JOURNAL OF AGRICULTURAL SCIENCE IN FINLAND
Maataloustieteellinen Aikakauskirja
Vol. 63: 435—441, 1991

Scanning electron microscopy of hyphal interaction between *Streptomyces griseoviridis* and some plant pathogenic fungi

EEVA TAPIO and ARJA POHTO-LAHDENPERÄ

Department of Plant Pathology, University of Helsinki, SF-00710 Helsinki

Abstract. The interaction between *Streptomyces griseoviridis* and the pathogens *Alternaria brassicicola, Botrytis cinerea, Fusarium oxysporum, Mycocentrospora acerina, Rhizoctonia solani* and *Sclerotinia sclerotiorum* was studied by SEM both on autoclaved seeds and living seedlings of turnip rape and carrot and the fungi *Phomopsis sclerotioides* and *Pythium ultimum* on cucumber seedlings. The samples were prepared by the standard method for examination by scanning electron microscope. The hyperparasitism of *S. griseoviridis* was clearly shown. *S. griseoviridis* tightly wound around *Alternaria* conidia and *Sclerotinia* hyphae, eventually disintegrating them. It grew along the hyphae of *B. cinerea, P. sclerotioides* and *M. acerina,* dissolving them. The hypha of *F. oxysporum* seemed to be slightly affected, and its conidia not at all. The hyperparasite grew only loosely on the hypha of *R. solani* and on the mycelium and oogonia of *Pythium* which seemed not to sustain much injury.

Index words: Hyperparasitism, biological control, *Streptomyces griseoviridis,* plant pathogenic fungi

Introduction

Streptomyces griseoviridis Anderson et al., isolated from Finnish light coloured *Sphagnum* peat, has been reported to be antagonistic to the plant pathogens *Alternaria brassicicola* (Schw.) Wiltshire, *Botrytis cinerea* Pers.: Fr., *Fusarium avenaceum* (Fr.: Fr.) Sacc., *F. culmorum* (W.G. Sm.) Sacc., *F. oxysporum* Schlecht. f. sp. *dianthi* (Prill. & Dol.) Snyd. & Hans., *Pythium debaryanum* auct. non Hess., *Phomopsis sclerotioides* van Kesteren, *Rhizoctonia solani* Kühn and *Sclerotinia sclerotiorum* (Lib.) de Bary (TAHVONEN 1982 a, b, LAHDENPERÄ 1987, TAHVONEN and AVIKAINEN 1987, TAHVONEN and LAHDENPERÄ 1988). *In vitro* tests with these fungi showed their growth to be inhibited by *S. griseoviridis* (TAHVONEN 1982 a). It controlled damping-off caused by *A. brassicicola* and *R. solani* on cauliflower and *P. debaryanum* on sugar beet in *in vivo* tests. It also reduced the mortality of barley sprouts and foot rot caused by *F. culmorum* (TAHVONEN 1982 b, TAHVONEN and AVIKAINEN 1987). Treatment of lettuce seedlings with a spore suspension prepared

from the *Streptomyces* isolate significantly reduced yield losses caused by *B. cinerea,* but had no effect on those caused by *R. solani.* The antibiotic effect against *R. solani in vitro,* was weaker than its effect against other tested pathogens (TAHVONEN 1982 a). It has been shown that the antagonistic effect is based on antibiosis, in which aromatic heptaene polyenes are the active substances (RAATIKAINEN et al. 1990). Many authors (COOPER & CHILTON 1949, JOHNSON 1954, RANGASWAMI & ETHIRAJ 1962) have shown that the antibiotics produced by *Streptomyces* spp. affect some pathogenic fungi. SKINNER (1956 b), LOCKWOOD (1959) and LOCKWOOD & LINGAPPA (1963) observed, in addition to antibiosis, a lytic effect for actinomycetes when these attacked the fungus mycelium directly. TU (1986, 1988) described the hyperparasitism of *Streptomyces albus* on *Nectria inventa* which itself is a mycoparasite of several fungi including *Sclerotinia sclerotiorum,* and the hyperparasitism of *S. griseus* against *Colletotrichum lindemuthianum.* The present study was done to elucidate the nature of the host-mycoparasite interactions between *Streptomyces griseoviridis* and some plant pathogenic fungi.

