JOURNAL OF AGRICULTURAL SCIENCE IN FINLAND
Maataloustieteellinen Aikakauskirja
Vol. 59: 199—208, 1987

The biological control of seed-borne *Alternaria brassicicola* of cruciferous plants with a powdery preparation of *Streptomyces* sp.

TAHVONEN, R. and AVIKAINEN, H.

Department of Plant Pathology, Agricultural Research Centre, SF-31600 JOKIOINEN, Finland

Abstract. The effectiveness of a powdery preparation of a *Streptomyces* sp. isolate as a seed dressing agent against seed-borne *Alternaria brassicicola* on different *Brassica* species was investigated in the study. The preparation was made by freeze-drying and milling the biomass produced in a fermentor into a form suitable for use as a dusting agent.

Seed dressing was 80—90 % successful in controlling damping-off from seeds artificially infected with *A. brassicicola*. The effectiveness of dressing remained unchanged on seeds stored under dry conditions for 5—6 weeks, but subsequently decreased slowly and was ca. 50 % six months after dusting. *Streptomyces* dressing controlled, in a manner comparable to chemical dressing with thiram, damping-off caused by *Alternaria* fungi on seedlings which were grown from commercial seed lots of different origin. The results of biological control of damping-off did not vary in the peat lots of different origin whose natural disease suppressivity varied considerably. The control result was the same or better than chemical dressing with thiram. The acidity of the substrate (pH 4.8—8.6) had no effect on the effectiveness of biological control. The results obtained against *Alternaria* damping-off were the same in other substrates — clay, fine sand and mull — as in peat.

Index words: *Streptomyces* sp. *Alternaria brassicicola,* biological control, *Brassica* sp.

Introduction

An isolate of *Streptomyces* sp., obtained from light-coloured, Finnish *Sphagnum* horticultural peat, has proved to be a potential biological control agent against plant pathogens (Tahvonen 1982 a, 1982 b, Tahvonen and Uoti 1983, Tahvonen 1985), and especially effective against seed-borne *Alternaria* on cruciferous plants. *A. brassicicola* is the most common seed-borne pathogen of *Brassica* sp. throughout the world (Neergaard 1945). It is also common and the most serious seed-borne damping-off fungus on cruciferous plants in Finland (Tahvonen 1979), and is routinely controlled by chemical dusting with thiram (Tahvonen 1985).

A research programme into the practical application of *Streptomyces* sp. isolated from peat was started at the beginning of 1985.

A dry, powdery preparation in which the number of living particles was of the same order of magnitude as in the spore suspensions used in earlier experiments (Tahvonen 1982 b), was developed by Kemira Co. The dry, powdery preparation was given the commercial name »Mycostop».

The aim of this study was to determine the effectiveness of the powdery *Streptomyces* preparation as a dressing agent in the control of *Alternaria* on cruciferous plants. The study was carried out at the Department of Plant Pathology, Agricultural Research Centre, during 1985—86.

Material and methods

A powdery preparation suitable for use as a seed dressing agent was prepared from a *Streptomyces griseoviridis* Anderson et al. isolate. Following culture in a fermentor, the cell mass was separated from the culture medium by centrifugation and then freeze-dried. Compounds designed to improve adhesion to the seeds and to maintain the vitality of the dry preparation, were added in connection with drying and milling. The final product contained 10^8—10^9 living particles/g. Details of the manufacture of the preparation are not given here.

Preliminary and methodological tests were carried out on »Erfurter 291» or »Suuri Tanskalainen» cauliflower seeds, which were artificially infected with *A. brassicicola* in order to ensure an even degree of infection (Tahvonen 1982 b). Commercial lots of cruciferous seed with a wide range of natural *Alternaria* infection (Fig. 4) were obtained from the State Seed Testing Institute and from a number of commercial seed suppliers. Ten lots of cabbage seed, 8 lots of cauliflower, and 9 lots of other *Brassica* species and their varieties were tested. A number of Chinese cabbage, swede and turnip seed lots were also included, but their results are not presented here because the degree of infection of the seeds was too low from the point of view of the experiment.

