotic effect on *S. trifoliorum* was studied. Most of the fungi which appeared were the same as those which had previously been included in the collections of the

Department of Plant Pathology; they were not further cultivated, with the exception of one isolate of *Acrostalagmus roseus*. Likewise, no cultures were made of the microbe isolates which had no antagonistic influence on the pathogenic fungi under study. As a rule, the bacteria which were isolated from the sclerotia had no significance as parasitic agents. Microbes were also isolated from the remains of dead clover plants on the field at different times of the year, as well as from the surface of sclerotia in laboratory trials, where they appeared as contaminants.

The isolated fungi were used to infect the sclerotia of *S. trifoliorum*, *S. sclerotiorum* and *Botrytis cinerea*. An isolate of *A. roseus*, which proved to have an exceptionally potent infective ability, was used as control (3, 6). The sclerotia were infected by immersing them for about two hours in a suspension of conidia and mycelial fragments of the parasite in distilled, autoclaved water. The sclerotia were then transferred to a moist, sterilized quartz sand medium in Petri dishes, which were kept at room temperature. The infective ability of two of the isolates, was studied at three different temperatures. In this case, the substrates were sterilized quartz sand and unsterilized soil.

Isolated microbes and their ability to infect sclerotia

In the years 1962—63 the following fungi found to have an antagonistic effect on phytopathogenic fungi were isolated.

Rhizopus nigricans EHRENB. (4, 14). Isolated from dead clover under the snow, 26. 3. 1962. The infection trials revealed that this isolate was a parasite of the sclerotia, infecting a greater number of sclerotia than *A. roseus* I (Table 1). However, only a small part of the infected sclerotia were destroyed during the 6-week trial period. The isolate grew rapidly and when cultivated on Henneberg agar it covered the entire surface of the Petri dish in two days. Colonies were formed by stolons, from which in certain spots branched hyphae attached themselves to the substrate by means of rhizoids. Sporangioophores arose from the mycelia in clusters. The sporangia and columellae were globose, the former having an average diameter of

Table 1. The ability of parasitic fungi to infect sclerotia on sterilized quartz sand substrate at room temperature. The number of sclerotia of each species varied from 10 to 30. Trial period 6 weeks.

Isolate	% of sclerotia infected			% of infected sclerotia destroyed during trial period		
	<i>S. trifoliorum</i>	<i>S. sclerotiorum</i>	<i>B. cinerea</i>	<i>S. trifoliorum</i>	<i>S. sclerotiorum</i>	<i>B. cinerea</i>
<i>A. roseus</i> I, control	36.7	100	100	13.3	90	100
<i>A. roseus</i> II	90	—	95	90	—	95
<i>Rhizopus nigricans</i>	60	100	100	10	30	100
<i>Sporotrichum carnis</i>	30	20	100	0	20	100

Table 2. The ability of *Coniothyrium minitans* isolate I to infect sclerotia on autoclaved quartz sand substrate at different temperatures. Trial period 6 weeks.

Isolate	% of sclerotia infected (out of 30)								
	<i>S. trifoliorum</i>			<i>S. sclerotiorum</i>			<i>B. cinerea</i>		
	20°C	9°C	3°C	20°C	9°C	3°C	20°C	9°C	3°C
<i>A. roseus</i> I, control	36.7	10	6.7	20	10	0	100	100	0
<i>C. minitans</i>	50	13.3	6.7	3.3	0	0	100	100	100

Table 3. The infective ability of *C. minitans* on different substrates and at different temperatures.

