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Some exploratory tests of the resistance of germinant seedlings and the virulence of some potential parasites

Rotparasiter på groddplantor av skogsträd

Några försök att testa resistens hos groddplantor respektive virulens hos några potentiella parasiter

by

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Root Parasites on Forest Tree Seedlings

It has long been known that a large number of different parasitic fungi attack coniferous seedlings. Among previous Swedish investigations a work by LINDFORS (1922) deserves special attention. Isolating a number of *Fusarium* species from diseased pine seedlings, he showed by infection experiments that healthy seedlings could be attacked.

Since it was considered desirable to acquire further knowledge of the parasitic fungi that may attack forest tree seedlings, a number of fungi that occurred at high frequency on dying seedlings were isolated mainly by gathering diseased seedlings from tree nurseries in central Sweden during the period 1954—58. Subsequently, the parasitic virulence on forest tree seedlings was tested by infection and re-isolation. Thus the following fungi have been isolated and tested with positive results: *Fusarium orthoceras, F. solani, F. culmorum, F. oxysporum, Pythium intermedium, P. debaryanum, Phytophthora cactorum, Botrytis cinerea, Rhizoctonia solani, Cylindrocarpon radicicola* and *Diplodia pinea*. Frequently, the following fungi have also been isolated from dead seedlings: *Trichoderma viride, Pullularia pullulans, Mucor ramannianus, M. hiemalis, Aspergillus spp.* and *Penicillium spp.* The latter group of fungi, however, have not caused damages at the infection experiments and they are probably secondary parasites. The fungi occurring most frequently in the material are: *Pythium intermedium, P. debaryanum, Botrytis cinerea* and *Rhizoctonia solani*.

The problem of preventing fungal attacks on coniferous seedlings has been elucidated by several investigations. The organic matter content of the seed beds, pH and the moisture conditions are environmental factors that have proved to be of great importance (STRONG 1952, VAARTAJA 1952, ROTH and RIKER 1942). Disinfection of seed before sowing by means of fungicides as applied in agriculture has appeared to be an inexpensive and simple method to reduce the frequency of damping-off on forest tree seedlings. Earlier, mainly toxic mercury compounds have been used for this purpose. However, in addition to being toxic to man and animals these agents also inhibit the germination of forest seeds. Lately, non-mercuric, organic fungicides have been applied with success. This treatment consists of a dry

Figure 1 A. Seedling of pine attacked by damping-off fungi.
Groddplanta av tall angripen av fallsjukesvamp.

disinfection that may act by killing fungal spores on the seed coat and by partially sterilizing the soil adjoining the seedling. Certain organic fungicides also stimulate the germination, which shortens the period of sensitivity when the germinating seeds are exceptionally susceptible to attacks. Disinfection of seed prior to sowing reduces the frequency of damping-off at a moderate infection potential of the parasitic fungi. Chemical treatment of seed, however, will not have the effect desired under circumstances where the fungal life conditions are at optimum. At low infection potential, too, the gain is slight.

In the case of valuable seed or heavily infected nurseries, additional measures must be tried to give the seedlings the best start possible in the struggle against parasites. Soil fumigation, i. e. a treatment of the soil with chemicals or heat before sowing, has been used for this purpose. Ever since the introduction of the method, the primary object of soil fumigation has been to improve the health of the crop, be it threatened by insects, nematodes, or fungi. In addition to providing control of weeds, the method also stimulates growth of the crop. Techniques used for partial disinfection of soils were recently reviewed by NEWHALL (1955). The effects of fungicides, insecticides and herbicides on the chemical, physical and micro-biological status of the soil have been summarized by MARTIN and PRATT, 1958, and ENO, 1958.