The seeds were dressed by carefully shaking them together with the dusting agent in a glass jar. When small lots of seeds were to be treated, a small batch was first dressed in order to ensure that there was a constant amount of the preparation on the wall of the jar. The seeds used in the experiment depicted in Fig. 2 were coated by a special method developed at Kemira Co's Vaasa factory in order to ensure that all the added amount of dressing agent had adhered to the seeds. According to tests carried out by Kemira Co, adhesion of the preparation in the other experiments varied from 50—70 %. In addition to undressed controls, the experiments also included undressed uninfected controls and a chemical control with a thiram dressing of 4 g/kg on naturally infected seeds.

Retention of the dressing effectiveness on seeds stored under dry conditions was studied over a period of 0—18 weeks after dressing. The dressed seeds were stored in the dark at +5 °C and at +20 °C. Since the storage temperature did not effect the effectiveness of control, the results presented in Figs. 1 and 2 are the means of two test factors.

In order to test the effect of substrate pH on the effectiveness of the *Streptomyces* isolate, the peat was limed with varying amounts of dolomite limestone and calcium hydroxide. The pH of the substrate following this treatment, as determined from a 0.02 N $CaCl_2$ extract, can be seen in Fig. 5. The effect of soil type on the effectiveness of the dressing agent was tested using the 6 different substrates listed in Table 2. In addition, the effectiveness of *Streptomyces* dressing was tested in 12 non-disinfected peat lots of different origin (Fig. 6).

Unless otherwise stated in connection with the results or in the tables, the seeds were sown in steam-disinfected light-coloured *Sphagnum* peat, which was given a basic fertilization of 10 g dolomite limestone/l, and 1.5 g of Peat Super Y fertilizer/l following steam disinfection. The seedlings were raised in 1 l plastic pots or in 30 × 40 cm plastic boxes. There were three or four replications, each replication

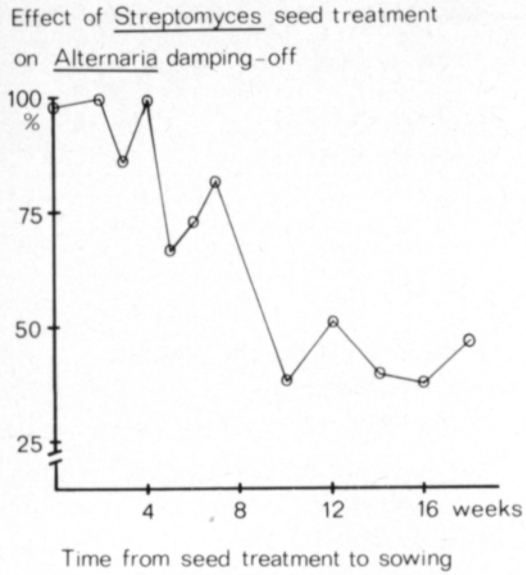

Fig. 1. Retention of *Streptomyces* seed dressing (15 g/kg) effectiveness on seed artificially infected with *Alternaria brassicicola* in seedling experiments.

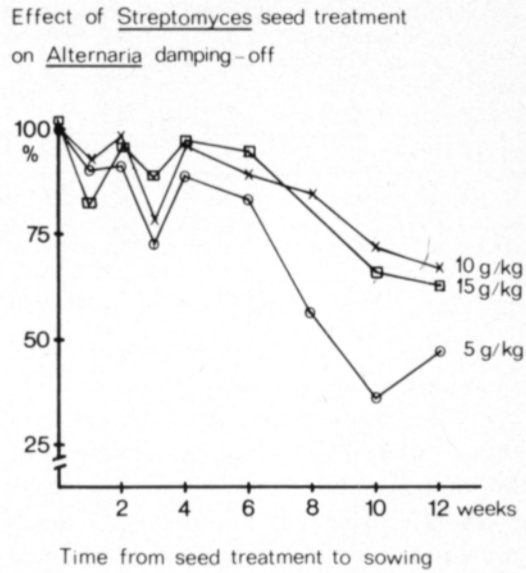

Fig. 2. Effectiveness of *Streptomyces* seed dressing on seed artificially infected to different extents with *Alternaria brassicicola.*

consisting of 50 seeds. The seedlings were grown from seed in thermostatically controlled greenhouses at 18—20 °C under artificial illumination of 8000 lux. This meant that the lots raised at different times were grown under as exactly the same conditions as possible. In addition to the substrate tests, 4×25 naturally infected seeds were sown on water agar in 9 cm petri dishes. The degree of fungal infection of the seedlings was determined under the microscope, as well as the mortality rate, two weeks after the seeds had been sown.

