THE FUNGI ASSOCIATED WITH BLIGHT OF ALEPPO PINE

PINUS HALEPENSIS MILLER

by

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A Thesis Submitted to the Faculty of the
DEPARTMENT OF PLANT PATHOLOGY
In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE
In the Graduate College
THE UNIVERSITY OF ARIZONA

1966
STATEMENT BY AUTHOR

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ACKNOWLEDGMENTS

The author is deeply indebted to Dr. A. M. Boyle for her inspiration and guidance during the research connected with this work. Special credit is given to Dr. G. A. Gries for his help with the spray program and to all of the members of the Department of Plant Pathology who have assisted in various ways with this problem.

The author wishes to acknowledge the tremendous support given by his wife, Eileen Rush, because without her help this work would not have been undertaken.
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Fungi, cultured from blighted Aleppo pines, were selected on the basis of consistency of isolation or previous report of pathogenicity to Pinus spp. and screened against P. halepensis for pathogenicity. None of the isolated fungi appeared to be primary parasites but representatives of several genera survived for 2 months or longer after inoculation into pine trees. The fungi associated with P. halepensis were apparently saprophytes or weakly parasitic in nature attacking tissues weakened by some other agent.

The effect of physiological drought on pine trees was investigated and blight symptoms similar to those on naturally blighted trees were produced by addition of 1.5 M sucrose or 2 M CaCl₂ solutions to the soil in which the trees were growing.

An investigation of the roots of blighted pines indicated the occurrence of unnatural root systems possibly affected by poor soil and moisture conditions, lack of mycorrhizial development, and the presence of fungi pathogenic to pine tissues.

The cause of Aleppo pine blight did not appear to be parasitic fungi attacking the upper portions of the
trees, but seemed related to physiological troubles stemming from some condition affecting the roots.
INTRODUCTION

The Aleppo pine (Pinus halepensis Miller) is an important landscaping conifer in the Southwestern United States which is widely used in commercial and home landscaping in Tucson and Phoenix, Arizona. As the tree is evergreen it is an excellent ornamental tree for motels, school grounds, parks, cemeteries, and other public areas. This pine, indigenous to the Mediterranean region, is adapted to the hot, dry desert climate of the lower elevations of Arizona. It is native to Spain, Portugal, France, Italy, Greece, Asia Minor, Cyprus, and Algeria; and has been introduced into South Africa as well as the United States (29).

The Aleppo pine is a medium to large tree with a maximum height of approximately 80 feet; usually conic, rectangular, or ovoid in outline with irregular branching. The bark of young trees or branches is silvery-gray shading to reddish-brown and smooth while the bark of mature trunks and limbs is scaly or vertically fissured. Young shoots and juvenile needles are glaucous gray and young trees have a gray-green color. Juvenile needles are single, 3/4-1 1/2 inches long, often subtending fascicles of mature needles. Mature needles are borne in pairs on fascicles with a 1/4-1/3 inch, persistent sheath. The needles are 2-5 1/2 inches long,
curved, slender, medium to dark green, with margins minutely toothed and the apex a short acute, horny point. Resin canals are marginal to median. Buds are conic, \( \frac{1}{3} \) to several inches long with fringed scales reflexed at the tips. The tree is monoecious with single, opposite or whorled cones; the cones are ovate-conic, reddish-brown to gray, 2-4\( \frac{1}{2} \) inches long, symmetrical, with reddish-brown ovuliferous scales about 1 inch long and 5/8 inch wide (29). The cones are persistent and seed from Arizona cones does not seem to be viable.

Aleppo pines have few insect or disease problems other than a needle and twig "blight" of unknown cause. The symptoms of Aleppo pine blight are expressed as needle blighting, twig and branch death, and branch cankers. Needle blighting ranges from a general bronzing or chlorosis of the foliage of an entire tree (Fig. 1) to sudden death of all the needles on a twig or branch (Fig. 2). The needles of the entire top of a severely diseased tree may be suddenly blighted or the needles of large branches may be killed (Fig. 3). Blighted needles initially are straw-colored or yellowish-green, the color changing in a few weeks to a bright reddish-brown. The dead needles are persistent on the trees for several months, to a year or longer.