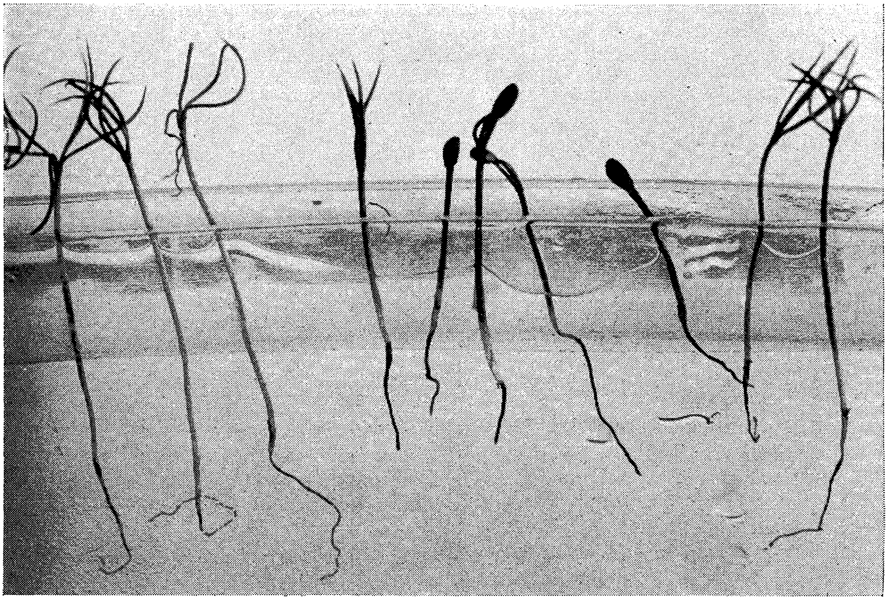


Figure 1 B. Early stage of damping-off infection. It may be noticed that the attack starts at the root tips and proceeds upwards.
Tidigt stadium av fallsjukeinfektion. Observera att angreppet startar vid rotspetsarna och fortsätter uppåt.

I. Methyl bromide disinfection of soil inoculated with damping-off fungi

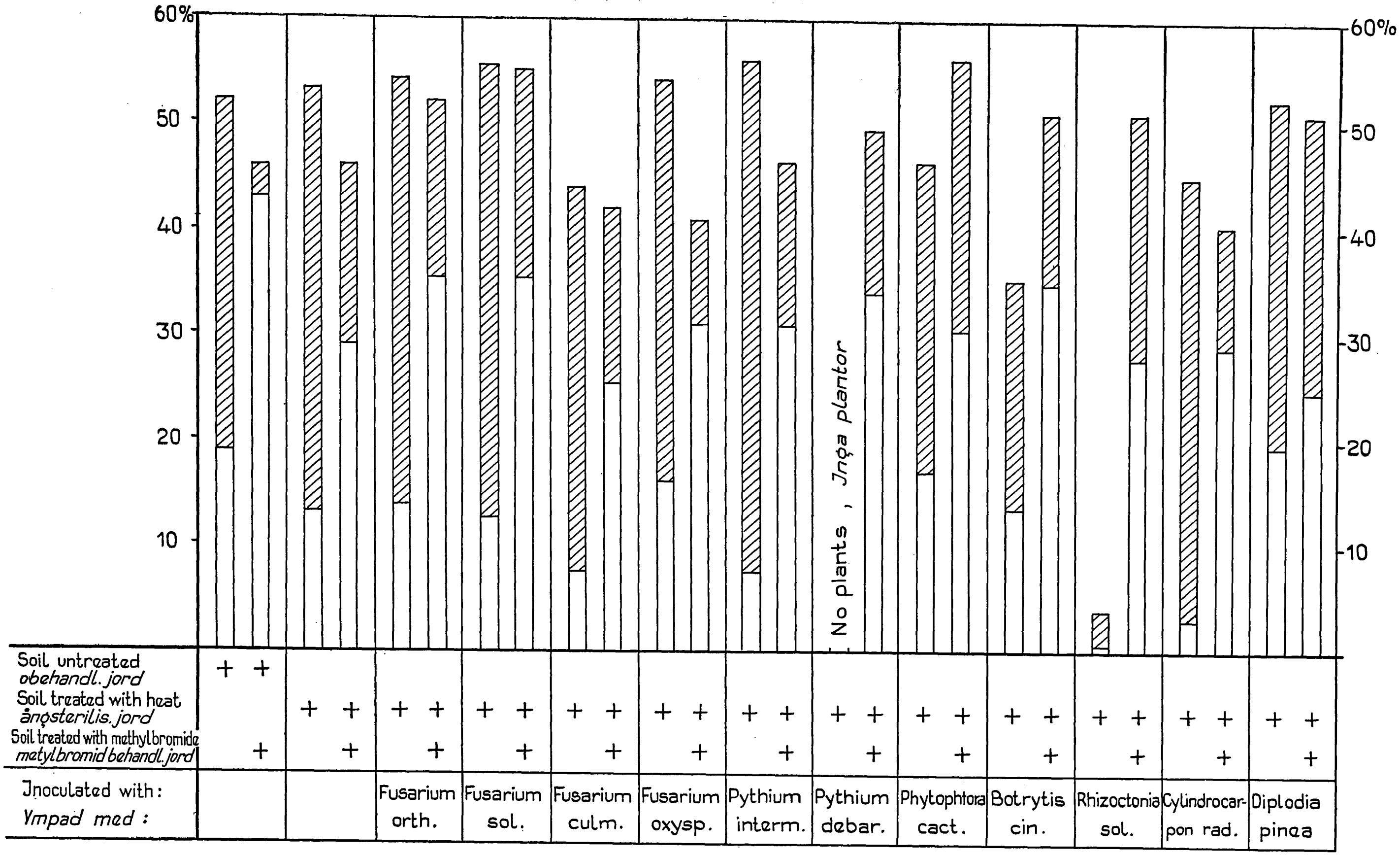
The purpose of this experiment was to study the influence of partial soil disinfection by means of methyl bromide on the infection potential of known damping-off fungi under controlled conditions. The experiments were carried out in greenhouses in artificial light. In glass jars (0.5 litre) a bottom layer of sand was covered with a mixture of sand and mull soil. After the jars had been closed and sterilized for one hour at one atm. two consecutive days a pure culture of a damping-off fungus was inoculated. Remaining closed at a constant temperature (22° C) for 14 days, half the number of jars was subsequently treated with methyl bromide. Three days later 100 Scots pine seeds were sown in each jar. The number of germinated and dead seedlings was recorded at various intervals. Isolations were performed on dead seedlings and in most cases the inoculated fungi could be re-isolated. *Fusarium spp.*, *Pythium spp.* and *Rhizoctonia solani* were isolated from dead seedlings found in control jars containing sterilized (heat) but uninfested soil.

