STUDIES ON DAMPING-OFF OF RED PINE

Thesis for the Degree of M. S.

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This is to certify that the

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STUDIES ON DAMPING-OFF

OF

RED PINE

By

ROBERT WILLIAM BRANDT

An Abstract

Submitted to the School of Graduate Studies of Michigan

State College of Agriculture and Applied Science

in partial fulfillment of the requirements

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Department of Botany and Plant Pathology

1955

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THESIS

An Abstract

This investigation of damping-off was mainly concerned with the penetration and subsequent breakdown of pine tissues by various causal agents.

Damped-off seedlings were collected from inoculated soils containing pure cultures of *Pythium*, *Fusarium*, *Rhizoctonia*, and a mixture of these three organisms. Stem lesions were serially sectioned and prepared slides observed for fungal development and possible anatomical barriers produced by the host. The growing of suitable plant material for histological sectioning presented an opportunity to study fungicidal control of damping-off by the organisms involved. Four chemicals; Manzate, Tersan, Fermate, and Orthocide-406 were used as dusts.

Orthocide-406 gave the best control on all the pathogens, and on a mixture of these pathogens. Tersan and Manzate gave fair control on all the infestations, but seedling survival was far below that produced with the use of the Orthocide.

Fusarium caused the worst pre-emergence and post-emergence damping-off under all treatments. The mixture of the three organisms gave no noticeable increase or decrease in the total damping-off over individual infestations.

It was shown that individuals of the three genera involved break down the host tissues in different ways. *Pythium*-infected stems had weakly stained tissues in the area of the hyphae, suggesting a chemical change produced

by the fungal invasion. Although showing extensive development within the host, Rhizoctonia infection did not seem to change the over-all structural appearance of diseased stems. Penetration by Rhizoctonia consistently involved the production of micro-sclerotia. The most destructive of the pathogens was Fusarium. This organism produced large expanses of laterally compressed, heavily stained tissues. More recently infected stems showed large areas dissolved out by some chemical extrusion produced by this fungus.

Complexes of organisms were rarely observed, and then only the two organisms, Rhizoctonia and Fusarium were found together in a single stem. A mixture seemed to be no more damaging to the structure of the seedlings than were the individual infestations.

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I. INTRODUCTION

Damping-off, the killing of very young seedlings by fungi, is still one of the most serious difficulties encountered in raising coniferous seedlings. Hartley (11) stated that the damping-off organisms are "specialized as to type and age of tissues which they attack rather than as to host". These experiments were concerned with the kinds of organisms attacking a specific host, and studies of the penetration and subsequent breakdown of tissues. An attempt to correlate the age of tissues with certain organisms was made.

A single host species, the red pine, Pinus resinosa Ait., a native American species of pine important on the light sandy soils of the upper Great Lakes region, was chosen. This tree species is assuming increasing importance as a timber tree in the reforestation plans of both state and federal forest agencies of the region. Mr. E.D. Clifford, Superintendent, Chittenden Forest Nursery at Wellston, Michigan, in a letter to the author, listed red pine as the most important tree for reforestation in Michigan. It is able to grow on sites which in the past were considered only jack pine sites. Again citing Mr. Clifford's letter, the planting plan of the Manistee National Forest (as an example) calls for approximately 95% red pine, and the rest white pine and jack pine. In 1954, Chittenden Nursery quoted the price of red pine seed at fifteen dollars a pound, and in the past recorded prices as high as thirty dollars a pound when the cone crop was poor.

Considering that Pinus resinosa seldom shows a germination of more than sixty percent under nursery conditions and that this species is known to be very susceptible to damping-off organisms (11), the losses may become substantial.

In view of the continued heavy losses, and the lack of damping-off control in the nursery, a series of experiments was designed to study possible anatomical barriers produced by coniferous seedlings in resisting infection by various damping-off organisms. Little information on the histological aspects of the problem is available. Diseased material was collected and sectioned to permit studies of the development of various pathogens in the host, and of subsequent breakdown of stem tissue. The same material permitted a study of the fungal complexities involved in the damping-off problem. Seed treatments were also run to determine their effects on the organisms under test.

II. REVIEW OF THE LITERATURE

Numerous early European plant pathologists and foresters emphasized the hazards of damping-off and its causal organisms (8). In the United States, Atkinson (1) was the first to make damping-off studies at Cornell. Spaulding (24) and Jones (17) independently published on control measures in 1908, with the former author contributing an account of the etiology of the disease and a method of control using sulfuric acid. Hartley (9) in 1910 began inoculations with both *Rhizoctonia* and *Pythium* to study their effect against conifers.

A little later, Gifford in Vermont (5), reported his studies in which he stated that *Fusarium* alone was the cause of damping-off. Shortly thereafter Hartley concluded his preliminary inoculations of conifers and found species of *Pythium*, *Rhizoctonia*, and *Fusarium* to be the only consistent pathogens (13). Spaulding (25) working with forty-four strains and species of *Fusarium* in pure culture, found them all capable of causing damping-off and well over half of these were very aggressive parasites. Graves (6) inoculated healthy seedlings with *Cylindrocladium scoparium*, but obtained no signs of pathogenicity. Hartley and Bruner's studies (12), on the causal agents and their relationships showed that *Rhizoctonia* was capable of attacking seedlings too mature to be killed by either *Pythium debaryanum* or *Fusarium moniliforme*.

Hartley (11) wrote an excellent review of damping-off. He discussed the economics of the problem, the value of disease as a selective agent versus the unpredictable losses, which in turn cause a lack of production stability. After this evaluation, many articles on control measures followed over a period of a few years. Most of these (7,14,16,19,27, 28,29) were published records of damping-off counts in various sections of the country, and the results of using variously applied chemical compounds.

Rathbun-Gravatt (18) demonstrated the ability of numerous strains of Pythium, Rhizoctonia, and Fusarium to reduce sprouting of coniferous seed. She concluded that the fungi reducing sprouting were the ones that caused decay of radicles. Eliason (4), testing the pathogenicity of Pythium, found that of twenty-two species and strains, all but three were able to cause damping-off in seedlings. Roth and Riker (21,22,23) succeeded in distinguishing between the field symptoms of damping-off exhibited by Pythium and Rhizoctonia, and made careful studies on the temperature and moisture relations of the two causative agents as they affected red pine. Their main conclusions were that factors favoring one fungus discouraged the other. Specifically Rhizoctonia, was encouraged by dry soil and warm weather, while Pythium was favored by more moist soil and cool weather. They excluded Fusarium from their studies as preliminary tests showed it to be negligible in the Plainfield soils with which they were working.

Tint (26) working with *Fusarium* found that he obtained greater pre-emergence losses with this organism than either *Pythium* or *Rhizoctonia*, and the post-emergence damping-off was highly comparable to these latter fungi. In temperature studies with *Fusarium* damping-off of red pine, the fungus damage was found to be directly related to temperature. Cox (3) investigated a disease situation in a Delaware State Forest nursery and found a complex of *Cylindrocladium scoparium*, *Rhizoctonia solani* and a species of *Pythium* causing severe losses in red and white pine beds.

III. CURRENT INVESTIGATIONS

Materials and Methods

One year old red pine seed was obtained from the Dunbar Forest Nursery of Michigan State College at Sault Saint Marie, Michigan. Dissected seeds proved one hundred percent viable and further tests demonstrated ninty-eight percent germination in sterilized soil.

Damped-off seedlings from Professor F.C. Strong's fungicidal test plots were collected and isolates of the damping-off organisms were obtained. The diseased stems were washed in tap water and then surface sterilized by placing them in a commercial sodium hypochlorate solution for three and one-half minutes. The stems were placed on potato dextrose agar, and incubated at room temperature. Pythiaceous cultures were purified by subsequent transfers to sterile water plates containing hemp seeds. Transfers were then made from the outer edges of all young, actively growing colonies to new plates which were incubated and stored under refrigeration.

To test the pathogenicity of the various isolates, three inch clay pots of nursery soil were sterilized for one and one-half hours on two successive days. All soil used throughout this investigation was collected from the Bogue Forest Nursery at Michigan State College. At the end of the second sterilization period, the sterile pots of soil were placed immediately in sterile petri-halves filled with sterile water. After the pots of soil were cool, an agar plug containing the fungus under test was placed in the approximate center of the potted soil. This was covered slightly with sterile soil, and fifteen red pine seeds were placed on this surface, and covered with one-quarter inch of sterile soil. The moisture content was kept up, to permit growth of the fungi, by frequent additions of sterile distilled water applied from below. Uninoculated checks were planted and treated the same as inoculated pots. After pathogenicity tests were completed, the non-pathogenic cultures were discarded.