Although the normal condition is a tip-blight of branches, needle death also occurs slowly from the base of
Fig. 1. Aleppo pine tree showing a general bronzing and chlorosis of the needles.
Fig. 2. Blighted branches showing typical "flagging" symptoms.
Fig. 3. Blighted tree showing dead top.
branches to the apex. Under these conditions the tips of the needles usually die and turn yellow-brown before the entire needle is killed. Some blighting may be observed throughout the year. Sudden needle blighting appears to be related to low soil moisture and drying winds or other dry conditions. The slower needle blighting, from the branch base toward the tip, occurs throughout the year.

Twigs or smaller branches may be killed when their needles are blighted or larger branches may die after repeated blighting of newly-formed needles. Usually top blighting of larger branches does not kill the terminal buds or the branch. The buds will initiate new growth under favorable conditions, often masking the original blight symptoms. Occasionally the needles of an entire tree will suddenly die (Fig. 4), but the tree will appear to completely recover when new growth is initiated.

Branch and twig cankers were observed on affected trees. The cankers begin as water soaked spots or elongated areas along a branch (Fig. 5). The spots turn a dark reddish-brown and the bark splits at right angles to the long axis of the cankers (Fig. 6 and 7). The dead bark pieces scale off and the underlying sapwood splits to the heartwood parallel to the length of the canker. The heartwood is exposed and becomes dry (Fig. 8). Callus tissue begins forming at the edges of the injured sapwood and eventually covers the exposed heartwood (Fig. 9 and 10).
Fig. 4. Aleppo pine tree with all of its needles blighted.

Fig. 5. Initiating branch canker.
Fig. 6. Branch canker showing drying and splitting of the bark.

Fig. 7. Cankers developing along a branch.
Fig. 8. Branch showing open canker exposing the heartwood.

Fig. 9. Branch cankers showing the beginning of callus formation.
Fig. 10. Completely callused over cankers.
The cankers range from 1/4 inch to 3 feet long and may completely callus over or continue extending toward the branch apex. Cankers 1/8 inch-3 inches in diameter were observed on branches. Often small twigs are girdled by cankers and the entire twig dies resulting in typical blight symptoms of the needles (Fig. 11 and 12).

The above symptoms are typical of blight expression. Another symptom that may be connected with the disease is that some trees retain their juvenile needle form and produced few or no mature needles even though they are 20-30 feet tall.

As the causal agent of this disease is not known, much confusion exists concerning control measures. Some commercial tree services and nurserymen have been recommending the use of several fungicide sprays for the control of symptom expression.

The purpose of this investigation was to study the fungi associated with blighted Aleppo pines and the role played by these fungi in disease expression. The areas covered include:

1. Culturing, identification of, and inoculations with fungi associated with diseased trees
2. Histopathology of tissues from diseased trees to determine the extent of fungus invasion or morphological changes due to fungus infection
Fig. 11. Severely cankered branch showing girdled twig with blighted needles.

Fig. 12. Twig girdled by a small canker and showing blighted needles.
3. Evaluation of two fungicides for control of blight symptoms.
A. The host: Aleppo pine, *Pinus halepensis* Miller

The Aleppo pine is indigenous to the Mediterranean region including the countries of Spain, France, Italy, Yugoslavia, Greece, Albania, Bulgaria, Turkey, Israel, Cyprus, Crete, and Sicily (29, 89, 42, 71, 91, 97). At one time it was an important forest species, covering hundreds of square miles of limestone formations, hilly and rocky regions, and desert or semi-arid regions (42, 97). Through centuries of misuse these natural forest stands have been nearly destroyed. Efforts are now being made to reforest large areas, not suited for crop production due to unsuitable soils or climatic conditions, with the Aleppo pine (89, 42, 71).