Table 1 and graph 1 show that a rather large number of seedlings have all succumbed due to fungal attacks in both untreated and sterilized (heat) soils inoculated with known damping-off fungi. The methyl bromide treatment

Table 1. Frequency of damping-off on seedlings of Scots pine growing in soil sterilized by heat and inoculated with isolates of known fungi. In all treatments half the number of jars have been treated with methyl bromide before sowing.

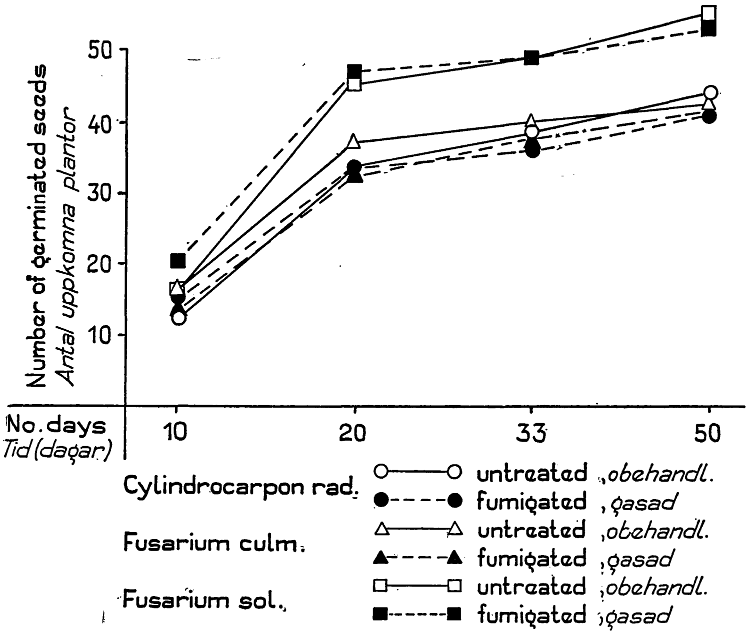
Frekvens fallsjuka hos groddplantor av tall i värmesteriliserad jord, som ympats med renkulturer av kända svampar. I samtliga försöksled har halva antalet burkar behandlats med metylbromid före sådd.

Disinfection of soil with Desinfektion av jord med	Soil inoculated with Jord ympad med	Sterilization of soil with Desinfektion av jord med	Emergence after 50 days per cent % uppkomna (50 dagar)	Mortality after 50 days per cent % döda (50 dagar)	Survival after 50 days per cent % överlevande (50 dagar)	Weight of seedling 50 days mg Vikt mg/planta (50 dagar)
Heat Värme	*Fusarium orthoceras*	—	54.0 ± 3.2	40.5	13.5 ± 2.0	135.0 ± 5.0
»	»	methyl bromide	52.0 ± 2.9	16.3	35.7 ± 0.3	135.0 ± 15.0
»	*Fusarium solani*	—	55.3 ± 3.4	42.7	12.7 ± 2.3	123.3 ± 12.0
»	»	methyl bromide	54.3 ± 3.4	18.7	35.7 ± 1.8	122.3 ± 3.3
»	*Fusarium culmorum*	—	43.7 ± 6.2	36.0	7.7 ± 1.2	110.0 ± 15.3
»	»	methyl bromide	42.0 ± 4.4	16.3	25.7 ± 4.3	133.3 ± 3.3
»	*Fusarium oxysporum*	—	54.0 ± 2.6	38.0	16.0 ± 6.6	126.7 ± 3.3
»	»	methyl bromide	41.0 ± 3.4	10.0	31.0 ± 2.5	153.3 ± 3.3
»	*Pythium intermedium*	—	56.0 ± 2.9	48.3	7.7 ± 0.7	93.3 ± 6.6
»	»	methyl bromide	46.7 ± 2.2	15.7	31.0 ± 3.6	140.0 ± 5.7
»	*Pythium debaryanum*	—	0	0	0	—
»	»	methyl bromide	49.7 ± 7.1	15.7	34.0 ± 4.5	136.7 ± 8.8
»	*Phytophthora cactorum*	—	46.7 ± 1.8	29.3	17.3 ± 1.5	120.0 ± 5.7
»	»	methyl bromide	56.0 ± 1.0	25.7	30.3 ± 2.3	156.7 ± 6.7
»	*Botrytis cinerea*	—	35.7 ± 0.7	22.3	13.3 ± 1.2	116.7 ± 3.3
»	»	methyl bromide	51.0 ± 4.0	16.0	35.0 ± 1.5	146.7 ± 3.3
»	*Rhizoctonia solani*	—	4.3 ± 0.9	3.7	0.7 ± 0.5	—
»	»	methyl bromide	51.3 ± 3.5	23.0	28.3 ± 7.2	140.0 ± 5.7
»	*Cylindrocarpon radicicola*	—	45.0 ± 3.4	41.7	3.3 ± 0.9	86.7 ± 6.7
»	»	methyl bromide	40.7 ± 5.8	11.3	29.3 ± 6.7	156.7 ± 8.8
»	*Diplodia pinea*	—	52.7 ± 5.0	33.0	19.7 ± 0.9	116.7 ± 3.3
»	»	methyl bromide	51.0 ± 4.4	26.3	24.7 ± 5.0	120.0 ± 15.3
»	Not inoculated Ej ympad	—	53.3 ± 4.7	40.0	13.3 ± 0.9	120.0 ± 5.7
»	»	methyl bromide	46.0 ± 2.1	17.0	29.0 ± 3.1	150.0 ± 5.7
Untreated soil Obehandlad jord		—	52.3 ± 4.5	33.3	19.0 ± 4.0	140.0 ± 15.3
»	»	methyl bromide	46.3 ± 4.7	3.3	43.0 ± 3.4	160.0 ± 5.7



