DAMPING-OFF ETIOLOGY ESPECIALLY IN FOREST NURSERIES

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SUMMARY

Inoculations of tree seedlings in aseptic test tubes with fungi showed great potential pathogenicity for many isolates of Rhizoctonia solani (mostly Pellicularia praticola), Pythium debaryanum, P. ultimum, Phytophthora cactorum, and several species of Fusarium and Cylindrocarpon. These comprised the most common isolates from diseased tree seedlings in several forest nurseries. Some less common isolates also demonstrated strong, moderate, or weak potential pathogenicity. Scots pine and caragana were inoculated with representative isolates in unsterilized soils. With light inoculum (culture suspension) the pathogenic effects of inocula were masked by those of natural soil floras. With heavy inoculum (infested seed-soil mixture), high mortality was caused by all 4 isolates, including Thielaviopsis basicola, which in laboratory tests was only moderate in potential pathogenicity and has no known association with damping-off. It is suggested that etiological studies on damping-off should be limited to tests of potential pathogenicity under aseptic conditions. The actual importance of isolates can be found through epidemiological and ecological approaches. Heavy artificial inoculations may perhaps be useful to simulate the most serious epidemics.

Mortality, mainly caused by naturally occurring Pythium, was increased by high soil moisture. The floras of loamy prairie soil types, including virgin sod, were pathogenic. Influences of temperature were variable and tended to be opposite in the two host species. Environmental effects were large with the natural floras and small with heavy inocula. Fusarium spp. were commonly isolated from healthy seedlings, mainly as harmless parasites or epiphytes. The partially discrepant results under sterile and unsterile conditions are thought to be caused by interactions of soil flora. Strong antagonism to the pathogens was demonstrated by bacteria, Penicillium spp., Trichoderma viride, and Streptomyces spp.

Problems in etiology of damping-off.—Pathogenicity is usually established by constant association and isolation of a parasite, reproduction of the disease by inoculation, and reisolation. Fulfilling just one or some of these requirements is not enough. Thus, Fusarium spp. were commonly associated with certain damping-off epidemics, but an inoculation experiment in unsterile soil indicated that the less common associates, Rhizoctonia solani Kühn and Pythium spp., were the important pathogens (43). Fusaria were associated even with healthy seedlings that would not have died. They either colonized dead tissues or failed to produce serious disease.

On the other hand, the association of many soil-borne pathogens with their hosts is difficult to establish. The association of Phytophthora cinnamomoni Rand with little-leaf disease of pines was not known until Tucker’s isolation technique, which utilizes apples as bait, was employed (7). Thousands of earlier isolations from roots and surrounding soil failed to yield the pathogen. In apple trees, P. cactorum (Leb. & Cohn) Schroet, colonizes only a narrow zone in living tissues because toxins from bacteria and decomposing bark kill it in dead tissues (6). For similar reasons Phytophthora fragariae Hickman are seldom isolated (9, 15). Aphanoomyces spp. are also extremely sensitive to mercury compounds commonly used to eliminate the epiphytes usually present on plants taken from soil (17). Certain strains of Phytophthora fragariae Hickman are seldom isolated from roots, because they are closely followed by Pythium sp. and do not grow if dextrose is present in the medium or if the temperature is as high as is common in laboratory rooms (25). Thielaviopsis basicola (Berk. & Br.) Ferr. is difficult to isolate because of its slow growth and sensitivity to bacterial antagonism (18, 34); bait is often used in its isolation. Reisolation of such fungi in experiments may for this reason fail if the soil used is unsterilized.

One problem in experimental reproduction of damping-off is the vagueness and variation of the symptoms. For instances, Pythium sp. killed the seeds in sterilized media but in unsterile seedbed media (43) it mainly attacked seedlings. On the other hand, a certain damping-off symptom can be caused by fungi of different taxonomic groups. Furthermore, under aseptic conditions, damping-off can be produced by fungi that are seldom or never isolated from diseased seedlings, and by fungi that are usually considered as saprophytes (12, 43).

Damping-off of tree species.—Because of these problems the etiology of damping-off in many plants may be less well known than is commonly thought, and new approaches are needed in future studies. For instance, the importance is unknown of such predisposing factors as fertilizers (38), weeds (38), heat, drought, hail (39), excess water (45), and salinity (4).