A survey of the pathogenic cultures indicated that _Pythium_ _debaryanum_, _Rhizoctonia_ _solani_ (_Corticium_ _vagum_), and species of _Fusarium_ were commonly isolated and most frequently proved pathogenic. Further experiments were limited to these three fungi. Inoculum was maintained on media consisting of corn meal and vermiculite instead of sand (20). The base material was steamed for one hour, 250 cc. aliquots were placed in liter erlenmeyer flasks, and autoclaved for one and one-half hours. After cooling, three one-half inch squares from a single pathogenic isolate were aseptically introduced into the medium. The prepared flasks were incubated at room temperature for a period of approximately five days.

The _Rhizoctonia_, _Pythium_, and _Fusarium_ inocula were mixed with thoroughly steamed soil at the rate of 100 cc.

to 15,000 cc. of soil. For mixed cultures about thirty-three cc. of each of the three fungal inocula, were mixed together in a flask and then mixed as above in a flat of soil. Chemically treated and untreated red pine seeds were planted at a depth of one-quarter inch in the various inoculated soils as well as in uninoculated checks and naturally infested field soil. As emergence began, careful notes were taken on the rate of emergence, density of stand, and occurrence of damping-off.

Damping-off checks were made at daily intervals, and diseased material was collected for histological studies. Seedlings which were damped-off, and those which were chemically injured were differentiated according to the discriptions of Hartley (11), and Roth and Riker (21). A few healthy stems were collected to serve as checks. Counts were continued for a month, after such time the seedlings were resistant to the damping-off disease.

The stems that were collected were immediately and carefully washed in flowing tap water, and killed and fixed in FAA (15). After a minimum of forty-eight hours in the above solution, dioxane (1,4-dioxan) was used at successive one-third, two-thirds, and full strength as the dehydration medium. The dioxane was replaced with absolute tertiary butyl alcohol in a series of changes over a two-day period, the last change using tertiary butyl containing eosin stain. Infiltration was started by using a 56° to 58° melting point parowax in a volume equal to that of the last tertiary butyl solution, and placing the open vials in the embedding oven for twelve hours. The solution was replaced with two complete changes of fresh parowax at three hour intervals, with the last such change being replaced by an equal melting point tissue mat. This solution was left for two hours after which the blocks were cast.

The embedded stems were then mounted on wooden blocks and serial sections were cut at twelve microns. The sections were mounted on three inch glass microscope slides with Haupt's adhesive (15) and numbered consecutively for each stem.

A modification of Conant's quadruple stain (15) was used for all staining. This stain contains thionin or Lauth's violet (C.I. No. 920) in place of the crystal violet in the Conant stain (2). The cover slips were cemented to the slides with HSR synthetic resin.

Experimental Data

Greenhouse studies. The fungal isolates showed differences in pathogenicity. Table I lists the isolates and results of the pathogenicity tests conducted with these isolates against

Table I

Fungus isolates and pathogenicity

Fungus line	Identity of Fungus	Soil treatment at time of isolation	Pathogen-icity
100	Rhizoctonia solani	TMTD	+
101	" "	Crag-658	+
102	" "	Manzate	+
103	" "	Manzate	+
104	" "	Manzate	+
105	" "	Ortho-75	+
106	" "	Crag-658	+
107	Fusarium species	Sulfur 150	+
108	" "	5400	-
109	" "	Crag-658	+
110	Pythium sp.	Ortho-75	+
111	Pythium debaryanum	Ceresan M	+
112	Unidentified ascomycete	Check	-
113	Pythium sp.	5400	-
114	Pythium debaryanum	Ortho-406	+
115	Cephalosporium sp.	Crag-658	+
116	Fusarium sp.	TMTD	-
117	Fusarium sp.	Tersan	+
118	Curvularia sp.	TMTD	-
119	Penicillium notatum	Manzate	-
120	Mucor sp.	Check	-
121	Rhizoctonia solani	Sulfur 150	+
122	Nigrospora sp.	Ceresan M	-
123	Unidentified fungus	Sulfur 300	-
124	Penicillium sp.	5400	-
125	Fusarium sp.	Tersan	-
126	Pythium sp.	Tersan	-

red pine. Only Rhizoctonia, Fusarium, and Pythium were pathogenic. Of the twenty-seven isolates nineteen were within these three generic groupings. All eight *Rhizoctonia* isolates, three *Fusarium* isolates, and four isolates of *Pythium* proved highly pathogenic on red pine. These fifteen isolates proved to be pathogenic when soil infested with cultured inocula was planted with red pine seed.

The growing of seedling materials for histological sectioning, provided an excellant opportunity to test a few seed treatment fungicides in soils infested with known pathogenic organisms. The fungicides used, their chemical compositions and their manufacturer are given in Table II. All

Table II

Name and chemical composition of fungicides used in treatment of seeds of *Pinus Resinosa*

Name	Chemical Composition	Manufacturer
Tersan	Tetramethylthiuram disulfide (50%)	E.I. duPont Co.
Fermate	Ferricdimethyldithiocarbamate	E.I. duPont Co.
Manzate	Manganeseethylenebisdithio-carbamate	E.I. duPont Co.
Orthocide-406	N- trichloromethylthiotetra-hydrophthalimide (50%)	California Spray Chemical Co.

compounds were used as dust, and excess dust was removed from the seed lots by screening. The results of these chemical tests are given in the following paragraphs. All results were computed at the end of four weeks following the first

Figure I

Comparison of survival of red pine six weeks after planting date. Similar seed dust treatments were used against each soil infestation.