The degree of damping-off infection was determined on the seedlings at the end of the substrate experiments using the scale 0—2, where 0 = healthy, 1 = slightly infected, 2 = dead or non-viable. In addition, the fresh weight of those seedlings which had developed from naturally infected seed was determined. These results are not presented here because there were no differences in the weights of the healthy seedlings between different dusting treatments, and the dryweight results for the infected seedlings were correlated with the degree of infection.

The infection index, viable seedling % (which includes healthy + slightly infected seedlings as a % of the number of seeds sown), and the control effectiveness % (calculated from the formula (A—B):(C—B)×100, where A is the infection index or vitality of the dressed seedlings, B that of the infected control and C that of the healthy control), are presented in the tables. Where necessary, the statistical significance of the results has been tested using variance analysis.

Results

Control of *Alternaria* damping-off with the *Streptomyces* preparation

Dressing the seeds with the *Streptomyces* preparation controlled artificial infection by seed-borne *A. brassicicola.* Dressing levels greater than 5 g/kg seed had no effect on the effectiveness of dressing when sowing was done on the same day or within one week after dressing (Table 1, Fig. 2). A dressing level of 1 g/kg seed resulted in significantly more healthy seedlings than the control, but was

Table 1. Effect of the *Streptomyces* seed dressing dose on damping-off caused by *Alternaria brassicicola* on cauliflower raised in peat.

Seed	*Streptomyces* dose, g/kg seed						
	0	1	5	10	15	20	
	Viable seedlings, %						
Alternaria-infected	30	62	90	94	90	86	F = 139.6***
Non-infected	88	—	86	78	—	86	F = 1.0

however poorer than dressing levels of 5 g/kg or more. Dressing had no effect on the number of healthy seedlings nor the dry weight in the case of healthy, uninfected seed in any of the experiments.

The effectiveness of the *Streptomyces* powder remained unchanged on dressed seed stored for a period of 4—6 weeks. The treatment was still effective on dressed seed stored for as long as 18 weeks, although less effective than after a short storage period (Figs. 1 and 2). Higher dressing levels of 10 and 15 g/kg seed had a better effectiveness after 6 weeks storage than the level of 5 g/kg seed (Fig. 2).

Dressing naturally infected *Brassica* sp. seeds with the *Streptomyces* powder completely controlled damping-off on the peat substrate. The result was fully comparable with that obtained with chemical dressing with thiram. A slight damage was found at the base of seedlings grown from some dressed seed lots. This was insignificant from the point of view of seedling growth, and in fact occurred with both biological and chemical dressing. The effect of dressing on *A. brassicicola* on seeds grown on the water agar medium also proved to be effective (Fig. 4). Both 5 g and 10 g of the *Streptomyces* preparation/kg seed were sufficent to prevent the growth of the pathogen. Almost the same result was obtained with thiram dressing, but the effectiveness of chemical dressing was lost in the case of some seed lots and there was more *Alternaria* in these petri dishes than in seeds dressed with *Streptomyces*. The mortality of the seedlings on the agar medium was completely dependent on the *Alternaria* content.

The effect of pH and the substrate on the effectiveness of the *Streptomyces* preparation

The acidity of the substrate had no effect on the ability of *Streptomyces* to control *Alternaria* damping-off over the pH range 4.8—8.6 (Fig. 5). There was more damping-off on undressed seeds grown on acidic or slightly alkaline substrates than on a neutral substrate. However, the result was not statistically significant.

Streptomyces dressing controlled damping-off on cauliflower on both the organic and inorganic substrates in a similar fashion to thiram dressing. There was no difference as regards the control of *Alternaria* between the steam-sterilized and unsterilized substrate (Table 2).