In the following, the main attention is given to damping-off of tree species. This is a serious nursery problem, many species being highly susceptible over an extended period. The main published findings on this subject were recently reviewed briefly by Vaartaja and Cram (43). Species of Rhizoctonia and Pythium appear as very important causes of damping-off of trees, but further study is required of their importance in different environments and the role played by other fungi.
Koch's postulates were followed in a large number of tests in test tubes with various fungi and host species. In each tube, organisms other than tree seedlings and a fungus isolate were excluded. The results of such tests, as of any inoculation done in sterilized medium, should be considered to show only what may be called "potential pathogenicity" of an isolate. (Many tests were made with R. solani and *Pythium* isolates by the senior author together with H. Saksena and P. Salisbury; details of these will be reported in other publications; the results generally agree with those reported here.)

Experiments were also made in the greenhouse in unsterile soils. These were alkaline soils, in which damping-off tends to be severe and persistent, especially on conifers. This common experience of nurserymen was recently confirmed by Schönhar (32).

Unsterile soils were used partly because the potential pathogenicity of an isolate does not indicate its actual importance in seedbeds, and partly because of the success of our earlier study (43). When tests with one type of inoculum were repeated in similar soils with similar hosts in the greenhouse, however, the results were widely varying in different years (mortality between 10 and 80%). To explore causes of such variation, the following factors were included in the experimental design: moisture, temperature, and modifications of soil types.


*Visual estimates of average relative growth when check seedlings = 100; 0 = no growth (all seeds killed).*

Table 2 illustrates the potential pathogenicity of 16 isolates on different host progenies and seed lots. This test showed that the particular fungus-host combinations used in tests in soils could result in at least moderate disease. Some variation in potential pathogenicity was indicated among strains within *P. cactorum* and within *T. basicola*. Some small variation of susceptibility to certain pathogens was indicated among seed lots within a host species. This test failed to indicate such conspicuous differences within a host species as were found earlier (43). The tests gave highly reproducible results that agree with the results of similar earlier studies (41, 42, 43). They demonstrate potential pathogenicity for various isolates and give a firm basis for further studies with these. The

**Potential pathogenicity.—**Representative isolates were tested for potential pathogenicity in tubes, with 5 aseptically germinated tree seeds in each tube. The method was essentially the same as used earlier (43) except that the medium was different (half-strength corn-meal agar) and the plants received more light (ca. 1500 ft-c). A number of *Rhizoctonia solani* isolates, mostly belonging to *Pellicularia praticola*, were highly virulent on various plant species; others showed considerable variation. Similarly, many *Pythium* isolates were highly virulent and a few less so. Examples of the results with these and other fungi are given in Tables 1 and 2. For comparison, isolates are included from diseases other than damping-off. For instance, *Thielaviopsis basicola* isolates were mostly from rotting roots of seedlings of *Pinus resinosa*, of *caragana* (no. 2617), or of *Populus* sp. cuttings (2732A).

Table 1.—Potential pathogenicity of various fungi on seedlings of *Pinus banksiana* (in test tubes).

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>Health index*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhizoctonia solani</em> (P. praticola)</td>
<td>1347</td>
<td>0</td>
</tr>
<tr>
<td><em>Rhizoctonia</em> sp.</td>
<td>2352 &amp; 2616</td>
<td>20</td>
</tr>
<tr>
<td><em>Rhizoctonia</em> sp. (from mycorrhizal rootlet)</td>
<td>2297</td>
<td>20</td>
</tr>
<tr>
<td><em>Rhizoctonia</em> sp.</td>
<td>1647</td>
<td>40</td>
</tr>
<tr>
<td><em>Rhizoctonia</em> sp. (top blight)</td>
<td>1590</td>
<td>70</td>
</tr>
<tr>
<td><em>Phytophthora cactorum</em></td>
<td>1722</td>
<td>0</td>
</tr>
<tr>
<td><em>Phytophthora cinnamomii</em></td>
<td>2006 &amp; 2009</td>
<td>0</td>
</tr>
<tr>
<td><em>Pythium debaryanum</em></td>
<td>1366</td>
<td>0</td>
</tr>
<tr>
<td><em>Pythium</em> sp.</td>
<td>2233</td>
<td>5</td>
</tr>
<tr>
<td><em>Aphanomyces</em> (? euteiches Drechs.)</td>
<td>1709</td>
<td>10</td>
</tr>
<tr>
<td><em>Fusarium oxysporum var. redolens</em></td>
<td>1684</td>
<td>0</td>
</tr>
<tr>
<td><em>F. pone</em></td>
<td>2380</td>
<td>2</td>
</tr>
<tr>
<td><em>F. roseum</em></td>
<td>2595</td>
<td>3</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>2767</td>
<td>21</td>
</tr>
<tr>
<td><em>F. oxysporum var. redolens</em></td>
<td>1912</td>
<td>60</td>
</tr>
<tr>
<td><em>Cylindrocarpon</em> sp. (rootlet)</td>
<td>2477</td>
<td>40</td>
</tr>
<tr>
<td><em>C. obtusispora</em> (top blight)</td>
<td>1595A</td>
<td>40</td>
</tr>
<tr>
<td><em>C. radicicola</em> (top blight)</td>
<td>1823A</td>
<td>60</td>
</tr>
<tr>
<td><em>Helminthosporium</em> sp.</td>
<td>2150 &amp; 1730A</td>
<td>5</td>
</tr>
<tr>
<td><em>Thielaviopsis basicola</em> (cuttings)</td>
<td>2017 B &amp; 2732A</td>
<td>80</td>
</tr>
<tr>
<td><em>T. basicola</em> (caragana root rot)</td>
<td>2617</td>
<td>20</td>
</tr>
<tr>
<td><em>Pestalotia truncata</em> (rootlet)</td>
<td>2496</td>
<td>30</td>
</tr>
<tr>
<td><em>Stachybotrys</em> sp.</td>
<td>2152</td>
<td>45</td>
</tr>
</tbody>
</table>