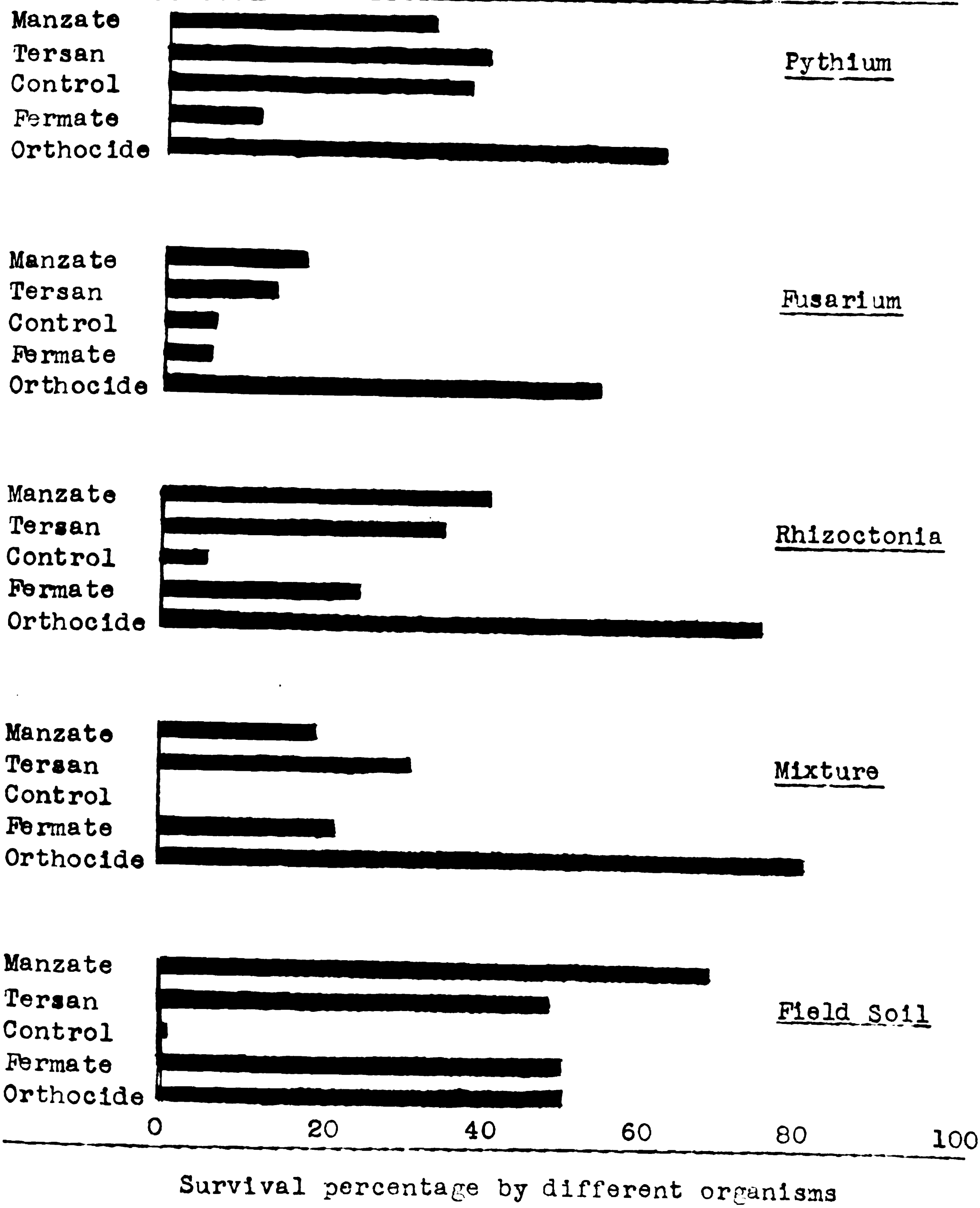
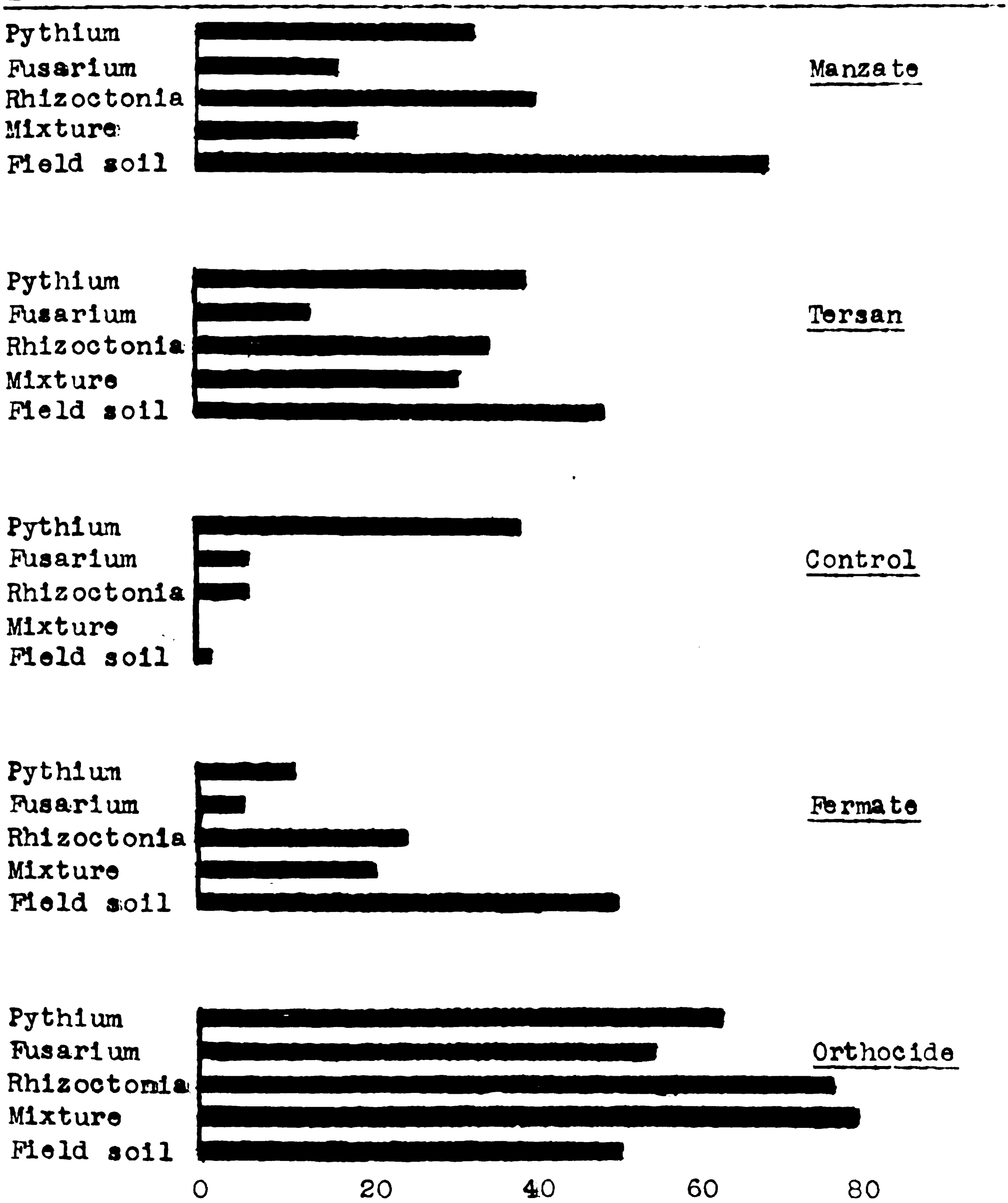


Figure II

Comparison of survival of red pine six weeks after planting date. Variation of survival in different soil infestations plotted for each seed dust treatment.



Survival percentage by chemical treatments.

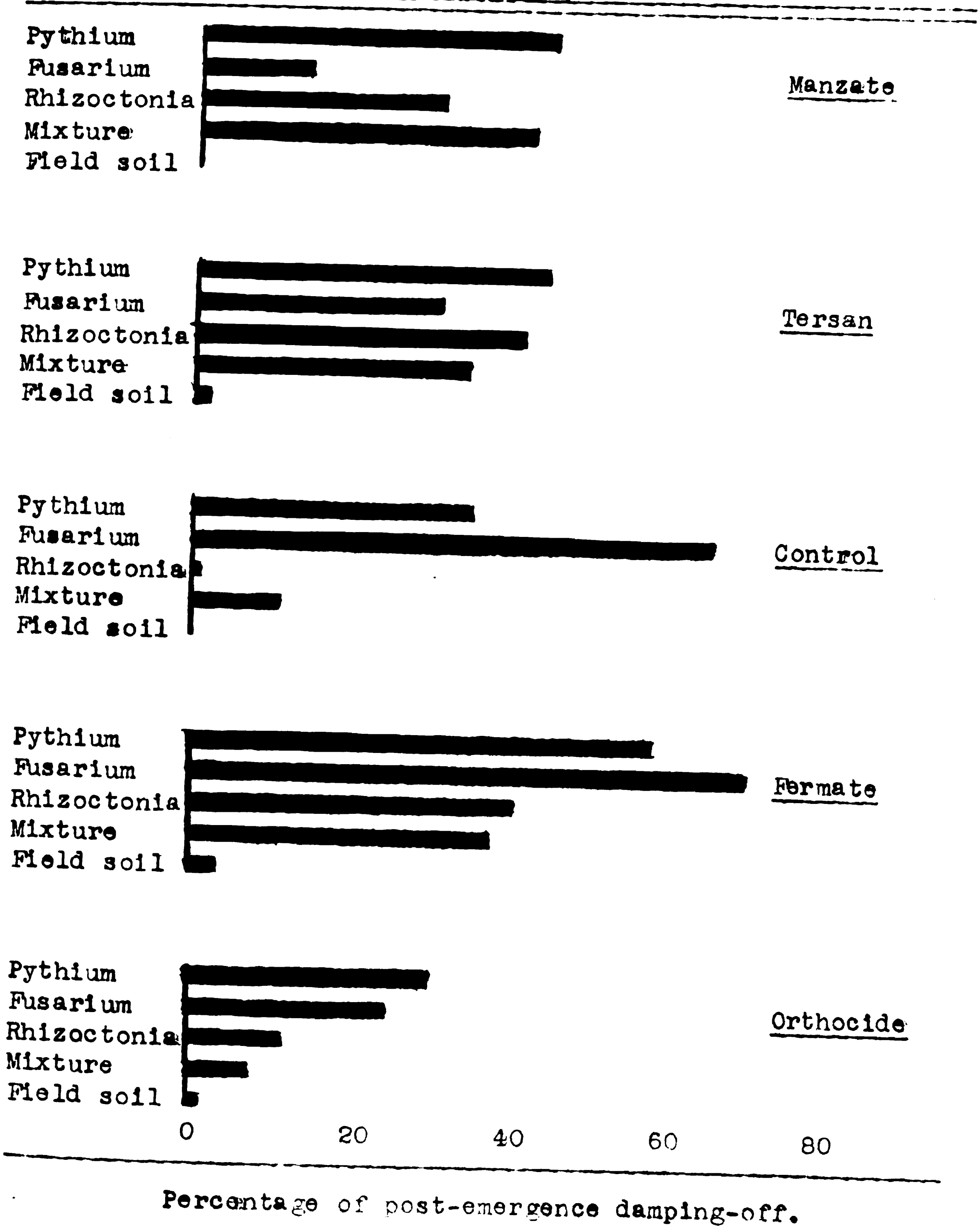
seed emergence and six weeks after the planting date. In a comparison of seedling survival, the percentages of stand at the termination of the greenhouse studies were plotted for the different fungicides and soil treatments (Figures I and II).