Because of its xerophytic nature *P. halepensis* is being used in arid regions of the world, where it is not native, in afforestation projects. The pine is being used in Australia, Africa, Argentina, and India to control soil erosion or to provide a source of low-grade lumber in areas where there are no natural forests (37, 49, 63, 82).

A taxonomic description is provided by Dallimore and Jackson (29) and a discussion of the ecological behavior and growth of Aleppo pine is described by Gindel (42).
The Aleppo pine appears to grow well in areas with light, porous soils, and annual rainfall of 15-25 inches, moderate temperatures, and high light intensities (42).

*P. halepensis* has been successfully introduced into the Southwestern part of the United States where it is used as an ornamental. In the Tucson and Phoenix, Arizona area, where the annual rainfall is 10-15 inches, rainfall is supplemented by irrigation.

B. The disease: Aleppo pine blight

Blight of *P. halepensis*, as described in the introduction, has not been reported in the literature. A similar disease in which the trees showed yellowing of the needles, twisting and curling of the shoots, and discoloration of the xylem in the branches, trunks, and roots was reported by Wahl and Reichert (97). They attributed the symptoms to abnormal ecological conditions reflected in defective mycorrhizal formation. The unfavorable ecological conditions mentioned were lack of litter and humus in the area around the trees and soil affected adversely by fires.

Fusion of young needles on Aleppo pine, a condition commonly observed in Arizona, was reported by Neilson-Jones (69). The symptoms were ascribed to a sudden shortage of water in the leaves at the time they are beginning to expand. The water deficiency was attributed to reduced root absorption due to failure of the trees to provide
functional feeder roots and a normal mycorrhizal association. The fused-needle symptoms expressed by Aleppo pines in Arizona, however, do not seem to be associated with pine blight as the fused needles do not necessarily become blighted and blighted needles were not necessarily fused before becoming blighted.

C. Fungi attacking *Pinus halepensis*

Fungi reported as pathogenic on *P. halepensis* are few. *Diplodia pinea* (Desm.) Kickx has been reported as attacking *P. halepensis* in Italy, Portugal, and Argentina (21, 22, 38, 85). *D. megalospora* Berk & Curt possibly synonymous with *D. pinea* was reported on *P. halepensis* in Brazil (100). These fungi cause a needle and twig blight with the new growth on trees being blighted for several years in succession until the trees die. *D. pinea* also infects through wounds in branches and twigs causing death of tissues around the wound and finally death of the branch or twig. The fungus has also been cultured from the collars and roots of infected trees. According to Capretti (21, 22) the disease caused by *D. pinea* in Italy is not serious unless the trees are growing in unfavorable conditions, but Saravi (85) showed that the fungus could directly attack buds, shoots, and leaves of *P. halepensis* and can attack twigs and branches through wounds.
Sphaeropsis elisii Sacc. was recorded by Oliveira (72) as a parasite of Aleppo pine causing a die-back and needle blight. It was also recorded from needles and twigs of *P. halepensis* in South Africa (100).

It appears that there is some confusion as to the identity of a fungus attacking *P. halepensis* and recorded as *Sphaeropsis elisii* Sacc. or *S. pinicola* Speg. According to Waterman (100) these names are synonymous with *Diplodia pinea* (Desm.) Kickx.

A fungus listed as *Thyriopsis halepensis* was recorded by Biraghi (10) as causing a die-back and blight of *P. halepensis* in a congested stand in Italy. The symptoms included blighting of needles, severe defoliation of mature needles, and persistent, blighted young needles. A branch die-back resulted from the severe blighting of needles. Dead mature needles were ringed with small black perithecia.