Substrate and isolate	% of sclerotia infected (out of 40)								
	<i>S. trifoliorum</i>			<i>S. sclerotiorum</i>			<i>B. cinerea</i>		
	20°C	9°C	3°C	20°C	9°C	3°C	20°C	9°C	3°C
Unsterilized soil:									
<i>C. minitans</i> I	17.5	0	0	72.5	37.5	75	100	90	85
<i>C. minitans</i> II	7.5	2.5	10	92.5	55	5	90	95	100
Sterilized quartz sand:									
<i>C. minitans</i> I	17.5	52.5	25	32.5	30	40	100	100	100
<i>C. minitans</i> II	47.5	70	42.5	67.5	62.5	67.5	100	100	100

225 μ and the latter 75 μ . The sporangiophores were dark-coloured, irregularly shaped or globose and furrowed. Their size ranged from $5.2 \times 8.0 \mu$ to $9.5 \times 12.7 \mu$. A few oval zygospores, having a diameter of 160–220 μ , also appeared in the mycelia. There were no chlamydospores. *R. nigricans*, which is a common soil fungus, can sometimes injure also cultivated plants (14). In some studies this species has been observed under certain conditions to hinder the growth of *Fusarium udum*, *Venturia inaequalis* and *Ophiobolus graminis* (13, 15, 19).

Sporotrichum carnis BROOKS & HANSFORD (4). Syn. *Aleurisma carnis* (BROOKS & HANSFORD) BISBY. Isolated on 15. 5. 1962 from a sclerotium of *S. trifoliorum* which had been outdoors the entire winter. The isolate had a somewhat weaker infective ability than *A. roseus* No. I. It was, however, definitely a parasite, since it completely destroyed the susceptible sclerotia of *B. cinerea* (Table 1). On Czapek's agar medium the colonies of this fungus were flat, initially whitish, later greyish. The hyphae were intertwined and branched, 1–2 μ in diameter. The conidiophores were short and branched, the conidia were oval and averaged 2.8–3.8 μ in size (Fig. 1). VARTIOVAARA (17, 18) found that *S. carnis* has effectively decomposed cellulose. It has also been established to grow even at a temperature as low as –6° C (2).

Coniothyrium minitans CAMPB. (1). Two isolates of which isolate I was isolated on 24. 5. 1962 from seed of Lakeland red clover imported from the U.S.A. and isolate



Fig. 1. *Sporotrichum carnis*. Conidiophore and conidia from a 10-day old culture on Czapek's agar medium. $\times 1000$.

II on 24. 11. 1962 from the surface of a sclerotium of *S. trifoliorum* which had been outdoors. In preliminary trials *C. minitans* isolate I was nearly as potent a parasite as *A. roseus* I (Table 2). Its infective ability was greatest at room temperature.

The relative infective ability of the two isolates of *C. minitans* was determined on two different substrates in Petri dishes: unsterilized soil medium and sterilized quartz sand. On both kinds of substrate, sclerotia of *S. trifoliorum*, *S. sclerotiorum* and *B. cinerea* were infected. It was found that on the unsterilized soil medium both isolates caused about the same amount of infection (Table 3). On the other hand, when the substrate was autoclaved quartz sand, isolate II infected considerably more sclerotia than isolate I.

On Czapek's agar medium both isolates formed a mycelium which was white, somewhat curled and wooly (Fig. 2). About two weeks after inoculation, pycnidia began to form in the centre of the colony; these were at first covered by a thin, wooly mycelial layer. The pycnidia were black, globose, 190—600 μ in diameter. The ostiole, from which the conidia were released in a black mass, was located in the centre of the upper surface of the pycnidium. The conidia were brown, globose or elliptical in shape, having either a smooth or rough surface and a size of $3.2\text{--}3.9 \times 3.9\text{--}4.8 \mu$. *C. minitans* is known as an effective parasite of *Sclerotinia* species (1, 16, 20).

Acrostalagmus roseus BAINIER (3, 4, 6, 12). A new strain was isolated on 7.6. 1963 from a sclerotium of *S. trifoliorum* in an infection trial, where it had appeared as a contaminant. It proved at least in preliminary trials to be an even more aggressive parasite than *A. roseus* isolate I, and therefore it was included in the collection of the Department of Plant Pathology for further studies. On Henneberg agar medium it had a slight antibiotic effect on *S. trifoliorum*. In preliminary trials *A. roseus* isolate II caused a more severe infection of sclerotia than the other antagonists studied (Table 1). Also in these trials, the sclerotia of *B. cinerea* were most readily attacked and destroyed. The most resistant sclerotia were those of *S. trifoliorum*, but even a considerable number of these were destroyed by *A. roseus* isolate II.