After six weeks, seeds treated with Orthocide - 406 showed the best survival rate averaging seventy percent against all five soil treatments. Fermate gave poorest results of the chemicals used but the untreated seeds suffered the worst damping-off, with an average survival of ten percent against the different organisms. All of the seed treatments afforded good damping-off protection in the field soil. Of the untreated seed in the field soil only one percent of the plants remained at the termination of the experiment. Fusarium infested soil yielded the least survivors. Only Orthocide gave moderate results with the other seed treatments never carrying over seventeen percent of the planted seed past the first six weeks.

The untreated checks sown in flats containing the different fungal infestations, failed to show a survival of six percent except in Pythium treated soils, which allowed a thirty-nine percent survival (Figure II). Survival of Manzate and Tersan treated seeds was spotty, both chemicals being fairly ineffective against Fusarium and the mixture of organisms. Fermate was also very ineffective and gave fair results only in the field soil where the potential inoculum

Figure III

Post-emergence damping-off in different soil infestations. Plotted for each seed dust treatment.



was very low in comparison to the manually infested flats and the soil seem to remain more moist.

Figure III shows the actual post-emergence damping-off in each treatment. Since pre-emergence damping-off was also important in this experiment, the survival graphs fail to show the cause of mortality. Seeds treated with Manzate for example showed a seedling survival of only seventeen percent against *Fusarium* and yet the post-emergence damping-off as shown by Figure III only amounted to fourteen percent, leaving a total of sixty-nine percent of the expected emergence unaccounted for. Pre-emergence damping-off was one of the responsible agents.

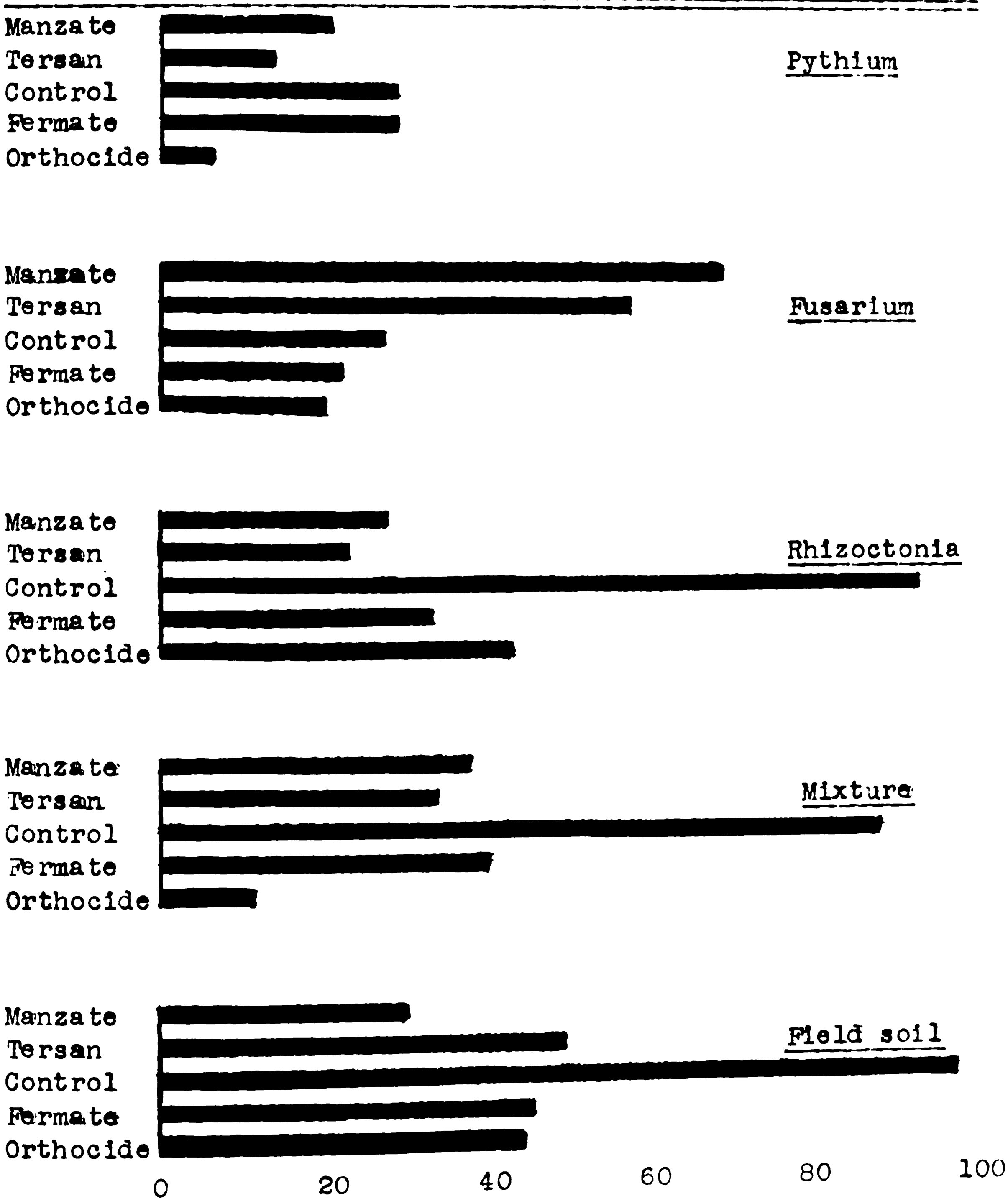
Post-emergence damping-off was most severe in Fermate-treated seedlings, with *Fusarium* and *Pythium* causing the greatest loss. Late damping-off was consistently higher in soil infested with *Pythium* (Figure IV). The two greatest losses in *Fusarium* infested soil were in seed dusted with Fermate and seed receiving no treatment. Total losses in the *Fusarium* infested soil were slightly lower than those caused by *Pythium.* *Rhizoctonia* and the mixture of infested soils produced losses up to twenty-five percent total crop. Field soil produced a negligible amount of post-emergence damping-off. However, pre-emergence damping-off was severe in some cases.

The consistent failure of any of the fungicides tested to prevent pre-emergence damping-off was noted (Table VII).

However, the extremely heavy losses in the untreated controls (Plates 1 and 2) shows the advisability of continuing chemical treatments for a partial check of losses. Pre-emergence damping-off losses were fairly uniform through-out the various soil infestations and fungicidal treatments. Only Pythium failed to reduce emergence drastically, and yet produced an average damping-off of twenty percent against all fungicides. Orthocide maintained a good stand of red pine through the first six weeks, but orthocide treated seed suffered an average pre-emergence loss of twenty-five percent against the different damping-off situations used.

Figure V

Comparison of the different seed dust treatments on pre- emergence damping-off of red pine planted in variously infested soils.



Graph of pre-emergence damping-off

Plate I

(Seed dust treatments reading downward).