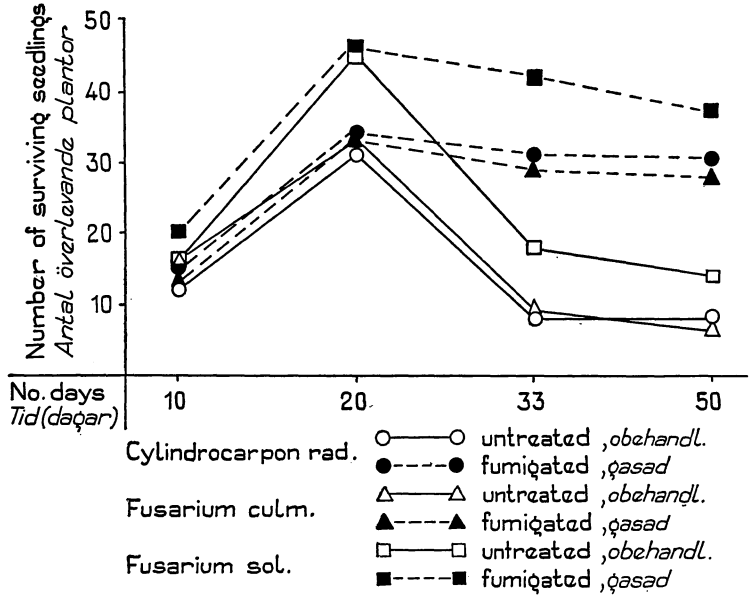
Graph 1. Frequency of damping-off on seedlings of Scots pine in sterilized soil, inoculated with known fungi ▨ dead ☐ survival.

Fallsjuke-frekvens på groddplantor av tall i steriliserad jord, som ympats med kända svampar. ▨ döda. ☐ överlevande.



Graph 2. Number of germinated seeds at various number of days.
Antal uppkomna plantor efter olika tider.

seems to have only a slight effect on the emergence of seedlings (graph 2). In some cases without methyl bromide treatment, viz. jars inoculated with *Pythium debaryanum* and *Rhizoctonia solani*, respectively, but a few or no seedlings emerged. This indicates that the fungi attack and kill the seedlings in the initial stage. Generally, the highest frequency of dead seedlings has been recorded 14—21 days after sowing. Only a few solitary seedlings die after 50 days (graph 3). A soil treatment with methyl bromide under the conditions controlled in the experiment increases the number of seedlings surviving after 50 days whether untreated or sterilized soil, or sterilized soil inoculated with parasitic fungi is used. Methyl bromide has reduced the attacks in all treatments. Yet, the effect was slight on *Diplodia pinea*. The greatest effect was attained after treatment of soil previously not sterilized by heat. This indicates that methyl bromide essentially affects the equilibrium between the populations of saprophytic and parasitic organisms, which may be considered as a biological control since the weakened parasites are suppressed by the less affected flora of saprophytes. Destruction of the major part of the microflora, parasitic as well as saprophytic, may be the reason for the severe attacks in soil sterilized by heat. When the re-colonization starts by airborne spores or by spores carried by the pine seed at the time of sowing, the parasitic fungi have successfully established themselves in advance of



Graph 3. Number of surviving seedlings at various number of days.
Frekvensen av överlevande plantor efter olika antal dagar.

the saprophyte flora and in the absence of antagonistic agents. Infection by seed or air has not occurred in the inoculated jars. The inoculated fungus has probably been able to prevent secondary spore infection from developing.