A. brassicicola caused damping-off to a varying degree on the peat substrate of different origins. Some of the peat lots inhibited *Alternaria* by 60—70 % compared to the steam-sterilized peat or peat lots susceptible to this pathogen. *Streptomyces* dressing controlled damping-off in the different peat lots in the same way as chemical dressing with thiram despite the natural antipathogenic properties of the substrate. Dressing resulted in similar numbers of viable seedlings as were obtained when healthy seeds were used (Fig. 6).

Discussion

The powdery preparation made from *Streptomyces griseoviridis* bacteria proved to be highly effective in controlling seed-borne *Alternaria brassicicola* growing on cruciferous plants. This is in good agreement with the results of earlier experiments in which a spore suspension was used in treating the seeds (TAHVONEN 1982 b). The effectiveness against damping-off was the same on both artificially infected and naturally infected seed. On the

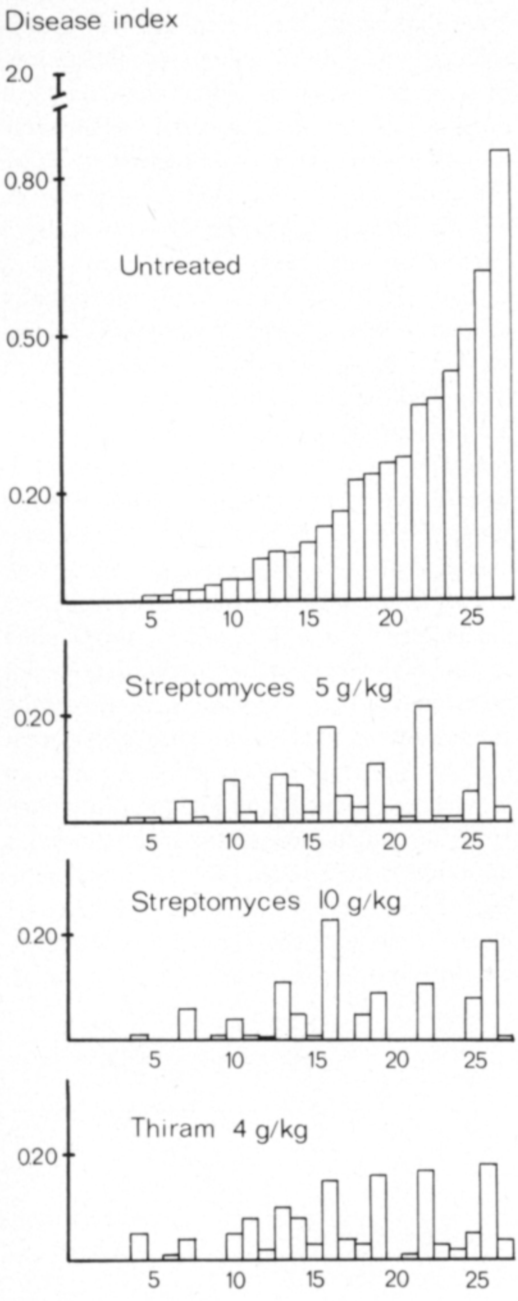

Fig. 3. Effect of seed dressing with thiram or different amounts of the *Streptomyces* preparation on the control of seed-borne *Alternaria brassicicola* in different lots of *Brassica* sp. seed. Naturally infected seed.