Pythium Control

Upper left	Lower left
Manzate	Orthocide-406
Manzate	Orthocide-406
Check	Check
Fermate	Tersan
Fermate	Tersan

Fusarium Control

Upper right	Lower right
Orthocide-406	Tersan
Orthocide-406	Tersan
Check	Check
Manzate	Fermate
Manzate	Fermate

Plate I

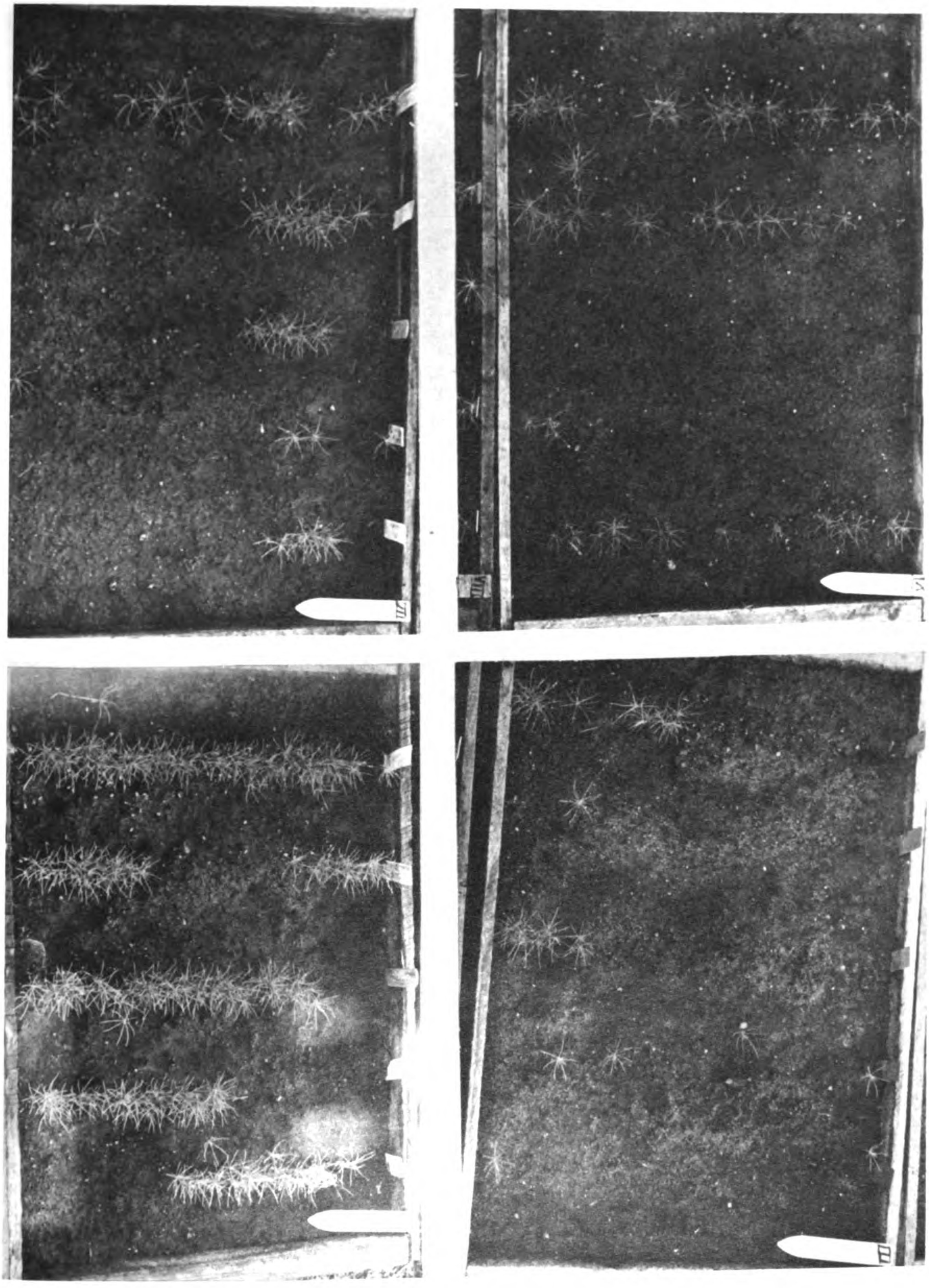


Plate II

(Seed dust treatments reading downward).

Rhizoctonia Control

Upper right

Tersan

Tersan

Check

Fermate

Fermate

Lower right

Manzate

Manzate

Check

Orthocide-406

Orthocide-406

Mixture of organisms

Upper left

Manzate

Tersan

Check

Fermate

Orthocide-406

Naturally infested soil

Lower left

Manzate

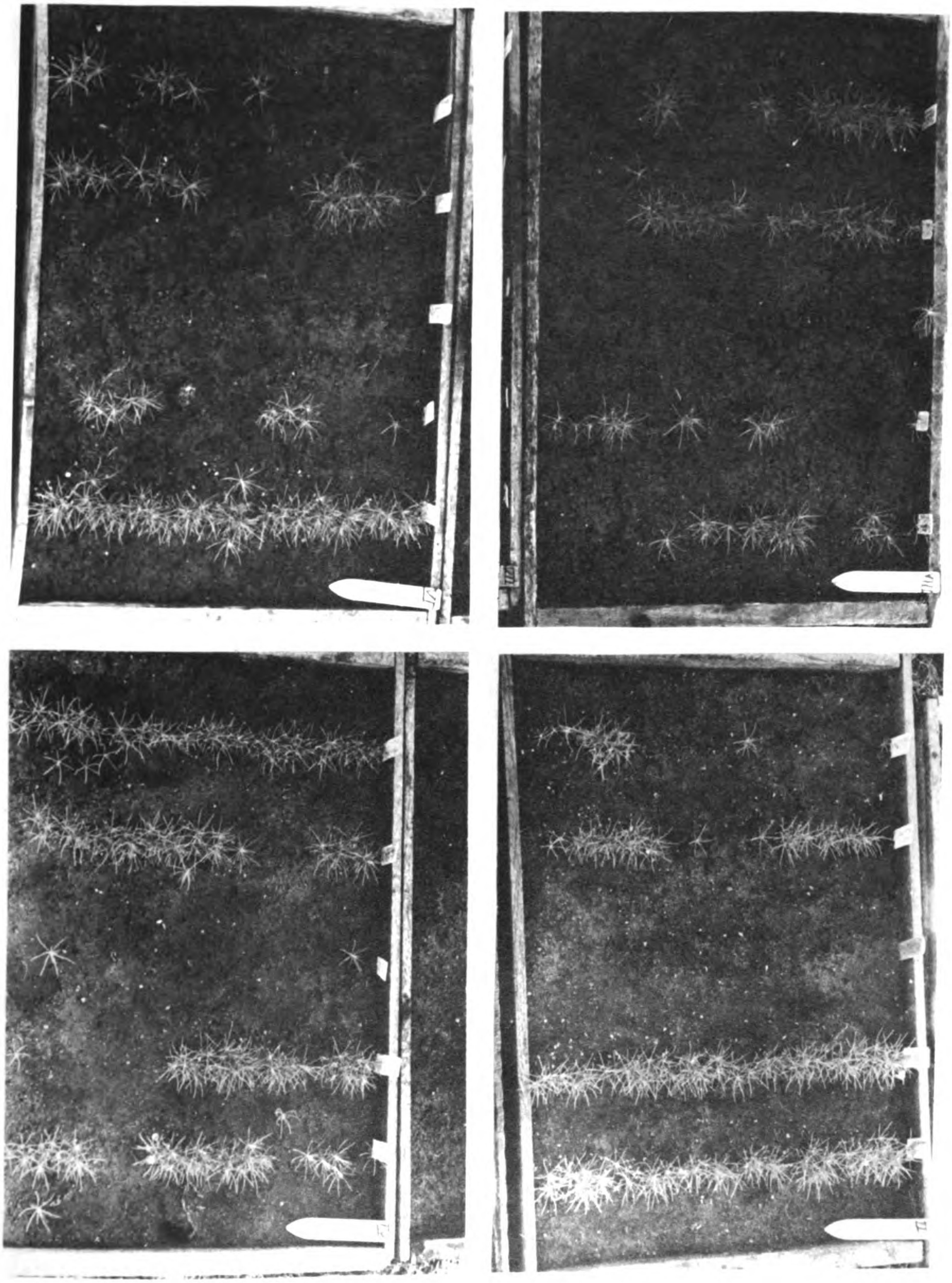
Tersan

Check

Orthocide-406

Fermate

Plate II



Histological studies. *Pythium* attacks the host plant just below ground line and grows upward through the stem. Initial penetration of the host tissues was not observed and the collected samples were always outwardly clean, with no hyphal attachments as was seen in *Rhizoctonia*. One instance was recorded in which a hypha was seen lying within a stomatal orifice and over the adjacent guard cell. It could not be determined whether this hyphal strand was growing out of, or into the stem, since the stem tissue beneath was already largely filled with mycelium.