II. The frequency of damping-off in soil sterilized by heat and inoculated with saprophytic fungi

Several investigators, e.g. FISHER (1941) and VAARTAJA (1956), have shown that a large number of fungi occurring as spores on seed of forest trees generally have no damaging effect on germinating seeds. This is contrary to what has been found regarding the flora, the spores of which occur on agricultural seeds. HUSS (1956) and GIBSON (1957), however, have shown that seed mechanically damaged in conjunction with the extraction process often may germinate poorly, probably because saprophytic fungi have gained access to the embryo through cracks in the seed coat. These fungi normally considered indifferent have a potential of great importance for the control of the parasitic fungi found on seedlings. The infection potential of the parasites may be indirectly affected by exudation of antibiotic agents and by competition for available nutrients. After partial disinfection the soil is first re-colonized mainly by these so called sugar fungi. *Trichoderma viride* is one of the fungi that predominate shortly after the disinfection, but *Penicillium spp.*, *Mucor spp.* and certain bacterias, too, grow very fast in treated soil. To investigate whether these fungi were

Table 2. Frequency of surviving seedlings after 50 days in sterilized soil inoculated before sowing with saprophytic fungi or bacteria.

Frekvens överlevande groddplantor efter 50 dagar i värmesteriliserad jord, som före sådd ympats med sapofytiska svampar respektive bakterie.

Treatment of soil Behandling av jorden med	Soil inoculated with Jorden ympad med	Emergence per cent % uppkomna	Survival after 50 days, per cent % överlevande efter 50 dagar
Heat Värme	Bacteria 53	26.0 ± 4.4	20.0 ± 3.8
	Pullularia pullulans	30.3 ± 9.3	20.3 ± 6.4
»	*Mucor ramannianus*	45.7 ± 3.2	37.0 ± 5.0
»	*Trichoderma viride* (viridin producer)	43.3 ± 2.7	31.3 ± 1.5
»	*Trichoderma viride* (gliotoxin producer)	33.3 ± 2.6	25.0 ± 2.7
»	*Trichoderma viride* (isol. 4—51)	34.3 ± 0.9	26.3 ± 3.4
»	Not inoculated Ej ympad	28.3 ± 1.8	21.0 ± 2.7
Untreated soil Obehandlad jord		32.3 ± 1.2	28.0 ± 0.6

able to attack seedlings under experimental conditions or possibly enhance the beneficial effect of soil fumigation by antagonism, supplementary tests were carried out by means of the technique described above. In this experiment the frequency of surviving seedlings after 50 days was investigated: in untreated soil, in soil sterilized by heat without or with subsequent inoculation of *Trichoderma viride* (viridine producer), *T. viride* (gliotoxin producer), *T. viride* (special isolation), *Mucor ramannianus*, *Pullularia pullulans*, or a bacterium isolated from soil sterilized by formaldehyde treatment. Table 2 shows that none of the jars inoculated with fungi produced higher frequency of attacks than those that were not inoculated. *Trichoderma*, however, and particularly *Mucor ramannianus* have reduced the frequency of dead seedlings. This consequently indicates that an inoculation or possibly a dry treatment of seed with inoculation material of e.g. *Mucor ramannianus* would enhance the effect of partial soil disinfection. Attempts along this line have been made for instance by WRIGHT (1956). A treatment of mustard seed with spores of *Trichoderma viride* prior to sowing in soil infected with *Pythium spp.* produced a relatively good control of the parasites. It was assumed that this effect was attained because *Trichoderma viride* produced gliotoxin.

III. The resistance of various provenances of Scots pine to damping-off

It is of great interest to know whether different provenances of forest trees in the seedling stage manifest various degrees of resistance to root

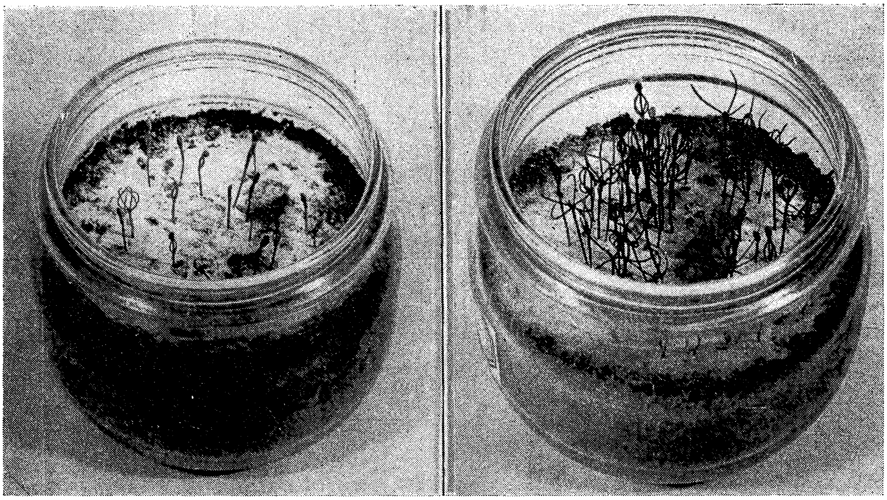


Figure 2. Jars containing soil sterilized by heat and inoculated with *Fusarium solani*.
Burkar innehållande jord, som steriliserats med värme och ympats med *Fusarium solani*.