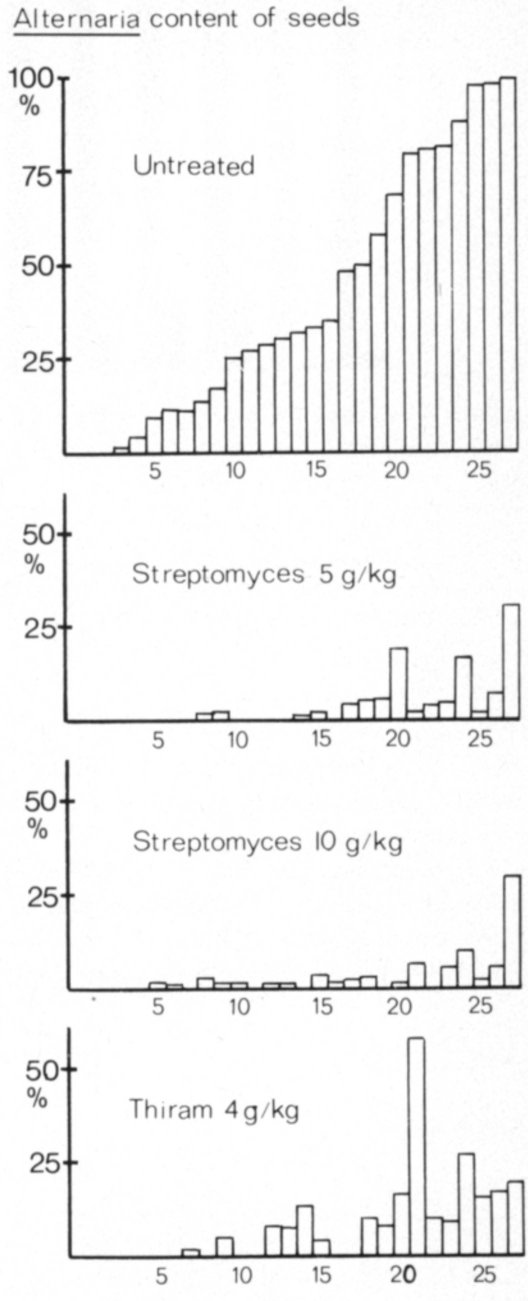

Fig. 4. Effect of seed dressing with thiram or different amounts of the *Streptomyces* preparation on the *Alternaria brassicicola* content of seedlings grown from seed on agar for two weeks. Different seed lots.

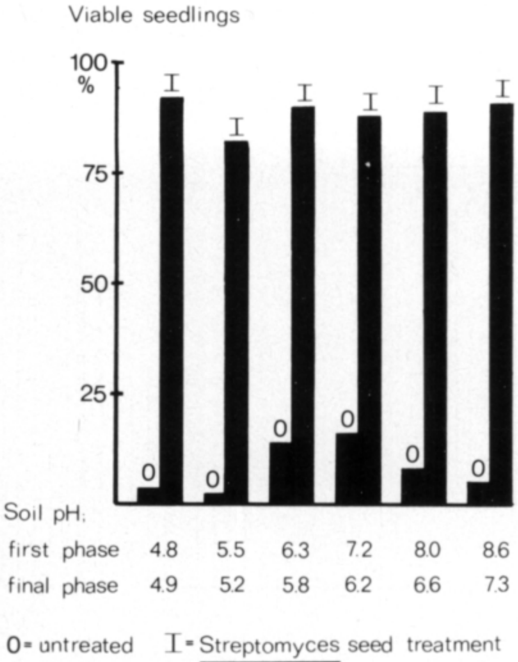

Fig. 5. Effect of *Streptomyces* seed dressing on the control of damping-off caused by *Alternaria brassicicola* on cauliflower seedlings grown in peat of different pH. F values: dressing = 151.7***; pH = 1.06.

basis of this, the results obtained with artificially infected seed can be generalized to cover normal commercial seed.

Alternaria fungi (*A. brassicae, A. brassicicola, A. raphani*) are the most common seed-borne pathogens on cruciferous plants (NEERGAARD 1945), and are controlled chemically by dressing the seed with dithiocarbamate (HEINZE 1974, DIXON 1981) or iprodione and fempropimorph fungicides (MAUDE et al. 1984). In the present experiments biological control of naturally infected seed was equally successful with the *Streptomyces* preparation, or in some cases even better, than chemical dressing with thiram. The result was the same in both *in vitro* and *in vivo* experiments. Since the antagonistic effect of *S. griseoviridis* against different pathogens is very wide *in vitro* (TAHVONEN 1982 a), there is no reason to assume that it would behave differently against *A. brassicae* and *A. raphani* as against *A. brassicicola*. The fungi in question are biologically and systematically very close to each other (NEERGAARD 1949). On the basis of this, *Streptomyces* dressing would appear to be effective against all seed-borne *Alternaria* fungi on cruciferous plants, as is the case with chemical dressing. However, *Phoma lingam* can act as a seed-borne pathogen of cruciferous plants (NEERGAARD 1977), and its susceptibility to this biological control method could not be estimated here. Nowadays, however, the fungus does not have to be controlled since it is of no significance as a seed-borne pathogen in seedling production in Finland (TAHVONEN 1979) for which most of the seed material is imported from Sweden, Denmark and Holland. In the experiments carried out here, for instance, none of the 33 seed lots examined was infected by *P. lingam*.