Once inside the stem, the fungus was inter and intracellular and moved both vertically and horizontally through the host. Penetration of cell walls occurred at any angle and place the hypha made contact. There was evidence of a pressure build-up, as the host walls occassionally became indented before the tissue was broken through by a small penetration peg. The host cell wall often became quite sharply indented over this peg, but usually nearly resumed its normal position after the puncture had been accomplished. Considerable swelling occurred in the hyphal tips before a wall was punctured, and immediately after the peg had passed through the wall, the hypha regained its normal size, leaving a definite constriction at the wall (Plate III, Lower right). Some hyphae passed through the cell from wall to wall. Series of four and five parallel walls were penetrated by a single hyphal strand with little change of direction,

Plate III

Histological figures

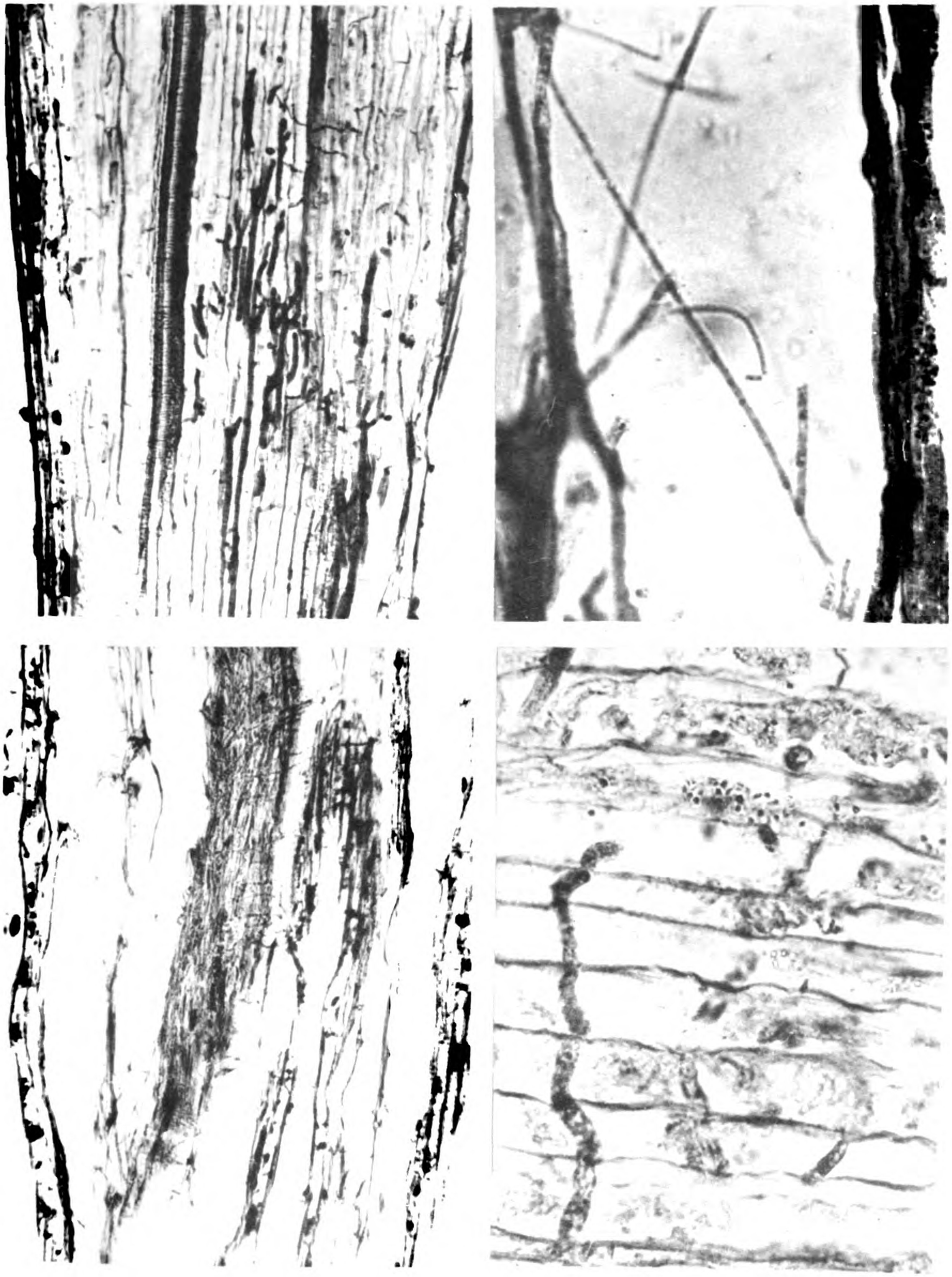
Lower right - Hyphal constrictions of *Pythium*. The hypha penetrated a series of cell walls with little change of direction. The faded nuclei in the region of activity is in direct contrast to the staining intensity of nuclei in *Rhizoctonia* infected stems.

Lower left - Photomicrograph of a red pine stem moderately infested with *Fusarium*. The central portion shows numerous hyphal strands and a heavy stain through-out. The remainder of the stem exhibits the large cavities typical of tissue breakdown by *Fusarium*.

Upper right - An enlarged view of one of the dissolved areas in the stem above. The dark staining protoplasm and white cross-walls of the hyphae are evident.

Upper left - Hyphal strands of *Fusarium* and *Rhizoctonia* in a diseased stem. The complexities of multiple infection existed only rarely. Here, *Fusarium* (left and upper right) and *Rhizoctonia* (center) are shown in the same stem.

Plate III



and without apparent loss of size or vigor. If the angle of approach is even slightly acute the wall of the host is penetrated, but the hypha then grows intercellularly until a wall is again hit directly.

Strong evidence of a host-parasite relationship within the invaded cells was shown by the disappearance of nuclei and chloroplasts in the areas of mycelial advancement, while normal check plants, and areas of infested plants that were not penetrated, showed brilliantly stained nuclei and easily observable chloroplasts. This condition was in direct contrast to Rhizoctonia infection, where such structures were always readily seen. The fungus was much branched and ramified the invaded area in all directions. The deposition of lignins and other hardening materials restricted the movement of the fungus however, for no hyphal strand was found to penetrate sclerotized tissue. Other than the natural hardening off that comes with age, there was no observable evidence of any incompatible reactions between host and pathogen. There was no collapse of host tissue in the attempt to seal or wall-off the penetrations. The hyphal tips were very dense and vigorous in appearance, and no dead or dying hyphae were present, which might indicate toxic substances being liberated by the deteriorating host cells.

Damping-off by Fusarium was the most damaging as far as actual structural disintegration was concerned. The fungus caused the formation of large cavities within the host stem, or in some instances resulted in collapsing and shredding of the tissues (Plate III, upper right and lower left). The hyphae themselves appeared to be less numerous through-out the host, but the actual numbers and spread were partially occluded by the intensity of the stain in the infected areas. The staining schedule times were varied considerably in an attempt to differentiate more clearly between the host tissue and fungal growth but the Conant's Quadruple stain used through-out the investigation continued to stain both host and pathogen a deep red. The chemical exudates from Fusarium apparently changed the nature of the host tissue so that normal staining reactions could not take place. Rarely, in isolated areas and on the fringes of decay areas, the hyphal strands were a light red or even pink, allowing for a more exacting study of the fungus and it's effect on the host tissues. The hyphae were approximately one-third to one-quarter of the size of the Rhizoctonia hyphae (Plate III, Upper left). Although the protoplasm of the fungus stained deep red, there were a few pale and quite vacuolate hyphal strands that showed unstained cross-walls. In such cases the nuclei of the fungus stained darker and were readily observed.

The deterioration of the cell walls and over-all break-down of structure of the tissue made it impossible to evaluate the fungal alignment in the host cells, association with vascular elements, or penetration of the relatively mature cells. The bulk of the stems collected for sectioning were completely disintegrated by the time the diseased seedling was recognized as infected. The material suitable for histological sectioning was limited. Only four of the one hundred and thirty-three slides made of Fusarium infected seedlings exhibited suitable detail for photomicrographing.

The pine stems collected from the Rhizoctonia infested soil were often covered with the hyphae of the fungus. These hyphae were quite discernible with the naked eye when the young seedlings were first removed from the soil. The base of the above ground portion of stems of such plants was usually white or yellowish-white, and so consistent in this characteristic that many of the damped-off seedlings from the flat of mixed inocula, and some from the field soil were marked as Rhizoctonia. Later, the slides prepared from these collections were observed and with one exception were found to contain Rhizoctonia only. The exception was from the check row of the flat of mixed inocula and this stem showed both Fusarium and Rhizoctonia (Plate III, Lower right).