*Visual estimates of average relative growth when check seedlings = 100; 0 = no growth (all seeds killed).*

Table 2 illustrates the potential pathogenicity of 16 isolates on different host progenies and seed lots. This test showed that the particular fungus-host combinations used in tests in soils could result in at least moderate disease. Some variation in potential pathogenicity was indicated among strains within *P. cactorum* and within *T. basicola*. Some small variation of susceptibility to certain pathogens was indicated among seed lots within a host species. This test failed to indicate such conspicuous differences within a host species as were found earlier (43). The tests gave highly reproducible results that agree with the results of similar earlier studies (41, 42, 43). They demonstrate potential pathogenicity for various isolates and give a firm basis for further studies with these. The
higher light intensity (1500 ft-c) than used earlier (250 ft-c) provided a more sensitive measure of the virulent isolates. Less virulent isolates are fairly pathogenic at very low light intensities (35); this is of importance in forest stands but perhaps not in open nursery beds.

The test tube method is recommended for wider usage, provided that tests in sterile conditions are considered as indicating only the potential pathogenicity. Very rich media should not be used since this may cause excessive accumulation of toxins. In high concentrations the excretions from innocuous fungi are known to be toxic to plants (33).

**Inoculations in unsterile soils.—Isolates and hosts.**

The following isolates were selected for further study:

- a) *R. solani* (*P. praticola*) (no. 1347);
- b) *Pythium debaryanum* (no. 1366);
- c) *Phytophthora cactorum* (no. 1722);
- d) *Thielaviopsis basicola* (no. 2732A). Isolate (d) was pathogenic but less virulent than (a), (b), and (c); it was included as a control but was found to be pathogenic under certain conditions. The high virulence of (a), (b), and (c) on a variety of hosts has been established in many tests in addition to those of Tables 1 and 2. The isolates were obtained from the following hosts: (a) from *Picea glauca*, (b) and (c) from *Pinus sylvestris*, and (d) from diseased poplar cutting.

Interaction of the isolates was explored by growing them in pairs at the opposing edges of corn-meal agar plates and observing the mutual effects in all combinations. Similarly, the interactions were explored with the 2 saprophytes commonly associated with damping-off in the soils used (*F. solani* and *Cylindrocarpon* sp.), and with 3 common antagonists (*Penicillium* sp., *Trichoderma viride* Pers. ex. Fr., and a bacterium).

The host species used were caragana and Scots pine. Seeds were collected from one tree of each (AIP and 54S, Table 2) so that the genetic variation among the test plants would be small.

**Methods.—1) Temperature.—**Three different temperature conditions were provided in greenhouse compartments, each with additional lights for 16 hours daily. The mean temperatures and average daily ranges were: 1) 19°C (14-24); 2) 26°C (24-30); and 3) 19°C (12-25).

2) **Soil and moisture.—**Six different "soils" were used: 1) virgin sod (Oxbow clay-loam) + 25% sand; 2) seedbed soil (originally as 1, used for 20 years with frequent manuring); 3) same as 2 but without weeds; 4) seedbed soil + 25% (sphagnum) peat; 5) seedbed soil sterilized with methyl bromide; 6) fungicide control, same as (2) but treated by mixing Orthocide [a wettable powder containing 50% captan (N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide)] and Tersan [a wettable powder containing 75% thiram (bis(dimethylthiocarbamoyl) disulfide)] into soil prior to sowing, and spreading these periodically on the soil surface after seeding. The ratio was 2 parts of both fungicides to 1000 parts of soil by weight. The surface application was with water at 0.2 g of the fungicides per sq ft.