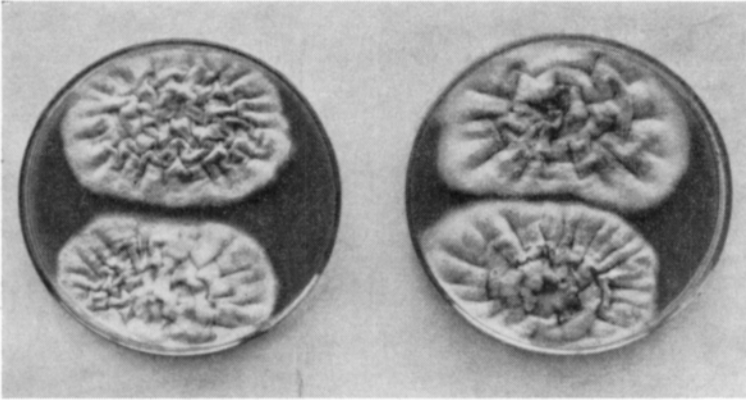


Fig. 2. *Coniothyrium minitans*. Isolates I and II on Henneberg agar medium.

When cultivated on Czapek's and on Henneberg agar media, the external appearance of this isolate differed from that of the previous isolate of *A. roseus* I in that its conidia were covered by only a very thin slime so that the mycelium as a whole appeared dry. The structure of the conidiophores and the conidia, however, were typically those of *A. roseus* (4, 12). The conidiophores were septate, verticillately branched, and had average dimensions of 3μ in diameter and $128-195 \mu$ in length (Fig. 3). The conidia were oval or globose, $2.1-3.5 \times 3.5-4.5 \mu$ in size.

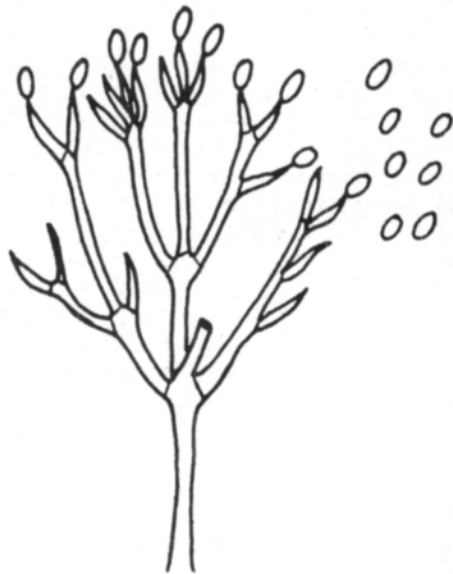


Fig. 3. *Acrostalagmus roseus*, isolate II. Conidiophore and conidia from a 14-day old culture on Henneberg agar medium. $\times 1000$.

Conclusions

Rhizopus nigricans EHRENB., *Sporotrichum carnis* BROOKS & HANSFORD, *Coniothyrium minitans* CAMPB. (two isolates) and *Acrostalagmus roseus* BAINIER proved to infect sclerotia of *Sclerotinia trifoliorum*, *S. sclerotiorum* and *Botrytis cinerea* in laboratory experiments. The most effective of these was *A. roseus*.

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SELOSTUS:

SIENIEN SKLEROOTIOIDEN ERÄISTÄ LOISISTA

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Helsingin yliopiston kasvipatologian laitos

Viljelykasvien patogeneenien antagonisteja koskevissa tutkimuksissa v. 1962—63 todettiin, että sienet *Rhizopus nigricans* EHRENB., *Sporotrichum carnis* BROOKS & HANSF., *Coniothyrium minitans* CAMPB. ja *Acrostalagmus roseus* BAINIER, isolaatti II, tuhosivat laboratoriokokeissa *Sclerotinia trifoliorum*in, *S. sclerotiorum*in ja *Botrytis cinerea* sklerootioita. Tehokkain näistä oli *Acrostalagmus roseus*.