A. Not treated with methyl bromide after sterilization. The seedlings are smaller probably because of the growth inhibiting effect of *Fusarium*.
Icke behandlad med metylbromid efter sterilisering. Groddplantorna äro mindre troligen på grund av tillväxthämmande verkan av *Fusarium*.

B. Treated with methyl bromide after sterilization.
Behandlad med metylbromid efter värmesterilisering.

parasites. The literature, however, is rather scanty in this respect. Works dealing with this problem have been published by THOMAS (1952), CORMACK and HARPER (1952) who reported varying resistance of *Carthamnus tinctcorius* to *Phytophthora* and *Pythium spp*. Resistance of certain varieties of pea plants to *Pythium* and *Rhizoctonia* are reported by MC CALLUM (1948). Additionally, VAARTAJA and CRAM (1956) found that of two provenances of white spruce (*Picea alba*) tested, one was relatively resistant to *Rhizoctonia* but very susceptible to *Pythium*. In material collected for other purposes EICHE (unpublished) found a great difference in resistance to fungi of pine seedlings cultivated from seed collected in the same locality but from two separate trees. Increased resistance with advancing age has been noticed in our experiments. But a few diseased seedlings were observed after 50 days. VAMOS and VIDA (1959) found antibacterial substances in coniferous seedlings above a certain age, but not in very young ones. This indicated that the resistance of older seedlings may be associated with the content of antibacterial substances in the seedlings.

The variation of resistance to one damping-off fungus, viz. *Fusarium solani*, has been tested in an experimental series of different pine provenances. The technique described earlier was used and six different seed provenances

Table 3. Survival of six different provenances of pine tested with *Fusarium solani*. Each treatment is represented by an average based on 1,200 seeds.

Frekvens överlevande av sex olika tallprovenienser testade med *Fusarium solani*. Varje försöksled utgör medeltal av 1 200 sådda frön.

Provenance Proveniens	Germinability in Jacobsen tank, per cent % grodda vid analys enl. Jacobsen	Soil sterilized by heat and inoculated with Fusarium solani Ångsteriliserad jord som ympats med Fusarium solani		
		emergence per cent % uppkomna	survival per cent % överlevande	survival in rel. to germination test per cent % överlevande rel. groningsanalys
4227 Gotland	85 ± 1.8	60.6 ± 1.4	27.6 ± 1.3	32.4 ± 1.5
4975 Halland	89 ± 1.6	35.3 ± 1.4	27.2 ± 1.3	30.5 ± 1.5
4966 Halland	92 ± 1.4	72.3 ± 1.3	23.3 ± 1.4	36.1 ± 1.5
9354 Västerbotten	69 ± 2.3	31.3 ± 1.3	28.2 ± 1.3	40.8 ± 1.9
5021 Gävleborg	91 ± 1.4	88.3 ± 0.9	30.4 ± 1.3	33.4 ± 1.4
F 213 Turkey		46.3 ± 1.5	21.7 ± 1.2	

were tested. The experiment was designed with 12 replications and 100 seeds were sown in each jar. After 50 days the number of germinated and surviving seedlings was recorded in the various cases. Table 3 shows relatively slight differences in number of surviving seedlings whereas the germination differences were quite considerable. The number of seedlings that germinated is not correlated with the germinability found in a Jacobsen tank. The result is not entirely conclusive depending on a deficiency of data regarding the seed lots. Processing and storage of seed, however, has been uniform for all the provenances. The experiment was intended as a study of techniques more than an attempt to ascertain a genetic variation of resistance of different provenances. The relatively moderate dispersion of the data seems to indicate that the method applied should be useful for a progeny test of seedling resistance to root parasites.

IV. Occurrence of toxins in exudates from disinfected spruce seed inocalated with known soil fungi

A prospective complement to the method of investigating seedling resistance just described is to test the seedlings not directly against the fungi but against exudates produced by these organisms.

It is well known that both the parasitic and saprophytic fungi in pure culture may form toxins that are sufficiently concentrated to become phytotoxic to germinant seedlings. These metabolic products may comparatively often be unstable in soil, but since they are continuously produced, it is likely that they are of ecological importance to microorganisms as well as plants. Consequently, it is reasonable to assume that fungal spores germinate on the

Table 4. Occurrence of toxins in extracts of spruce seed inoculated with known soil fungi.
Förekomst av toxiner i extrakt av granfrö, som ympats med kända marksvampar.