Clay pots containing the soils were packed in peat in a flat. The flats were maintained at 2 moisture levels by adding small amounts of water daily. The levels were ca. 70 and 100% as indicated by the Bouyoucos blocks and scale ("Moisture available to plants").

3) **Inoculum potential.—**The pots were inoculated either "lightly" or "heavily." Light inoculum contained chopped cultures grown on corn-meal agar, applied in suspension in water 2 days after sowing. Heavy inoculum contained chopped cultures grown in flasks in mixtures of vermiculite, sawdust, and dead conifer seeds (volume ratios 4:4:2), and water, sugar, and minerals; the cultures were mixed in the surface soils when a month old, at 1 teaspoonful per pot, 3 days before sowing. In particles such as sawdust, the fungi were well protected. They could be cultured from the flasks left for a year in laboratory rooms.
Phytophthora cactorum and T. basicola were recovered from the medium after this was allowed to dry until only 4% water (by weight) could be extracted from it at 110°C. The other species required somewhat moister media to survive.

Results.—1) General.—Seedlings survivals were recorded 2 months after sowing, and calculated as percentages of seeds sown. Separate statistical analyses were made for the 2 host species and for the 2 inoculum potentials. Significant differences were calculated for P level .05 for data in Tables 4, 5, and 7.

In addition to damping-off, which caused low survival, caragana seedlings exhibited retarded growth and foliar discoloration in 2–3 months in soils treated with the fungicides. The edges of leaves and sometimes the whole leaves become chlorotic or brown, and died.

2) Light inoculum.—Results with light inocula were surprising. In contrast to results in earlier tests of Vaartaja and Cram (43), inocula in suspension now had little or no obvious effects on seedling survival.

The earlier tests (43) showed differences in seedling survival between Rhizoctonia solani, Pythium debaryanum, Fusarium oxysporum var. redolens, and Alternaria tenuis. In the new test, inoculation with fungi of obviously different pathogenic potential caused no significant differences. The effects of the inocula on the seedlings were perhaps masked by the greater effects of soil floras. Apparently the inoculum potentials of soil flora were weak in the earlier test (43). As a result the virulence indicated was great for the R. solani inocula and medium for the P. debaryanum inocula (isolates similar to those used in the present test). Weak virulence was indicated for F. oxysporum, and no conclusion could be drawn for A. tenuis.

On the other hand, there were great and significant differences between different soils, temperatures, and moisture in the present tests. Seedling survival was much higher in sterilized soils than in the unsterilized soils, indicating that light inocula caused less disease alone than when combined with soil flora. Survival was highest when both sterilization and fungicides were applied. These general results are illustrated by the examples of effects of moisture and temperature in Tables 4 and 5. Similarly to caragana (Table 5), pines were adversely affected by high moisture. The influence of temperature on caragana was rather small and interacted with moisture.

Survival in unsterilized soils differed only slightly, as exemplified by these means for caragana: seedbed soil, 35%; seedbed soil + peat, 38%; seedbed soil without weeds, 41%; virgin soil soil, 49%. For pine the order was the same.

3) Heavy inoculum.—Analyses of variance for heavy and light inocula on pine are compared in Table 6. Heavier inocula increased the effects of fungi (including F × S interaction) and cancelled those of other factors. This trend was still more obvious with caragana.

Heavy inoculum of each fungus had its own peculiar influence on seedling survival (Table 7). R. solani induced low survivals under all conditions. With P. debaryanum, survivals were relatively high, yet consistently lower than for light inoculum. P. cactorum and T. basicola were intermediate, but T. basicola allowed high survivals in the sterilized soil.

Survivals were generally lower with heavy inocula than with light inocula, including unsterilized soils; soil flora was relatively less important, and differences between unsterilized and sterilized soils were small, with certain exceptions. With T. basicola, survivals were high in the sterilized soil, and low in both unsterilized soils and controls (sterilized soil + fungicides).

With heavy inocula, differences in seedling survival between unsterilized soils were small and inconsistent. Similarly, differences between the 2 moisture levels were small and inconsistent except that survivals with P. cactorum were significantly lower in the moister soils.

In general, survival of caragana seemed to be favored by high temperatures, and that of pine by low temperature. This is in accordance with the germination speed of these species. One of the several exceptions was pine with Thielaviopsis inocula (no temperature effects). Survival of caragana with Thielaviopsis inocula was 61% at high temperatures, and 22% at low.

4) Reisolation.—Two diseased and two healthy pine seedlings were taken from each of the high-temperature pots each week and treated with 0.5% HgCl₂. A piece of root collar of the border area of healthy and diseased tissues was cut off, rinsed, and plated on agar. Table 8 summarizes the records of fungi isolated.