Materials and methods

The interaction of *Streptomyces griseoviridis* isolated from light coloured peat and some plant pathogenic fungi was studied on autoclaved seeds and living seedlings of turnip rape and carrot. The seeds were placed on water agar in Petri dishes and inoculated with pathogens, *Alternaria brassicicola,* isolated from cauliflower seeds, *Botrytis cinerea* and *Sclerotinia sclerotiorum,* isolated from carrots or *Fusarium oxysporum* and *Rhizoctonia solani,* isolated from turnip rape. These were inoculated three days later with *Streptomyces griseoviridis.* The dishes were incubated in room temperature. After three days the samples were fixed in a mixture of 2 % glutardaldehyde and 1 % formaldehyde in 0.1 M phosphate buffer, pH 7.3, rinsed in buffer and dehydrated in a graded ethanol series. The specimens were then criticalpoint dried in a Balzers CPD 020 Critical point dryer using carbon dioxide, coated with gold in a vacuum evaporator with a Jeol Fine Coat JFC-1100 Sputtering device, and examined under a scanning electron microscope (Jeol JSEM-820) at the Department of Electron Microscopy, University of Helsinki.

The hyperparasitism of *S. griseoviridis* on the pathogens *Phomopsis sclerotioides* and *Pythium ultimum,* isolated from cucumber, was studied in the same way on cucumber seedlings.

The interaction between *Mycocentrospora acerina* Deighton, isolated from carrot and *S. griseoviridis* was examined directly on mycelia grown on potato dextrose agar (PDA) in Petri dishes. The antagonist was inoculated on the one-week-old mycelia of *M. acerina* and the samples were prepared three days later. Agar blocks with mycelia were frozen in liquid nitrogen at —180°C (Hexland CT 100), dehydrated at —80°C in vacuum in a cryonit, coated with gold and examined under a scanning electron microscope (Cambridge Instrument S 360) at the Kemira Research Center.

Results

The antagonist, *S. griseoviridis,* was easily distinguished from its hosts by its fine (0.5 µm in diameter) sporulating hyphae in contrast to the coarse hyphae of the host fungi. The hyperparasite grew epiphytically on the hyphae of the plant pathogenic fungi.

The conidia of *A. brassicicola* were heavily colonized by *Streptomyces* and almost totally destroyed (Fig. 1). Its hyphae were tightly coiled around the *Sclerotinia* hyphae, dissolving and disintegrating them (Figs. 2 and 3).

The hyperparasite also grew on the hyphae of *Botrytis cinerea* causing their lysis and destruction (Fig. 4). At the early state of parasitism, single strands of *S. griseoviridis* hypha growing closely pressed to the host hypha seemed to secrete cell wall-dissolving enzymes (Fig. 5). The hyphae of the hyperparasite

seems also to grow inside the host hypha of *B. cinerea* (Fig. 4).

The sporulating hyphae of *S. griseoviridis* grew along and coiled around the hyphae of *Fusarium oxysporum* slightly dissolving them, but the conidia did not seem to be affected (Fig. 6).

The hyphae of *Phomopsis sclerotioides* were collapsed in the presence of *S. griseoviridis,* but the sclerotia did not seem to be affected (Fig. 7).

The mycelia of *Mycocentrospora acerina* were dissolved and flattened by *S. griseoviridis* in a similar manner to those of *P. sclerotioides* (Fig. 8). The hyphae of the hyperparasite grew loosely on the hypha of *Rhizoctonia solani* at the early stages of infection. Later on, *Streptomyces* hyphae seemed to be closely associated with the collapsed host hyphae (Fig. 9).

Most of the mycelia and oogonia of *Pythium* were not visibly affected at the moment of fixing the preparation, although the hyperparasite seems to have penetrated its mycelial wall (Figs. 10, 11). A portion of the host hyphae abundantly covered with the hyperparasite showed some disintegration (Fig. 11).