Solution Lösning	Extract con- centr. Konc. av extrakten	Germinat- ed seeds after 20 days per cent % grodda frön efter 20 dagar	Size distribution in per cent of total germ. Olika storl.klasser i % av tot. ant. grodda		
			<1 cm	1—4 cm	>4 cm
Water Vatten		59.7 ± 5.5	10.5	79.0	10.5
Seed extract Fröextrakt	C_1	66.3 ± 5.3	19.0	77.0	4.0
»	$C_1/10$	72.5 ± 5.0	7.0	70.5	22.5
»	$C_1/100$	68.7 ± 5.2	13.0	72.5	14.5
» + *Fusarium culmorum*	C_2	16.2 ± 4.0	46.0	54.0	0
» + » »	$C_2/10$	56.2 ± 5.5	15.5	82.5	2.0
» + » »	$C_2/100$	66.2 ± 5.3	5.5	73.5	21.0
» + » *solani*	C_3	52.5 ± 5.6	43.0	57.0	0
» + » »	$C_3/10$	70.0 ± 5.0	14.5	80.0	5.5
» + » »	$C_3/100$	71.2 ± 5.1	5.5	70.0	24.5
» + *Trichoderma viride*	C_4	52.5 ± 5.6	57.0	43.0	0
» + » »	$C_4/10$	70.0 ± 5.0	14.5	80.0	5.5
» + » »	$C_4/100$	73.7 ± 4.8	20.5	56.0	23.5
» + *Mucor ramannianus*	C_5	62.5 ± 5.3	40.0	60.0	0
» + » »	$C_5/10$	61.2 ± 5.5	18.5	79.5	2.0
» + » »	$C_5/100$	81.2 ± 4.4	4.5	58.5	37.0
» + *Pythium debaryanum*	C_6	57.5 ± 5.5	28.5	71.5	0
» + » »	$C_6/10$	67.5 ± 5.1	15.0	85.0	0
» + » »	$C_6/100$	71.2 ± 5.1	7.0	56.0	37.0
» + *Diplodia pinea*	C_7	56.2 ± 5.5	9.0	91.0	0
» + » »	$C_7/10$	67.5 ± 5.1	15.0	83.0	2.0
» + » »	$C_7/100$	75.0 ± 4.7	3.5	53.0	43.5

surface of submerged seeds or in their immediate vicinity and that the fungi exert direct influence on the germination of seed by exudates. The fungi may also affect the resistance of germinant seedlings since the sprout absorbs antibiotics that inhibit the attack of parasites. The seeds, too, contain substances that in addition to being influential on the germination of spores also may be of importance for the resistance of the seedling. The following experiment has been carried out to elucidate this complex problem.

Spruce seed (100 g) was weighed in Erlenmeyer flasks of one litre capacity and 100 ml water was added. The flasks were closed by cotton stoppers and put in an autoclave for half an hour at one atm. The autoclave treatment was repeated once after two days. A piece of mycelium of the fungus the exudate of which was to be tested was inserted in all flasks and in the uninfested controls. After 15 days in culture 150 ml water was added and the flasks were again put in an autoclave for half an hour at one atm. Seed and mycelium were separated by a filter funnel and the extract was diluted with 150 ml

Table 5. Germination of pine seed in contact with seed extracts of various concentrations. Groning av tallfrö i kontakt med fröextrakt av olika koncentration.

Extract concentr. Koncentration av extrakten	Provenance A Proveniens A				Provenance B Proveniens B			
	Germinated seeds after 20 days per cent % grodda frön efter 20 dagar	Size distribution in per cent of total germ. Olika storl.klasser i % av tot. ant. grodda			Germinated seeds after 20 days per cent % grodda frön efter 20 dagar	Size distribution in per cent of total germ. Olika storl.klasser i % av tot. ant. grodda		
		<1 cm	1—4 cm	>4 cm		<1 cm	1—4 cm	>4 cm
C	32.3	71.0	19.0	—	44.7	35.0	65.0	—
C/10	59.5	25.5	63.5	11.0	49.0	7.5	57.0	35.5
C/20	64.0	14.0	62.6	23.4	57.7	10.0	59.0	31.0
C/40	63.6	17.0	50.5	32.5	56.3	6.5	51.5	42.0
C/80	58.0	10.0	63.0	27.0	52.3	4.5	37.5	58.0
C/100	73.6	9.5	62.5	28.0	49.3	6.0	51.0	43.0
C/200	—	—	—	—	49.0	3.5	46.0	50.5
Control Kontroll (H_2O)	75.0	14.5	70.5	15.0	49.0	4.5	52.5	43.0