Fusarium oxysporum and especially F. solani were isolated very frequently from diseased seedlings. Because they were commonly isolated even from healthy seedlings, they did not seem to occur as virulent pathogens. The same is true with bacteria and miscellaneous fungi that were fairly frequent in seedlings from all soils. Although the same Fusarium isolates, according to the pure-culture inoculation test, possessed high potential virulence, they did not cause symptoms, at least in many seedlings that they had parasitized or on which they occurred epiphytically.

In soils with Rhizoctonia inocula, R. solani was frequently isolated from diseased seedlings and rarely from healthy seedlings. Similarly, in soil with Pythium inocula, Pythium spp. were frequently isolated from diseased seedlings and rarely from healthy seedlings. The rare occurrence of Rhizoctonia and Pythium in "healthy" seedlings was assumed to be a result of unavoidable errors in selecting healthy seedlings; a few were selected that had an incipient or arrested symptomless infection.

Some seedlings from unsterilized soils in treatments other than Rhizoctonia and Pythium, particularly with light inocula, nevertheless contained these fungi, especially Pythium. Such naturally occurring Pythium was not isolated from seedlings in sterilized soils. This suggests that in sterilized soils the mortality was caused not by the reinfection flora but by the inocula. The reisolation percentage from diseased seedlings
Table 3.—Interaction of pairs of fungal cultures in various combinations in corn-meal agar dishes.

<table>
<thead>
<tr>
<th>Effect by</th>
<th>1347</th>
<th>1366</th>
<th>1722</th>
<th>2732A</th>
<th>2767</th>
<th>2771</th>
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<tbody>
<tr>
<td>R. solani</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. debaryanum</td>
<td>1347</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. cactorum</td>
<td>+</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. basicola</td>
<td>-</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. solani</td>
<td></td>
<td></td>
<td>+?</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cylindrocarpon sp.</td>
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<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillium</td>
<td></td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterium</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Trichoderma</td>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strepomyces</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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* = growth reduced slightly; -- = growth reduced considerably; --- = growth stopped; + = stimulation; L = lysis; 0 = no effect.

2732A was a dark type of T. basicola known to be especially sensitive to bacterial antagonism (34).

Upper line shows the effect before the colonies contacted each other; lower line shows the effect after contact.

was somewhat higher with heavy than light inocula. *Thieliopsis basicola* was not reisolated.

An attempt was also made to isolate the root pathogens causing the leaf symptoms. In addition to commonly found species of *Fusarium*, only a few isolations of *Rhizoctonia* spp. and *T. basicola* were obtained. In a more elaborate attempt during the previous winter, *T. basicola* was commonly isolated from roots of *Carragana* showing similar symptoms. This fungus is possibly the pathogen, but perhaps it can be isolated only at a certain stage of the disease.

Interpretation of the results.—The lack of effects by fungi, the increased tree survival by soil sterilization, and the reisolation data indicated that with light inocula the mortality was caused mainly by soil flora, especially *Pythium* spp. Therefore, the various environmental effects (Tables 4 and 5), at least in the unsterilized soils, should be attributed chiefly to the responses of *Pythium* spp. No conclusions or generalizations could be made on the responses of the inoculum fungi.

With heavy inocula, Koch's postulates were fulfilled for *Rhizoctonia solani*, *Pythium debaryanum*, and *Phytophthora cactorum*. *P. cactorum* is slow-growing, easily suppressed by other fungi and bacteria (Table 3), and usually very difficult to isolate. Therefore, the relatively low reisolation incidence of this fungus should not invalidate the conclusion about its pathogenicity, based on high mortality of seedlings (Table 7).

Conclusions about the pathogenicity of *T. basicola* are postponed since it showed only moderate potential pathogenicity in test tubes, is not known to be associated with damping-off and was not reisolated in tests in soils. The high mortality with *T. basicola* may have been an artifact due to excessive inoculum and conditions favoring disease. A less likely possibility is that *T. basicola* is an important cause of damping-off but is commonly overlooked because of isolation difficulties.

The potentials of the heavy inocula were probably stronger than is usual in seedbeds (perhaps especially

Table 4.—Survival* of *Pinus sylvestris* in different soils under three temperature conditions with light inocula (results of all fungi and both moistures combined).

<table>
<thead>
<tr>
<th>Soil</th>
<th>Temperature</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsterilized soils (av.)</td>
<td>High 25 Low 14 Variable 31</td>
<td>23</td>
</tr>
<tr>
<td>Sterilized seedbed</td>
<td>37* 61* 51*</td>
<td>50*</td>
</tr>
<tr>
<td>Fungicide control</td>
<td>68* 61 64 65*</td>
<td>Mean</td>
</tr>
<tr>
<td>Mean</td>
<td>29 39** 42</td>
<td></td>
</tr>
</tbody>
</table>

* As % of seed sown.
* Significantly greater than for preceding soil.
** Significantly greater than for high temperature.