Rusts have been reported on *P. halepensis* several times. A blister rust of Aleppo pine was reported in Italy by Moriondo (65) who listed *Cronartium asclepiadeum* as the causal organism. *Coleosporium inulae* was also recorded in Italy on *P. halepensis* as causing severe needle death in a stand of young pines (43).

In the South of France *P. halepensis* was attacked by *Trametes pini* (34). Trees of all ages were affected and fructifications were formed on tree trunks.
In Argentina *Fusarium vasinfectum* was isolated from the roots of *P. halepensis* and was found to be virulent towards pine seedlings (61).

D. Fungi causing blight symptoms or cankers on *Pinus* spp.

Several genera of fungi in the Sphaeropsidales, Melanconiales, and Ascomycetes are known to cause blights, diebacks, or needle casts on various members of the genus, *Pinus*. *Diplodia pinea* causes a severe blight and dieback of the current seasons growth on *P. nigra*, *P. sylvestris*, *P. mugo* var. *mughus* in the United States and *P. radiata* and *P. muricata* in Australia and New Zealand (78, 100). The symptoms of the disease caused by *D. pinea* include dieback of the current seasons growth for several years until the tree becomes stunted or killed, formation of cankers which spread from needle scars and completely girdle twigs, the death of young trees, and the presence of dark fruiting bodies on dead needles, fascicles, and twigs (100). Waterman's experiments with the fungus showed that it would attack *P. nigra*, *P. sylvestris*, and *P. mugo* var. *mughus* when inoculated into stems and buds, or when spores were sprayed onto the plants (100). Waterman reviews the literature concerning diseases of pines caused by *Diplodia* sp. and concludes that *Sphaeropsis ellisii* is synonymus with *D. pinea*. Previous to 1943 Waterman and Miller had described a twig and needle blight on the current seasons
growth of *P. nigra* and *P. strobus* caused by *Sphaeropsis ellisii* (99). *Diplodia pinea* causes a dieback or "blight" on many *Pinus* spp. It is believed to attack pine trees weakened by drought or other conditions adverse to growth. This belief was supported by a New Zealand Forest Service report (70). A dieback of *Pinus* spp. in Queensland was reported to be caused by *Diplodia pinea* and *D. natalensis* (107). *Diplodia pinea* was reported to attack pines in Italy, France, Belgium, Argentina, South Africa and Australia causing a "red top" or bud wilt (11). In South Africa *D. pinea* causes a serious dieback on *P. radiata* when soil moisture is low or very high (58). Haddow and Newman (46) reported a twig and branch dieback, with the development of cankers, on *P. sylvestris*. The fungus appeared to be associated with injury by the pine spittle bug. A root and collar disease of *P. resinosa* seedlings caused by *Sphaeropsis ellisii* was reported by Crandall (26) and in France a dieback of *P. sabiniana* was reported to be caused by *S. pinastri*. Another possible synonym of *D. pinea*, *Botrydiplodia pinea*, was reported by Curtis (28) to cause a dieback of *P. radiata* and *P. muricata*.

Species of *Phoma* have been reported to cause or be associated with diseased pines (51, 78). In England *Phoma acicola* was widely recorded on Scots and Corsican pines which were dying back due to unsuitable climatic conditions. *P. acicola* was also reported in Britain associated with a
dieback of *P. nigra calabrica* caused by *Crumenula pinea* and *Phoma* spp. were reported as widespread on pine in the United States (51, 78).

A dieback and canker disease of Douglas fir caused by *Phomopsis* spp. may spread to pine trees causing similar symptoms (13, 106).

*Crumenula pinea*, the imperfect stage of which is *Brunchorstia pinea*, attacks several *pinus* spp. causing the loss of older needles, a progressive dieback of shoots and twigs, and the formation of small cankers on the trunk (78). *C. pinea* attacks *P. nigra calabrica*, *P. cembra*, *P. montana*, and *P. pinaster* in Britain and *P. pinaster* in Spain (78). Boyce (12) observed a needle and twig blight of *P. monticola* Dougl., *P. cembra*, and *P. montana* in Britain caused by *Brunchorstia destruens*. Several instances of dieback or cankers on *Pinus* spp. in Norway, Spain, Europe, and the United States have been reported (53, 54, 60, 62, 73).