Discussion

The first observations made by TAHVONEN (1982 a) about the antagonism of *Streptomyces* spp. against many plant pathogenic fungi *in vitro* demonstrated their antibiotic and growth inhibiting effect. The hyperparasitism of one of these antagonists was clearly shown in the present study. The lytic activity of actinomycetes has been earlier shown by LOCKWOOD (1959). According to TU (1986, 1988), *Streptomyces albus* could act as

Fig. 1. Thin hyphae (width 0,5 µm) of *Streptomyces griseoviridis* are covering and gradually dissolving the hyphae and especially the conidia (arrow) of *Alternaria brassicicola.*

Fig. 2. The sporulating *Streptomyces* hyphae are coiling around the hyphae of *Sclerotinia sclerotiorum* disintegrating them (arrow).

Fig. 3. The hyperparasite is dissolving the hyphae of *Sclerotinia sclerotiorum.*

Fig. 4. Thin hyphae and spores of *S. griseoviridis* are growing on the broad hyphae of *Botrytis cinerea.* The hyperparasite are growing both epiphytically and internally (arrow) on the pathogen, which is disintegrated and dissolved.

Fig. 5. Eroded wall of hyphae of *Botrytis cinerea* adjacent to hyphae of *S. griseoviridis.*

Fig. 6. The sporulating hyphae of *S. griseoviridis* only slightly affecting the hyphae of *Fusarium oxysporum* (arrow). The conidia do not seem to be affected.

Fig. 7. Streptomyces hyphae degrading the hyphae of *Phomopsis sclerotioides,* but seem not to affect the sclerotia of the pathogen.

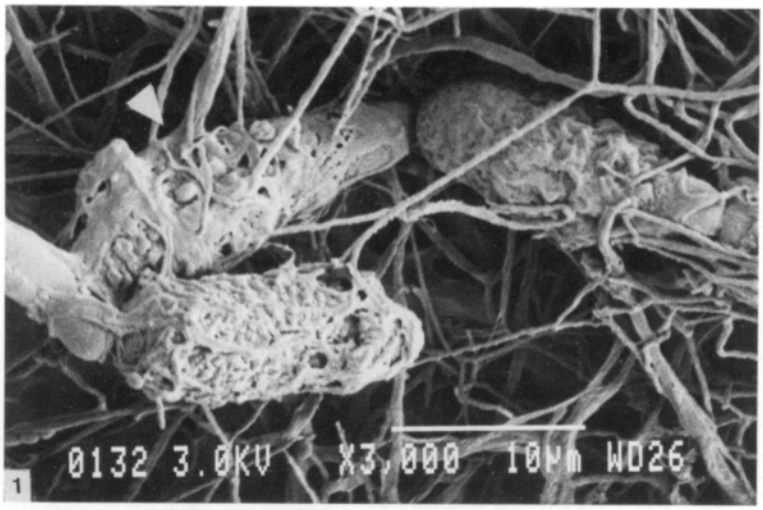
Fig. 8. S. griseoviridis hyphae are growing from the right towards *Mycocentrospora acerina* hyphae, which are collapsing and disintegrating.

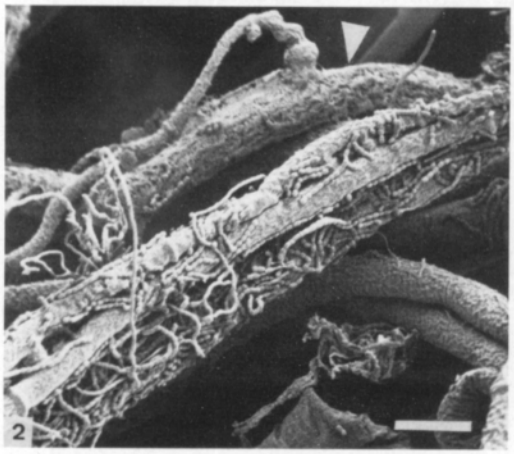
Fig. 9. Streptomyces hyphae are growing on the hyphae of *Rhizoctonia solani* (arrow), which are only slightly affected. The hyperparasite seems, however, be associated with sunken area of some collapsed host hyphae (double arrow).