Table 5.—Survival* of *Carragana arborescens* in different soils of low and high moisture with light inocula (results of all fungi and temperatures combined).

<table>
<thead>
<tr>
<th>Soil</th>
<th>Moisture</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsterilized soils (av.)</td>
<td>High 23 Low 59 Variable 41</td>
<td></td>
</tr>
<tr>
<td>Sterilized seedbed</td>
<td>72* 80*</td>
<td>76*</td>
</tr>
<tr>
<td>Fungicide control</td>
<td>92* 92*</td>
<td>92*</td>
</tr>
<tr>
<td>Mean</td>
<td>43 68**</td>
<td>55</td>
</tr>
</tbody>
</table>

* As % of seed sown.
* Significantly greater than for preceding soil.
** Significantly greater than for high moisture.
so with *T. basicola*, which produces masses of endo-
conidia in culture). Therefore, the results from tests
with heavy inoculum should be generalized not to
normal seedbed conditions but perhaps to unusually
poor sanitation conditions after heavy epidemics have
developed without application of control measures.

Seeding survival was expected to be higher in un-
sterilized than in sterilized soils as a result of biolog-
ical control by soil flora (Table 3) (43). Soil flora-
s were more virulent than any of the light inocula, how-
ever, and no biological control could be demonstrated.
With heavy inocula, soil flora mostly did not make
much difference except that *T. basicola* was controlled
more effectively by the reinfestation flora than by the
original flora.

Soil floras, original or new, prevented the mortalities
from reaching the same incidences as in the test tubes,
except with the heavy *Rhizoctonia* inoculum. The
sterilized soils were soon colonized by bacteria, *Fusar-
ium* spp., and *Penicillium* spp., which appear antag-
onistic to the pathogens (Table 3).

The fungicide treatment with captan and thiram
gave considerable increase in seeding survival. This
was about as large (av. 14–16%) with light as with
heavy inocula, but at a higher level with light inocula.
Control was greatest on *P. cactorum* (caragana sur-
vivals increased from 11 to 63%); with *Rhizoctonia*,
fungicides increased survivals from 3 to 30%; with *T. basicola*, fungicides decreased survivals, perhaps by
interfering with useful antagonism in soil.

Heavy inoculum may be used to produce enough
mortality for significant differences in fungicide trials.
The conclusions may not apply to normal field condi-
tions, however, except perhaps to very serious epide-
mics such as occurred at one nursery in 1953, when
95% of the conifer crop was lost.

**Discussion.**—**Etiology.**—There is a paradox: to study
what actually happens in epidemics in seedbeds, inocu-
lation experiments should be done in undisturbed, un-
sterilized seedbed soil; but when a naturally infested
soil is inoculated with additional pathogens, the dis-
ease may not increase significantly. In this study, high
mortality was caused only by very heavy inocula, which
of course disturbed the soil. This, together with the
problems discussed in the introduction, suggests that
studies of the etiology of damping-off should be limited
to testing the potential pathogenicity of isolates. To
avoid unpredictable effects of soil floras and aerial
contaminants, the tests should be done in sterilized
media in jars or test tubes. With such standardized
screenings many fungus strains can be rapidly tested
on many host strains. This seems necessary considering
the recent findings of variability in pathogenicity and
susceptibility (Tables 1 and 2) (18, 20, 34, 43). The
etiological method based on Koch's postulates can
hardly go further in damping-off. Applying heavy
inocula will be a useful research tool in screenings of
fungicides that will later be tested in seedbeds.

To learn what actually happens under different
seedbed conditions requires comprehensive epidemi-
ological studies. It becomes necessary to develop new
methods to investigate the natural inoculum in undis-
turbed soils and on the surfaces of the plants. This
is gradually being realized in studies on many root
diseases (12). It is especially true with damping-off,
which may be caused by various fungi.

Results in Table 6 emphasize the importance of
inoculum potential (as discussed by Garrett, 12) and
agree with other recent reports. For instance, *Fusarium*
and *Gliocladium* inocula are more virulent on clover
seedlings if grown in a medium of seeds than if grown
in agar or liquids (27). New isolation methods (5),
especially with various types of bait (3, 19, 22, 28),
may greatly facilitate studies of the natural inoculum
in soils.