Histologically, the hyphal strands along the stem surfaces were seen to consist of loose and appressed stringy hyphae and small knots of closely compacted, septate hyphae having the appearance of micro-sclerotia. These sclerotial structures were very abundant in the slide preparations and apparently play a large roll in mycelial penetration of the pine host. No special organs of attachment, such as appressoria or cementing materials were observed. Sizeable

Plate IV

Histological figures

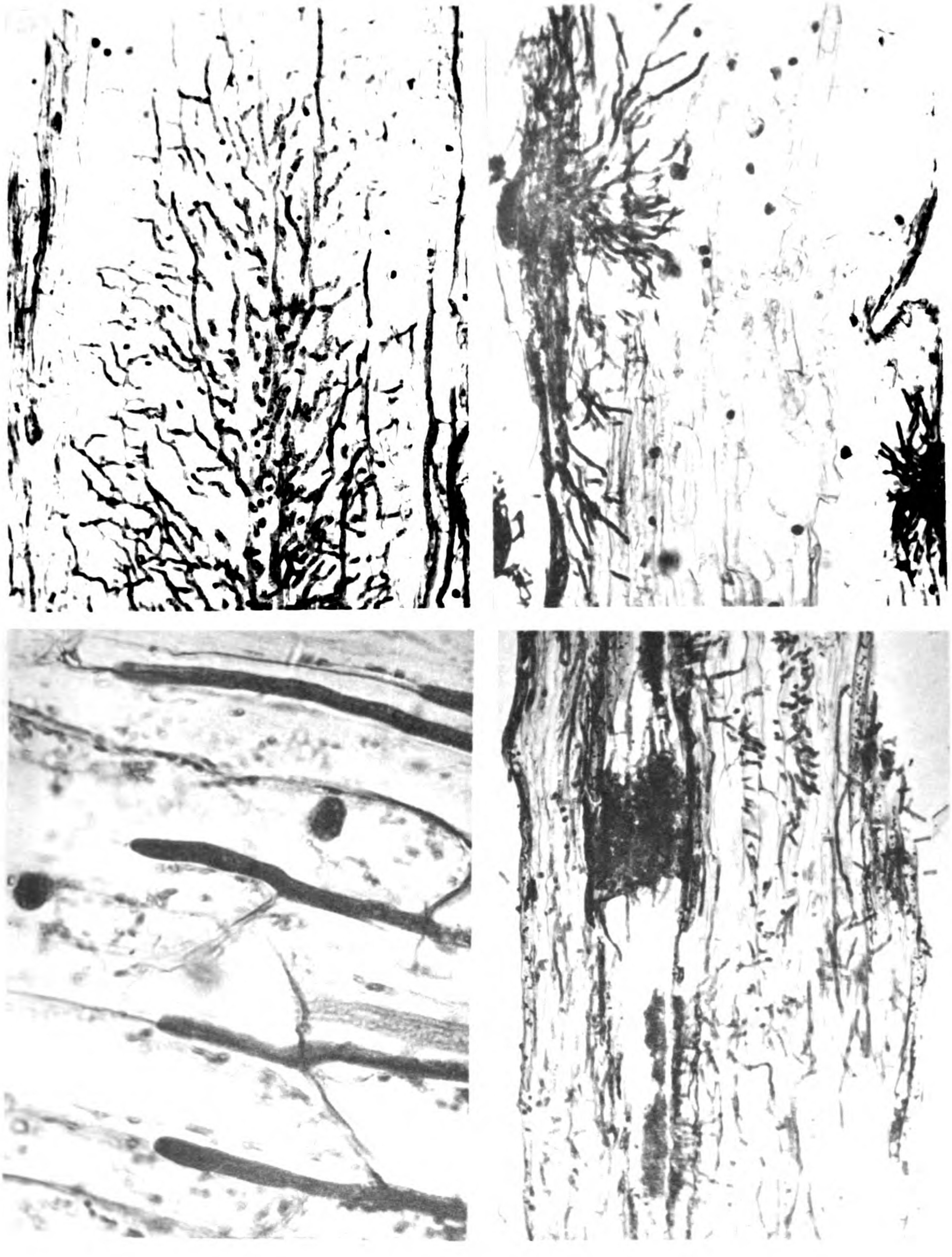
Upper left - Ramification of Rhizoctonia hyphae. The christmas tree growth effect was very common.

Lower left - The intercellular spread of the Rhizoctonia hyphae. The picture is oriented at right angles to the normal. The longest hyphal extensions were usually aligned with the long axis of the host. Note the intensity of stain in the nucleus.

Upper right - Sclerotial attack in Rhizoctonia solani. A stem showing penetration at opposite surfaces.

Lower right - A second example of sclerotial build-up, this time in the interior of the infected stem.

Plate IV



penetration forces must exist for mass penetrations as shown (Plate IV, Right upper and lower). The number of sclerotial bodies attacking a single stem was very variable. Some areas of the surface were densely packed with six or seven small sclerotia, while other surfaces had none, one, or several widely scattered. Occasionally a stem was invaded from both sides (Plate IV, Upper right). Some areas around penetration points stained densely.

After the fungus was established within the host, it appeared to penetrate the host cell walls mechanically. There were no signs of abnormal staining reactions or dissolution of host parts that might indicate chemical changes except breakdown of chlorophyll in the stem. A fading of the chloroplasts to a pale yellow-green occurred in certain stems, which accounted for the chlorosis of the stem at ground line.

The Rhizoctonia hyphae were usually the same size but variations occurred which were explained by age differences. Penetration appeared mechanical, and constrictions at the wall punctures were observed in some instances but not in others. The fungus ramified through the host with no apparent inhibitions. The spread was accomplished horizontally and vertically through the host cells with equal readiness. A christmas-tree-like growth occurred in some stems (Plate IV, Upper left) but in others great vertical extensions of dense hyphae traveled intercellularly (Plate IV, Lower left). The growth of the fungus through the stem as portrayed above occurred, as in Pythium, only through succulent tissues. Any cells showing secondary thickening in spiralar deposition or accumulation were not penetrated. Certain parenchymatous cells contained a hypha which curled inside the cell until a corner of the cell was reached, where penetration occurred. Often the pressure of the penetration hypha would displace or indent the host cell wall, but again as in Pythium, the wall distension would decrease considerably but not entirely after penetration was affected.

The fungus branched less in host tissue than in sclerotia or cultures. Septation was not as evident, but the distinguishing characteristic of Rhizoctonia, the slightly bulbous base of the new branch, closely followed by a septum, was always observed. Differential staining of fungal strands was apparently due to differences in protoplasmic densities.

In Rhizoctonia infection, as well as with the other two organisms used, the lack of any antagonistic reactions on the part of the host was noted. There were no hyphal fragments or unstained portions of mycelia (representing dead or senescent fungal tissues) which might indicate an incompatible host-parasite relationship. No evidences of walling off or cell collapse to stop further penetrations were observed.

As explained in the materials and methods section, inocula of the three organisms, Pythium, Fusarium, and Rhizoctonia were mixed in equal portions to yield a total inoculum equal to that induced into the other experimental flats.

Treated seeds were planted and the damping-off results were recorded in the above greenhouse studies. Observation of the slides made from the damped-off seedlings from this flat showed that the bulk of the damping-off was caused by *Rhizoctonia* alone or *Rhizoctonia* and *Fusarium* together. In the latter case infection seemed to take place at quite widely divergent locations and the two fungi to grow toward each other through the host stem. Although they approached very closely, there was no general intermingling between the hyphal strands of the two. Destruction of the stem tissue occurred in each area of infection as it did in stems having but a single infection of a specified fungus. *Pythium* was not observed in a single processed stem, out of a total of sixty lesioned stems collected and sectioned from the mixed inoculum tests.

Discussion and Conclusions

Orthocide-406 dust gave the best control of damping-off when based on survival, seedling vigor and lack of injury to seedlings. The data showed a survival of fifty-one percent in the field soil and a survival of eightly percent of seedlings in the soil with the mixed inoculum. Post-emergence damping-off usually occurred immediately after emergence when seedlings were most succulent. Good plants were produced without chemical injury when Orthocide dust was used. Orthocide gave the best protection of seeds and seedlings against the pathogenic organisms used both in pure cultures and mixtures.

Tersan and Manzate gave about the same amount of control against the test organisms. Both chemicals produced fair survival in *Pythium* and *Rhizoctonia* inoculated soils. *Fusarium* caused very severe losses both as pre-emergence and post-emergence damping-off in Tersan and Manzate treatments. The apparent percentage of damping-off in Manzate treatments may be partially due to chemical injury.

Fermate treatments gave poor control of damping-off with a final survival of fifty-one percent against the naturally infested field soil, but a high survival of twenty-five percent in soils infested with all of the pathogens. On seed dusted with this chemical, *Fusarium* caused seventy-two percent post-emergence losses, and *Pythium* caused sixty percent loss, based on expected field emergence.