Table 2. Effectiveness of *Streptomyces* seed dressing in controlling *Alternaria brassicicola* on cauliflower raised in different types of soil.

Seed dressing	Type of soil					
	Peat	Steam-sterilized peat	Fine sand	Fine sand + 30 % peat	Clay	Mull
	Healthy, viable seedlings					
Healthy seeds	86a	90a	86a	91a	75a	85a
Untreated	26b	35b	58b	25b	38b	72a
Streptomyces 5 g/kg	84a	83a	74a	85a	70a	78a
Streptomyces 10 g/kg	79a	80a	80a	86a	71a	88a
Thiram 4 g/kg	85a	83a	90a	80a	77a	85a

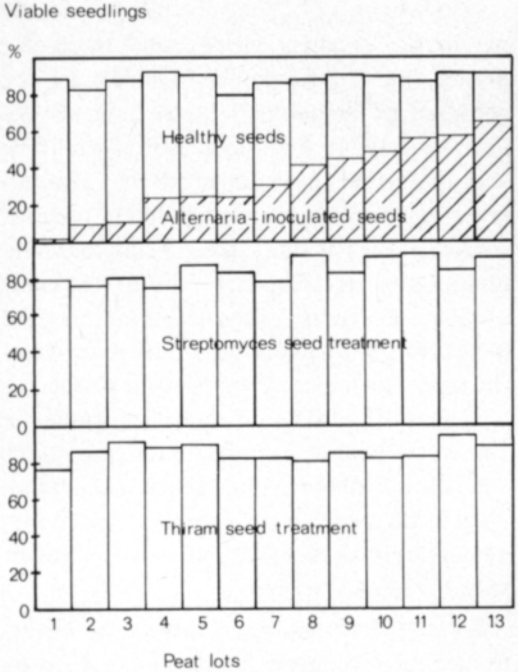

Fig. 6. Effect of seed dressing with thiram or different amounts of the *Streptomyces* preparation on the control of damping-off caused by *Alternaria brassicicola* on cauliflower grown in different peat lots. Peat lots: 1 = steamed peat, 2—13 peat lots of different origin.

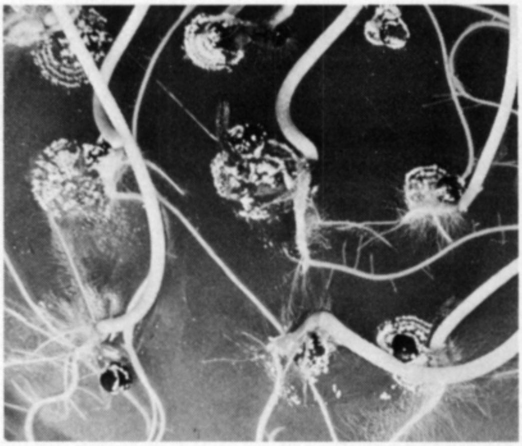

Fig. 7. Growth of *Streptomyces* sp. on cauliflower seeds and agar.

Fig. 8. Control of *Alternaria* damping-off with *Streptomyces* seed treatment. At the bottom untreated seeds and at the top seeds treated with *Streptomyces*-preparate.

The *Streptomyces* isolate used in these experiments was strongly antagonistic on agar against all the pathogens tested earlier (Tahvonen 1982 b). Turhan and Grossmann (1986) reported that their *Streptomyces* isolates were always antagonistic to a number of pathogens if they were strongly antagonistic towards *Rhizoctonia* and *Alternaria.* This would indicate that the dressing method employed here could also be effective against other fungi, such as *Fusarium* spp., *Phoma* spp. and *Stemphylim* spp, which are all significant seed-borne fungi on a number of plants (Neergaard 1977).