water. A series of concentrations C, C/10, C/100 was prepared of each extract. Spruce seeds were then grown in a Jacobsen tank in contact with the extracts via a filter paper strip. Record was kept of the germination of pine seeds connected with the various concentrations. The number of seeds germinated after 15 days in each treatment was recorded separately for the number of sprouts shorter than 1 cm and those longer than 4 cm. Results of the experiment are shown in table 4 where it appears that the seed-extract itself has had a tendency to affect the germination of seed. Besides the seed extract also the various fungal exudates have had an effect. Although heavily inhibiting, as in the case of *Fusarium culmorum,* the influences may in other cases stimulate the germination of seed considerably. Concerning the lowest concentrations of *Mucor ramannianus* and *Diplodia pinea* the stimulating effects are statistically significant. The concentration of exudates will determine whether a stimulation or a suppression is to be expected.

Still another experiment with germination of pine seed in contact with seed extracts has been carried out. Pine seed from the province of Halland (A) and from northern Sweden (B) has been used. The results are presented in table 5. The tables 4—5 show that germination of seed probably is influenced by the fungal flora on the seed coat and in the immediate vicinity of submerged seeds. The effect may be either negative or positive, depending on the physiological status of the fungi and the severity of infection. It is worth mentioning that *Mucor ramannianus* inoculated in soil sterilized by heat provided a considerable protection against damping-off and also that it may produce substances that have a beneficial effect at suitable concentrations on the germination of pine seed.

Summary

Soil sterilized by heat in glass jars has been inoculated with cultures of fungi isolated from diseased coniferous seedlings. Pine seeds were sown in the jars 14 days later and the parasitic virulence of the fungi in relation to germinant seedlings was recorded. The following fungi have been isolated and re-isolated from infected seedlings: *Fusarium orthoceras*, *F. solani*, *F. culmorum*, *F. oxysporum*, *Pythium intermedium*, *P. debaryanum*, *Phytophthora cactorum*, *Botrytis cinerea*, *Rhizoctonia solani*, *Cylindrocarpon radicicola* and *Diplodia pinea*. Presowing treatment of the soil with methyl bromide has reduced the frequency of attacks in all cases, and considerably larger and more vigorous seedlings (table 1) than those obtained in untreated soil have been produced.

Heat-sterilized soil inoculated with cultures of saprophyte fungi produced in several cases a higher frequency of surviving seedlings than that obtained on non-inoculated soil. Particularly *Mucor ramannianus* and *Trichoderma viride* have significantly increased the yield of healthy seedlings after 50 days (table 2).

An exploratory test of pine seed of various provenances with respect to resistance to *Fusarium solani* has also been carried out in soil sterilized by heat and under controlled conditions. Considerable differences in germination and survival, not correlated with differences in germinability observed in a Jacobsen tank, have been recorded (table 3).

Various saprophytic and parasitic fungi cultivated on substrates of sterilized spruce seed have been studied with respect to their effect on germinating pine seed. Inhibitive as well as stimulative effects have been recorded depending on the concentration and origin of the extract (table 4).

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Sammanfattning

Rotparasiter på groddplantor av skogsträd

Värmesteriliserad jord i glasburkar har ympats med renkulturer av svampar, som isolerats från sjuka barrträdsplantor. I dessa burkar såddes tallfrö efter 14 dagar, och svamparnas parasitiska kapacitet gentemot groddplantor noterades. Följande svampar har isolerats och reisolerats från infekterade groddplantor: *Fusarium orthoceras*, *F. solani*, *F. culmorum*, *F. oxysporum*, *Pythium intermedium*, *P. debaryanum*, *Phytophthora cactorum*, *Botrytis cinerea*, *Rhizoctonia solani*, *Cylindrocarpon radicicola* och *Diplodia pinea*. Behandling med metylbromid har i samtliga fall minskat angreppsfrekvensen i förhållande till obehandlade kontroller och har även givit avsevärt större och kraftigare plantor (tabell 1).

Ympning av värmesteriliserad jord med renkultur av saprofytiska svampar gav i flera fall ökad frekvens överlevande plantor i förhållande till kontrollburkar. Speciellt *Mucor ramannianus* och *Trichoderma viride* har signifikativt ökat utbytet av friska plantor efter 50 dagar (tabell 2).

Ett försök har även utförts att testa tallfrö av olika proveniens i avseende på sin resistens mot *Fusarium solani* i värmesteriliserad jord och under kontrollerade betingelser. Avsevärda skillnader i uppkomst och överlevandeprocent, som icke kunde korreleras med skillnader i groningsprocent på Jacobsens groningslåda, har noterats (tabell 3).

Extrakt av värmesteriliserat granfrö på vilket odlats olika svampar, såväl saprofyter som parasiter, har undersökts med avseende på sin effekt på groende tallfrö. Såväl groningshämningar som groningsstimuleringar har noterats, beroende på extraktets koncentration och ursprung (tabell 4).