The untreated checks showed variations in damping-off. *Fusarium* caused a post-emergence loss of sixty-seven percent. One reason for the large losses was the relatively heavy inoculum used in proportion to the amount of soil to be infested. These consistently heavy losses in all the fungicide tests, indicated that the amount of inoculum used was much greater than that normally found in infested nursery soil. However, such heavily inoculated soils resulted in severe tests which showed differences in the effectiveness of the chemicals tested.

The effects of environment on these pathogens was reported by Tint (26), and Roth and Riker (21,22,23). Under different soil and environmental conditions, both organisms and chemical compounds might have reacted quite differently.

Pythium and *Rhizoctonia* caused little or no structural differences in infected stems in the earlier stages of the disease. *Pythium* infested stems failed to show brilliantly stained nuclei in the areas of heavy penetration, the visible nuclei being pink-gray in color and highly granular. In the lesions caused by *Pythium* the number of chloroplasts had decreased, and nuclei failed to stain normally. This staining reaction, and the water soaked appearance of the lesions demonstrate physiologic changes.

Stems containing *Rhizoctonia* showed typical red-staining nuclei even when these structures were in close proximity to the hyphal strands. A chemical breakdown of the chloroplasts was apparent in the majority of the slides. In the areas of advanced hyphal penetrations the chloroplasts were a pale yellow-green. When seen in the field the overall effect is one of chlorosis.

A characteristic of the Rhizoctonia infection was the production of micro-sclerotia. These minute knots of hyphae appear to be a common mode of establishment of the pathogen in the host, and were observed singly and in large numbers on various diseased stems. An occasional sclerotium was observed with the pine tissue.

Fusarium caused extreme breakdown of host tissues. Heavily stained disintegrated masses of tissue were found in slides prepared from stems showing only incipient decay upon collection. All the various tissues were attacked by the fungus. In the few places where the attacking pathogen was observed, the host was largely dissolved away, leaving the Fusarium hyphae free in the large open cavities. The cell walls were the last structures to disintegrate, but this resistance might be caused by their mass rather than any certain chemical resistance.

A complex of organisms was observed in stems grown in soil infested with a mixture of Pythium, Fusarium, and Rhizoctonia. This complex, however, was not as involved as some seen earlier in sections of naturally infected Austrian pine, prepared by the author for classroom use. The damped-off seedlings above showed unknown hyphae of two definite or

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organisms inter-twining and ramifying through-out the host cells. In the few cases, where more than one organism was observed in sections of diseased red pine stems, the mixture was always one of Rhizoctonia and Fusarium. They exhibited no interplay of hyphae strands. Breakdown of tissue containing such a fungal mixture was accomplished by individual action of organisms and not by collective action.

Summary

A summarization is made, with full realization that the results are dependent on the inherent variables in such an experiment.

1. Orthocide-406 gave the best control against all pathogens, and against a mixture of these pathogens.

2. *Fusarium* caused the worst pre-emergence and post-emergence damping-off against all treatments.

3. A mixture of the test organisms did not increase or decrease the total damping-off as compared to individual infestations.

4. Individuals of the three genera used in this experiment break down plant tissue in different ways.

5. In soil infested with a mixed 1:1:1 inoculum, *Rhizoctonia* was most frequently found in diseased plants grown under environmental conditions presiding at the time the experiment was preformed.

6. Only rarely were *Rhizoctonia* and *Fusarium* observed together in the same diseased stem. None of the other possible combinations of organisms were observed.

7. It is highly desirable that further histological studies be made with different host species and with various other known damping-off organisms.

Bibliography

1. Atkinson, G. F.
1894. Damping-off. New York (Cornell) Agr. Expt. Sta. Bul. 94: 301-346, illus.

2. Conn, H. J.
1946. Biological Stains. Geneva, New York.

3. Cox, R. S.
1954. Cylindrocladium scoparium on conifer seedlings. Agr. Expt. Sta. U. of Delaware Tech. Bul. 301.

4. Eliason, E. J.
1928. Comparative virulence of certain strains of Pythium in direct inoculation of conifers. Phytopath 18: 361-367.

5. Gifford, C. M.
1911. The damping-off of coniferous seedlings. Utah Agr. Expt. Sta. Bul. 157: 143-171, 10 figs., 4 pl. Bibliography p. 171.

6. Graves, A. H.
1915. Root rot of coniferous seedlings. Phytopathology 5: 213-217.

7. Hansen, T. S.
1923. A study of the damping-off disease of coniferous seedlings. Minn. Univ. Agr. Expt. Sta. Tech. Bul. 15: 1-35.

8. Hartig, R.
1894. Textbook of the diseases of trees. Translation by W. Somerville. Rev. and ed. by H. M. Ward. 331p., illus. London.

9. Hartley, C.
1910. Notes on some diseases of coniferous nursery stock. Abstracted in Science, n.s., v. 31, no. 799, p. 639.

10. Hartley, C.
1915. Injury by disinfectants to seeds and roots in sandy soils. U.S.D.A. Bul. 169: 1-35.

11. Hartley, C.
1921. Damping-off in forest nurseries. U.S.D.A. Bul. 934: 1-99.

12. Hartley, C., and Bruner, S. C.
1915. Notes on Rhizoctonia. Phytopathology 5: 73-74.

13. Hartley. C., and Merrill, T. C.
1914. Preliminary tests of disinfectants in controlling damping-off in various nursery soils. Phytopathology 4: 89-92.

14. Hartley, C., and Pierce, R. G.
1927. The control of damping-off of coniferous seedlings. U.S.D.A. Bul. 453: 1-32.

15. Johansen,
1940. Plant Microtechnique. McGraw-Hill Book Co. Inc. New York and London.

16. Johansen, L. P. V., and Linton, G. M.
1942. Experiments on chemical control of damping-off in Pinus resinosa Ait. Canad. Jour. Res. Sect. C., Bot. Sci. 20: 559-569.

17. Jones, L. R.
1908. The damping-off of coniferous seedlings. Vt. Agr. Exp. Sta. Ann. Rpt. (1906-1907) 20: 342-347.

18. Rathbun-Gravatt, Annie E.
1931. Germination loss of coniferous seeds due to parasites. Jour. Agr. Res. 42: 71-92.

19. Riker, A. J., Gruenhagen, R. H. and Roth, L. F.
1943. Control of damping-off of nursery pine seedlings. Abs. in Phytopathology 33-11.

20. Riker, A. J., and Riker, Regina, S.
1936. Introduction to research on plant diseases. Planographed by John S. Swift Co., Inc. New York.

21. Roth, L. F., and Riker, A. J.
1943 (a). Life history and distribution of Pythium and Rhizoctonia in relation to damping-off of red pine seedlings. Jour. Agr. Res. 67: 129-148, illus.

22. Roth, L. F., and Riker, A. J.
1943 (b). Influence of temperature, moisture, and soil reaction of the damping-off of red pine seedlings by Pythium and Rhizoctonia. J. Ag. Res., 67: 273-293.

23. Roth, L. F., and Riker, A. J.
1943 (c). Seasonal development in the nursery of damping-off of red pine seedlings caused by Pythium and Rhizoctonia. Jour. Agr. Res. 67: 417-431.

24. Spaulding, P.
1908. The treatment of damping-off in coniferous seedlings. U.S.D.A. Bur. Plant Indus. Cir. 4: 1-4.

25.

26.

25. Spaulding, P.
1914. The damping-off of coniferous seedlings. Phytopathology 4: 73-88.

26. Tint, H.
1945. Studies in the *Fusarium* damping-off of conifers. I. The comparative virulence of certain Fusaria. II. Relation of age of host, pH and some nutritional factors to the pathogenicity of Fusarium. III. Relation of temp. and sunlight to pathogenicity of *Fusarium*. Phytopathology 35: 421-439, 440-457, 498-510.

27. Toumey, J. W., and Li, T. T.
1924. Nursery investigations with special reference to damping-off. Yale Univ., School Forestry Bul. 10, 1-36.

28. Wiant, J. S.
1929. The *Rhizoctonia* damping-off of conifers, and its control by chemical treatment of the soil. N.Y. (Cornell) Agr. Expt. Sta. Mem. 124: 1-64.

29. Young, G. Y., Davis, W. C., and Latham, D. H.
1937. Control of damping-off of conifers (abstract) Phytopathology 27: 144.