Bacteria proper and *Actinomycetes* usually thrive best in neutral or slightly alkaline substrates (Baker and Cook 1974). For this reason, it would be expected that *Actinomycetes* would be an ineffective biological control agent in acidic substrates such as peat, where the pH at which cultivation normally takes place is in the range 5.5—6.5 (Puustjärvi 1974). In the experiments described in this paper, the *Streptomyces* sp. isolated from naturally acidic peat was effective against *Alternaria* over the wide pH range of 4.8—8.6. This is a very satisfactory result as regards the practical application of the control method in substrates of varying acidity. However, the question of whether it is a case of *Actinomycetes* thriving in the favourable conditions of the plants' rhizosphere, or an example of adaptation to different acidity levels in the soil, can only be answered through more detailed investigations.

Fig. 9. Effect of *Streptomyces* and thiram seed treatment on seed-borne *Alternaria brassicicola.* At the top *Streptomyces,* in the middle thiram treated seeds and at the bottom naturally infected cauliflower seeds.

The properties of the substrate, especially pH, moisture content, airspace and the organic matter, have a fundamental effect on the activities of antagonists in the soil (Baker and Cook 1974). For this reason it would be very important to demonstrate that *Streptomyces* sp. also functions in different types of substrate, such as fine sand and clay soils, and in mull mixtures. Similarly, the effectiveness of biological control of the pathogen was good in peat lots of different origin, irrespective of the peat's own disease suppressive properties which have been found in earlier studies to vary considerably from lot to lot (Tahvonen 1982 a). It is obvious that the natural antagonists in the peat do not inhibit the activities of the *Streptomyces* antagonist carried by the seed.

It has been possible in these experiments to demonstrate, using a wide range of strategies, that the *Streptomyces* sp. isolated from peat is effective in controlling seed-borne *A. brassicicola* on *Brassica* sp. plants. Other antagonists, such as *Gliocladium, Trichoderma* and *Penicillium* spp., have also given good results in similar applications (Wu and Lu 1984).

Other species of *Streptomyces* have been successfully used on cabbage (Kundu and Nandi 1984) and pea (Rothrock and Gottlieb 1984) in the control of *Rhizoctonia* damping-off. In preliminary trials the *Streptomyces* sp. used here also controlled *Rhizoctonia* damping-off (Tahvonen 1982 b). The *Streptomyces* antagonist clearly has a good potential in commercial applications as an alternative to the chemical dressing of cruciferous plants. The present results suggest that more extensive applications to other plants and pathogens are also possible (Tahvonen and Uoti 1983, Tahvonen 1985).

References

Baker, F.B. & Cook, R.J. 1974. Biological control of plant pathogens. 433 p. San Francisco.

Dixon, G.R. 1981. Vegetable Crop Diseases. 404 p. Salisbury, Wiltshire.

Heinze, K. 1974. Leitfaden der Schädlingsbekämpfung. Band I. Schädlinge und Krankheiten im Bemüsebau. 360 p. Stuttgart.

Kundu, P.K. & Nandi, B. 1984. Control of cauliflower damping-off by using antagonist coated seeds. Pedobiol. 27: 43—48.

Rothrock, C.S. & Gottlieb, D. 1984. Rise of antibiosis in antagonism of *Streotimyces hygroscopicus* var. *geldanus* to *Rhizoctonia solani* in soil. Can. J. Microbiol. 30: 1440—1447.

Maude, R.B., Humpherson, J.F.M. & Shuring, C.G. 1984. Treatments to control *Phoma* and *Alternaria* infections of brassica seeds. Pl. Path. 33: 525—535.

Neergaard, P. 1945. Danish species of *Alternaria* and *Stemphylium*. 560 p. Cobenhagen.

— 1977. Seed Patholgy. 1187 p. Surrey.

Puustjärvi, V. 1974. Kasvuturve ja sen käyttö. 172 p. Helsinki.

Tahvonen, R. 1979. Seed-borne fungi on cruciferous cultivated plants in Finland and their importance in seedling raising. J. Scient. Agric. Soc. Finl. 51: 327—379.