**Effects of soils.**—The different soils, other than the
sterilized ones, were associated with slight differences
in disease. With light inocula the differences, although
of the anticipated order between the soils, were not
significant. The naturally present pathogens were fairly
potent even in virgin sod soil. Similarly, both *Pythium*
and *Rhizoctonia* damping-off have been found in a
prairie nursery the first year after beds were cleared
from aspen forest on slightly acid sand (39). On the
other hand, very acid types of forest humus reduce
damping-off (3, 26, 35). Powdered leaves and bark
from various tree species have reduced *R. solani*
damping-off, whereas humus from certain forest stands did
not (40). The antibiotics of the leaves and bark may
exert a protecting effect, but this probably lasts for
a significant duration only under very acid conditions
that reduce decomposition. This would explain the
published (37) and similar unpublished results during
7 years in a nursery where incorporation of a 1/2-in.
layer of slightly acid forest humus in seedbed soil
failed to give adequate control.

Similarly, the protective effect of peat was insuffi-
cient in the greenhouse test; it has also been insuffi-
cient in laboratory (40) and field tests (37, and un-
published results).

This does not mean that utilizing virgin soils or
plant materials, including acid sphagnum peat, should
not be recommended. Indeed, under conditions less
conducive to damping-off, they may exert worthwhile
effects in various ways. These are: a) protection by

---

**Table 6.**—Analyses of variance for survivals of *Pinus*
*sylvestris* with light and heavy inocula.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>D. F.</th>
<th>Light</th>
<th>Heavy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi (F)</td>
<td>3</td>
<td>1.6</td>
<td>43.4**</td>
</tr>
<tr>
<td>Soils (S)</td>
<td>4</td>
<td>67.3**</td>
<td>51.5**</td>
</tr>
<tr>
<td>Moisture (M)</td>
<td>1</td>
<td>43.3**</td>
<td>0.1</td>
</tr>
<tr>
<td>Temperatures (T)</td>
<td>2</td>
<td>18.0**</td>
<td>8.6**</td>
</tr>
<tr>
<td>F X S</td>
<td>12</td>
<td>1.2</td>
<td>5.3**</td>
</tr>
<tr>
<td>F X M</td>
<td>3</td>
<td>2.8*</td>
<td>2.0*</td>
</tr>
<tr>
<td>F X T</td>
<td>6</td>
<td>1.1</td>
<td>1.4*</td>
</tr>
<tr>
<td>S X M</td>
<td>4</td>
<td>4.5**</td>
<td>2.6*</td>
</tr>
<tr>
<td>S X T</td>
<td>8</td>
<td>4.3**</td>
<td>1.3</td>
</tr>
<tr>
<td>M X T</td>
<td>2</td>
<td>5.6**</td>
<td>0.6</td>
</tr>
<tr>
<td>Triple intera. + residual</td>
<td>74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M.S.: 119

P levels: *.05; **.01
Table 7.—Survival of Pinus sylvestris and Caragana arborescens in different soils with heavy inocula of four fungi (results of different temperatures and moistures combined).

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>R. solani</th>
<th>P. cactorum</th>
<th>T. basicola</th>
<th>P. debaryanum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pinus sylvestris</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsterilized soils (av.)</td>
<td>2</td>
<td>13</td>
<td>7</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Sterilized seedbed soil</td>
<td>6</td>
<td>34*</td>
<td>47*</td>
<td>22</td>
<td>27*</td>
</tr>
<tr>
<td>Fungicide control</td>
<td>34*</td>
<td>49*</td>
<td>43</td>
<td>45*</td>
<td>43*</td>
</tr>
<tr>
<td>Mean</td>
<td>9</td>
<td>25**</td>
<td>22</td>
<td>29**</td>
<td></td>
</tr>
<tr>
<td>2. Caragana arborescens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsterilized soils (av.)</td>
<td>0.3</td>
<td>13</td>
<td>33</td>
<td>63</td>
<td>36</td>
</tr>
<tr>
<td>Sterilized seedbed soil</td>
<td>0</td>
<td>11</td>
<td>72*</td>
<td>72</td>
<td>52</td>
</tr>
<tr>
<td>Fungicide control</td>
<td>25</td>
<td>63*</td>
<td>59</td>
<td>82</td>
<td>68*</td>
</tr>
<tr>
<td>Mean</td>
<td>4.4</td>
<td>21**</td>
<td>43**</td>
<td>68**</td>
<td></td>
</tr>
</tbody>
</table>

* As % of seed sown.
* Significantly greater than for preceding soil.
** Significantly greater than for preceding fungus.

Table 8.—Isolations a from diseased (D) and healthy (H) conditions (both types of inocula combined).