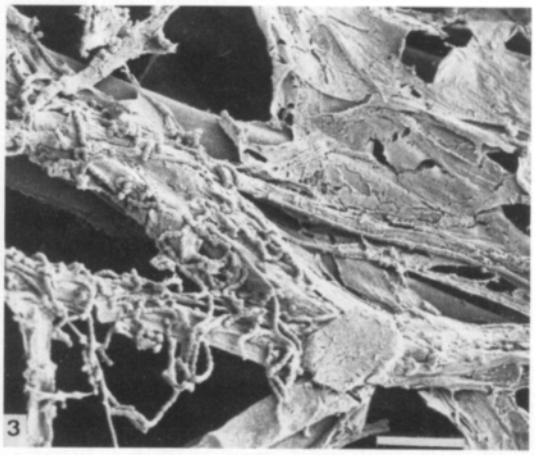
Fig. 10. The hyphae and oogonia of *Pythium* are not much affected, although the *Streptomyces* hyphae seem to be able to penetrate (arrow) its hyphal wall.

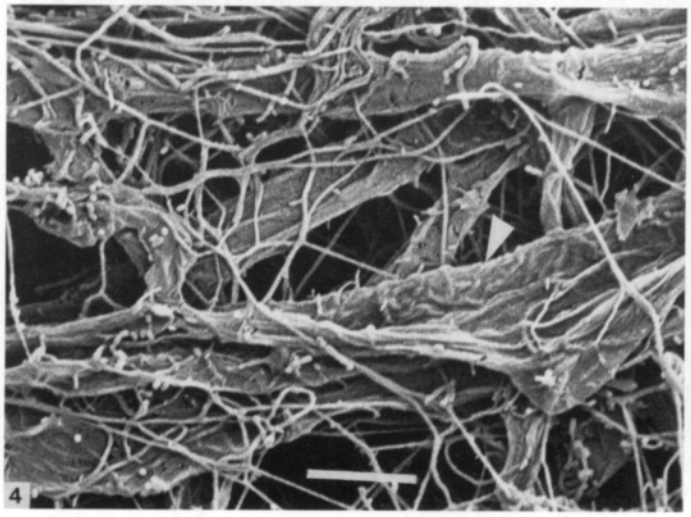
Fig. 11. The hyphae of *Pythium* sp. are penetrating the cucumber root cells (arrow), although the *Streptomyces* hyphae are abundant. In some portions of the preparation, the *Pythium* hypha are clearly disintegrated (double arrow).

In figure 5 the scale bar is 1 µm, in all other figures it is 10 µm.