— 1982 a. The suppressiveness of Finnish light coloured *Sphagnum* peat. J. Scient. Agric. Soc. Finl. 54: 345—356.

— 1982 b. Preliminary experiments into the use of *Streptomyces* spp. isolated from peat in the biological control of soil and seed-borne diseases in peat culture. J. Scient. Agric. Soc. Finl. 54: 357—369.

— 1985. Mycostop — ett biologiskt bekämpningsmedel mot svampsjukdomar. Växtskyddsnotiser 49: 86—90, 93.

— & Uoti, J. 1983. The use of *Streptomyces* as a biological control agent. 10th Intern. Congr. Pl. Protect. 1983: 2, 795.

Turhan, G. & Grossmann, F. 1986. Investigation of great number of actinomycete isolates on their antagonistic effect against soil-borne fungal plant pathogens by an improved method. J. Phytopath. 116: 238—243.

Wu, V.S. & Lu, J.H. 1984. Seed treatment with antagonists and chemicals to control *Alternaria brassicicola*. Seed Sci. and Technol. 12: 851—862.

Ms received August 19, 1987

SELOSTUS

Alternaria Brassicicola -sienen biologinen torjunta kaalikasvien siemeniltä jauhemaisella *Streptomyces*-bakteerivalmisteella

Risto Tahvonen ja Hanna Avikainen

Kasvitautiosasto
Maatalouden tutkimuskeskus,
31600 Jokioinen

Turpeesta eristetystä *Streptomyces*-bakteerista valmistettua jauhemaista bio-peittausainetta tutkittiin kaalikasveilla siemenlevintäisen *Alternaria brassicicola* -sienen torjumiseksi. Valmiste oli tehty fermentaattorissa kasvatetusta biomassasta pakastekuivaamalla ja jauhamalla peittausaineeksi soveltuvaksi pulveriksi, jonka kaupallinen nimi on Mycostop. Ainetta käytettiin peittauksissa 1—15 g/siemenkilo. Taimipoltteen torjuntatehoa verrattiin kemialliseen tiraami-peittaukseen sekä keinosaastutetuilla että luontaisesti saastuneilla siemenillä ravintoalusta ja taimikasvatustestein. Valmisteen biologisen tehokkuuden säilyminen varastoiduilla, peitatuilla siemenillä tutkittiin 0—6 kk peittauksen jälkeen tehdyillä kylvöillä höyrytettyyn turpeeseen. Eri alkuperää olevien turpeiden, eri kivennäisalustojen sekä turpeen pH:n vaikutusta torjuntatehoon selvitettiin.

Siemenen peittaus *Streptomyces*-valmisteella torjui 80—90 %:sti taimipoltteen voimakkaasti *Alternaria*-sienellä saastutetuilta siemeniltä turvealustalla kasvatettuna. Peittauksen teho säilyi kuivana varastoiduilla siemenillä 5—6 viikkoa muuttumattomana, minkä jälkeen teho laski hitaasti ollen noin 50 % 6 kk:n kuluttua peittauksesta. *Streptomyces*-peittaus torjui taimipoltteen samalla tehokkuudella kuin kemiallinen tiraami-peittaus luontaisesti saastuneilta kauppasiemeneriltä. Taimipoltteen biologinen torjuntatulos ei vaihdellut eri alkuperää olevissa turve-erissä suoritetuissa taimikasvatuksissa, vaikka turpeiden luontainen taudinestokyky vaihteli voimak-

kaasti. Torjuntatulos oli sama tai parempi kuin kemiallisella tiraami-peittauksella. Kasvualustan happamuudella pH-alueella 4.8—8.6 ei ollut vaikutusta biologisen peittauksen tehoon. Muissa kasvualustoissa, savi, hieta ja multaseos, oli *Alternaria*-taimipoltteen torjuntatulos sama kuin turpeessa.

Tehdyt tutkimukset osoittivat, että kaalikasveilla voidaan kemiallinen peittaus korvata biologisella peittausmenetelmällä, joka ei käytännön toteutukselta kuitenkaan poikkea perinteisistä työtavoista.