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Soil groups</th>
<th>R. solani D</th>
<th>Pythium spp D</th>
<th>P. cactorum D</th>
<th>Fusaria D</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. solani</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsterilized</td>
<td>++</td>
<td>(--)</td>
<td>++</td>
<td>(--)</td>
<td>++*</td>
</tr>
<tr>
<td>Sterilized</td>
<td>+++</td>
<td>(++)</td>
<td>++</td>
<td>(--)</td>
<td>++*</td>
</tr>
<tr>
<td>Pythium debaryanum</td>
<td>Unsterilized</td>
<td>-</td>
<td>+</td>
<td>(--)</td>
<td>+</td>
</tr>
<tr>
<td>Sterilized</td>
<td>-</td>
<td>(--)</td>
<td>+</td>
<td>(--)</td>
<td>+</td>
</tr>
<tr>
<td>Phytophthora cactorum</td>
<td>Unsterilized</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterilized</td>
<td>-</td>
<td>(--)</td>
<td>+</td>
<td>(--)</td>
<td>+</td>
</tr>
<tr>
<td>Thielaviopsis basicola</td>
<td>Unsterilized</td>
<td>+</td>
<td>+</td>
<td>(++)</td>
<td>+</td>
</tr>
<tr>
<td>Sterilized</td>
<td>+</td>
<td>(--)</td>
<td>+</td>
<td>(++)</td>
<td>+</td>
</tr>
</tbody>
</table>

Effects of temperature.—The effects of temperature varied greatly and interacted with other factors. The effect was greatest with heavy T. basicola inoculum on caragana; survivals at lower temperatures were 1/3 of those at higher ones. The cool humid greenhouse conditions may favor this fungus in some way. Martin and Ervin (24) reported great increases of T. basicola in pots with citrus seedlings in the greenhouse. This caused foliar symptoms on citrus leaves (23), resembling those reported here on caragana.

Antagonistic flora.—According to Boosalis and Scharen (5), 100 g of soil may contain 6800 debris particles infested with living R. solani 7 months after sugar beet crops. With such inoculum and with 100% disease potential demonstrated in test tubes (Tables 1 and 2), all seedlings should be killed by R. solani in seedbeds. This obviously is not the case, and damping-off incidence varies from time to time and from spot to spot in a bed. Factors regulating this variation are perhaps found in antagonistic flora (Table 3) (11, 12, 34, 36, 43) and in the peculiar lytic (21) and fungistatic effects (16) commonly found in undisturbed soils. These effects, in turn, must be influenced by weather factors, but probably in a complex, perhaps unpredictable, way. This situation may also apply to damping-off caused by such common isolates as Pythium spp. and, in certain nurseries, P. seedlings of Pinus sylvestris from the high temperature

Isolations a from diseased (D) and healthy (H) conditions (both types of inocula combined).

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<th>R. solani D</th>
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<th>P. cactorum D</th>
<th>Fusaria D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizoctonia solani</td>
<td>Unsterilized</td>
<td>++</td>
<td>(--)</td>
<td>++</td>
<td>(--)</td>
</tr>
<tr>
<td>Sterilized</td>
<td>+++</td>
<td>(++)</td>
<td>++</td>
<td>(--)</td>
<td>++*</td>
</tr>
<tr>
<td>Pythium debaryanum</td>
<td>Unsterilized</td>
<td>-</td>
<td>+</td>
<td>(--)</td>
<td>+</td>
</tr>
<tr>
<td>Sterilized</td>
<td>-</td>
<td>(--)</td>
<td>+</td>
<td>(--)</td>
<td>+</td>
</tr>
<tr>
<td>Phytophthora cactorum</td>
<td>Unsterilized</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterilized</td>
<td>-</td>
<td>(--)</td>
<td>+</td>
<td>(--)</td>
<td>+</td>
</tr>
<tr>
<td>Thielaviopsis basicola</td>
<td>Unsterilized</td>
<td>+</td>
<td>+</td>
<td>(++)</td>
<td>+</td>
</tr>
<tr>
<td>Sterilized</td>
<td>+</td>
<td>(--)</td>
<td>+</td>
<td>(++)</td>
<td>+</td>
</tr>
</tbody>
</table>

a Isolation incidence: -, 5%; +, 5-10%; ++, 10-30%; +++, 30%. Reisolations are boxed.

b Mostly F. solani.
cactorum. Furthermore, the importance of many other fungi that are less commonly isolated, but potentially pathogenic (Tables 1 and 2), may be governed by these biological factors. Thus, Arndt (2) found that a large number of common fungi are potentially capable of killing cotton seedlings at a wide temperature range, but, obviously, most of them are usually inhibited from doing so in unsterile soil.—Forest Biology Section, Canada Agriculture Research Station, Saskatoon, Saskatchewan, Canada, and Forest Nursery Station, Indian Head, Saskatchewan, Canada.

LITERATURE CITED