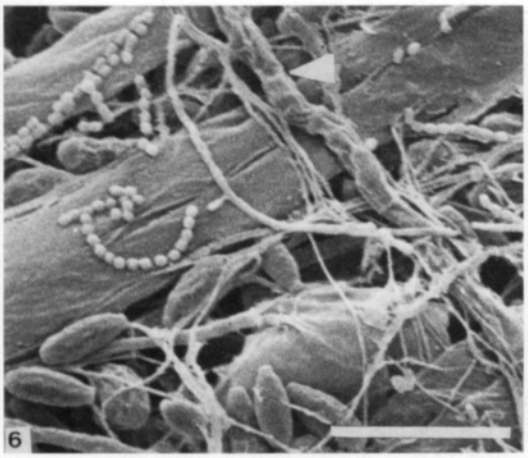

0132 3.0KV X3,000 10µm WD26
1

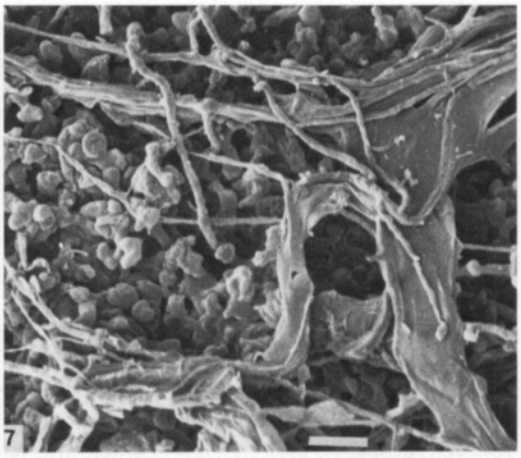

2

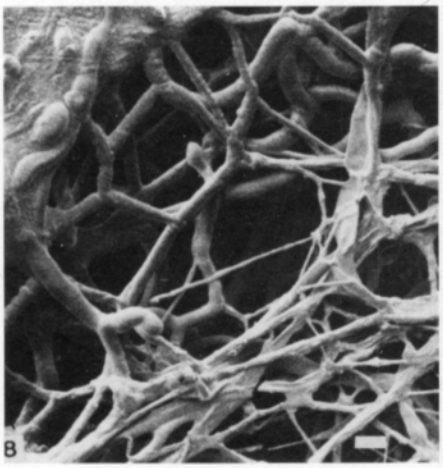

3

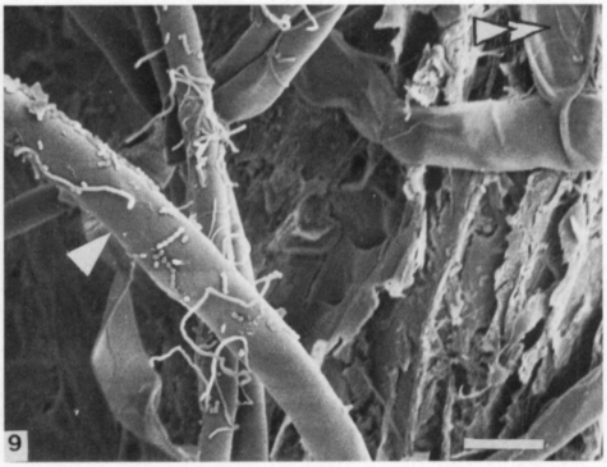

4

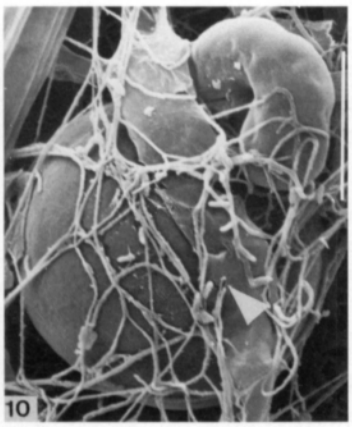

5

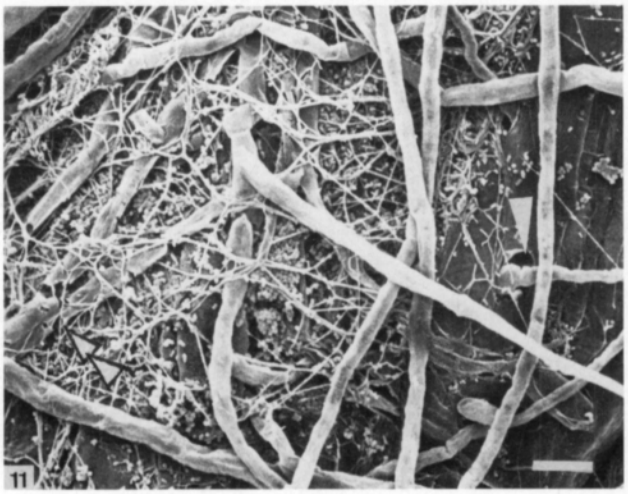

6


7


8


9


10


11

a surface parasite with or without the formation of appressoria-like structures and *S. griseus* produced appressorium-like swellings on the hyphal surface of *Colletotrichum lindemuthianum*. Appressoria were not observed in our studies at *S. griseoviridis*. The internal parasitism of *A. brassicicola, S. sclerotiorum, B. cinerea* and *P. sclerotioides* was evidenced by the fact that hyperparasitic hyphae were frequently found inside partly fractured host hyphae, similar to the findings at TU (1986, 1988) who found parasitism of hyphae of *N. inventa* and *C. lindemuthianum* by *Streptomyces albus*. *S. griseoviridis* seemed also to grow inside the collapsed *Rhizoctonia*-hypha. There is some doubt as to whether the cells were penetrated before or after death. The hyperparasitized hyphae of *F. oxysporum* were abnormal, but not clearly disintegrated. SKINNER (1956 a) found that *Streptomyces albidoflavus* limited early growth of *Fusarium culmorum* by antibiotic action and that it also directly attacked preformed fungus mycelium. He also observed that the actinomycete lysed the contents of its host hyphae (SKINNER 1956 b). The conidia of *F. oxysporum* seemed not to be affected at least in three days these were hyperparasitized. LOCKWOOD (1959) observed that the germination of *Fusarium solani* f. *pisi* conidia was not affected by *Streptomyces* isolates, although the mycelia of *F. oxysporum* f. *pisi* and *F. solani* f. *pisi* were lysed and disappeared.

In our studies the parasitized hyphae of *P. sclerotioides* and *M. acerina* were collapsed, dissolved and flattened without disruption of the cell walls. *S. griseoviridis* only slightly parasitized on *R. solani,* at least at the beginning of the infection. According to TAHVONEN (1982 a) and TAHVONEN and LAHDENPERÄ (1988) its growth inhibiting effect *in vivo* tests was weaker on *R. solani* than on the other pathogens studied. TAHVONEN (1982 a) observed a strong antibiotic effect of *S. griseoviridis* against *P. ultimum,* as did KNAUSS (1976) for two other *Streptomyces* species. In our study oospores of *P. ultimum* were not parasitized by *S. griseoviridis*. SNEH et al. (1977) and SUTHERLAND and LOCKWOOD (1984) found a zoospore-producing actinomycete, *Actinoplanes missouriensis* Couch, frequently infecting oospores of *Pythium* sp. and some other Oomycetes primarily on flooded soil. *S. griseoviridis* seems, however, to be able to penetrate the hyphae of *Pythium* sp. and disintegrate them.

References

COOPER, W.E. & CHILTON, S.J.P. 1949. Antibiosis of Actinomycete strains to *Pythium arrhenomanes, P. ultimum* and *Rhizoctonia solani*. Phytopathology 39, 5.

JOHNSON, L.F. 1954. Antibiosis in relation to *Pythium* root rot of sugarcane and corn. Phytopathology 44, 69—73.

KNAUSS, J.F. 1976. In vitro antagonistic activity of several *Streptomyces* spp. against species of *Pythium* and *Phytophtora* Plant Disease Reporter 60, 846—850.

LAHDENPERÄ, M-L. 1987. *Streptomyces* -sädesienivalmiste ja sen käyttö *Fusarium oxysporum* f.sp. *dianthi* -sienen aiheuttaman neilikanlakastumistaudin torjunnassa. Licentiate-thesis. Department of Plant Pathology, University of Helsinki.

LOCKWOOD, J.L. 1959. *Streptomyces* spp as a cause of natural fungitoxicity in soils. Phytopathology 49, 327—331.

LOCKWOOD, J.L. & LINGAPPA, B.T. 1963. Fungitoxicity of sterilized soil inoculated with soil microflora. Phytopathology 53, 917—920.

RAATIKAINEN, O., TAHVONEN, R., TUOMISTO, J. and ROSENQVIST, H. 1990. *In vitro* production of heptaene polyene by suppressive *Streptomyces* sp. isolated from *Sphagnum* peat. In 5th Europea Congress on Biotechnology, Copenhagen, July 8—13, 1990. Abstract Book, ed, by Christiansen, C., Mucsk, L. and Villadsen, J.

RANGASWAMI, G. & ETHIRAJ, S. 1962. Antibiotic production by *Streptomyces* sp. in unamended soil. Phytopathology 52, 989—992.

SKINNER, F.A. 1956 a. Inhibition of the growth of fungi by *Streptomyces* spp. in relation to nutrient conditions. Journal of General Microbiology 14, 381—392.

SKINNER, F.A. 1956. The effect of adding clays to mixed cultures of *Streptomyces albidoflavus* and *Fusarium*

culmorum. Journal of General Microbiology 14, 393—405.

Sneh, B., Humble, S.J. & Lockwood, J.L. 1977. Parasitism of oospores of *Phytophthora megasperma* var. *sojae, P. cactorum, Pythium* sp., and *Aphanomyces euteiches* in soil by Oomycetes, Chytridiomycetes, Hyphomycetes, Actinomycetes, and Bacteria. Phytopathology 67, 622—628.

Sutherland, E.D. & Lockwood, J.L. 1984. Hyperparasitism of oospores of some Peronosporales by *Actinoplanes missouriensis* and *Humicola fuscoatra* and other Actinomycetes and fungi. Canadian Journal of Plant Pathology 6, 139—145.

Tahvonen, R. 1982 a. The suppressiveness of Finnish light coloured *Sphagnum* peat. Journal of the Scientific Agricultural Society of Finland 54, 345—356.

Tahvonen, R. 1982 b. Preliminary experiments into the use of *Streptomyces* spp. isolated from peat in the biological control of soil and seedborne diseases in peat culture. Journal of the Scientific Agricultural Society of Finland 54, 357—369.

Tahvonen, R. & Avikainen, H. 1987. The biological control of seedborne *Alternaria brassicicola* of cruciferous plants with a powdery preparation of *Streptomyces* sp. Journal of Agricultural Science in Finland 59, 199—208.

Tahvonen, R. & Lahdenperä, M-L. 1988. Biological control of *Botrytis cinerea* and *Rhizoctonia solani* in lettuce by *Streptomyces* sp. Annales Agriculturae Fenniae, 27, 107—116.

Tu, J.C. 1986. Hyperparasitism of *Streptomyces albus* on a destructive mycoparasite *Nectria inventa* Journal of Phytopathology 117, 71—76.

Tu, J.C. 1988. Antibiosis of *Streptomyces griseus* against *Colletotrichum lindemuthianum*. Journal of Phytopathology 121, 97—102.

Ms received April 15, 1991

SELOSTUS

Pyyhkäisyelektronimikroskooppitutkimukset *Streptomyces griseoviridis*-sädesienen ja eräiden kasvitauteja aiheuttavien sienten vuorovaikutuksesta

Eeva Tapio ja Arja Pohto-Lahdenperä

Kasvipatologian laitos, Helsingin yliopisto, 00710 Helsinki

Helsingin yliopiston kasvipatologian laitoksella on tutkittu pyyhkäisyelektronimikroskoopilla (SEM) sädesienen, *Streptomyces griseoviridis,* ja useiden kasvitautia aiheuttavien sienten vuorovaikutusta. *S. griseoviridis* eristettiin 1980-luvun alussa vaaleasta rahkaturpeesta ja sillä todettiin olevan antagonistisia eli kasvitauteja ehkäiseviä ominaisuuksia. Se erittää antibioottisia aromaattisia hepteenipolyeenejä. Tässä tutkimuksessa selvitettiin pystyykö se myös loisimaan kasvipatogeeneilla sienillä. *Alternaria brassicicola* (kaalikasvien taimipoltesieni), *Botrytis cinerea* (harmaahome), *Fusarium oxysporum* (lakastumistautisieni), *Mycocentrospora acerina* (porkkananmustamätäsieni), *Rhizoctonia solani* (mm. seittirupi- ja taimipoltesieni) ja *Sclerotinia sclerotiorum* (pahkahome) -sienillä tartutettiin rypsin ja porkkanan siemeniä ja siementaimia. *Phomopsis sclerotioides* ja *Pythium ultimum* -sienet, jotka aiheuttavat mm. kurkun tyvi- ja mustajuurimätää, levitettiin kurkun siementaimiin. Kolmen vuorokauden kuluttua ne käsiteltiin *S. griseoviridis*-suspensiolla. Sen jälkeen ne inkuboitiin kolme vuorokautta huoneenlämmössä ennenkuin niistä valmistettiin tavanomaisia menetelmiä käyttäen SEM-preparaatit elektronimikroskopointia varten.

Kuvauksissa oli nähtävissä, että sädesienirihmat loisivat useiden sienten rihmoilla. *S. griseoviridis* kietoutui tiukasti *Alternaria* -kuromien ja *Sclerotinia* -rihmojen ympärille hajoittaen ilmeisesti niitä. Sädesieni kasvoi *B. cinerea, P. sclerotioides* ja *M. acerina* rihmoilla liuottaen niitä. *S. griseoviridis* vioitti vain vähän *F. oxysporum* -sienirihmaa eikä lainkaan sen kuromia. Se kasvoi löyhästi *R. solani*-rihmalla ja *Pythium* sienen rihmalla ja munaitiöllä, jotka eivät paljoakaan vioittuneet.