A needle blight, caused by *Dothistroma pini* on Austrian and Ponderosa pines occurs in Oklahoma. Chlorotic spots appear on infected needles during the fall and winter, the spots spread, and the needles are girdled causing the distal ends to die while the base remained green. In the spring black stromata are visible erupting through the needle epidermis (92).
Cucurbitaria pithyophila, Cylindrocladium scoparium, Monochaetia pinicola, and Pestalozzia funerea are all reported to cause diebacks or foliage blights on Pinus spp. (15, 16, 24, 51, 78). The fungi above are either the causal agent or closely associated with the blights.

Leptographium spp. have been found associated with blight of western white pine, P. monticola Dougl., causing lesions on and death of small roots, usually in soils with low available moisture and shallow soil depths. The blight symptoms were not reproduced by inoculations with the fungi (50, 59).

A brown-spot needle blight of several species of Pinus has been reported in the United States. Dearness (32) described a needle blight of two Pinus spp. caused by Septoria pinicola, and Siggers (86) reported a brown spot needle blight of P. palustris caused by S. acicola. Siggers also reported that S. acicola is the imperfect stage of Scirrhia acicola. A serious needle blight of P. palustris, P. taeda, and P. caribaea was reported to be caused by Septoria acicola by Hedgcock (48). Boyce (17) reported extensive needle dieback on P. taeda in Georgia and the Carolinas caused by Scirrhia acicola. Boyce later recorded Scirrhia acicola on blighted P. strobus (18).

A group of hypodermateous fungi including Lophodermium spp., Hypoderma spp., Elytroderma spp.,
Hypodermella spp., and Naemacyclus spp. are reported to cause needle casts and blights on many different Pinus spp. (78). Usually these fungi attack individual needles and cause a needle dieback and thinning with black hysterothecia formed on dead or dying needles (16, 78). Weir (103) reported an epidemic needle blight on western white pine caused by Lophodermium pinastri. The trees turned brown and from a distance looked scorched. A blight and needle cast on P. banksiana, P. contorta, P. echinata, P. edulis, P. jeffryi, and P. ponderosa in the western United States was reported to be caused by Elytroderma deformans. Fink (39) reported a blight on needles of the lower branches of P. strobus caused by Cenangium abietis after a long drought period. Cenangium dieback of Pinus spp. was reviewed by Boyce (16). The disease is caused by C. ferruginosum which is found associated with injury by a gall midge Cecidomyia brachyntera that mines the lower part of needles through the fascicle to the stem. In the Southwestern United States the fungus has been found associated with extensive flagging on Ponderosa pine caused by a scale insect Matsucoccus verillorum (16, 51, 78).

Species of three genera of fungi, Atropellis, Cytospora and Tympanis, are reported to cause blighting and cankers on pines in the United States (16, 108).

Pullularia pullulans has been reported to cause blighting or forking of Pinus spp. or to be associated with
diseases and deterioration of pine trees (4, 7, 45, 55, 74, 76, 81, 84). The organism appears to have a sphaeropsidaceous form described by Robak (84) as Dothichiza pithyophila. It was reported to be associated with injury from a gall midge (Cecidomyia baeri), injury by drought, and frost injury (7, 45, 76, 81). Sclerophoma pityella has been reported in New York and Pennsylvania on dead needles and twigs of various Pinus spp. (51). The perfect stage of Pullularia pullulans has been reported to be Anthostomella pullulans (51).

Macrophoma magnifructa and Pestalotia funerea have both been reported on pines in Texas (51). P. funerea is found on blighted needles and twigs and causes a seedling rot while P. peregrina was reported on blighted needles and twigs.

E. Physiological causes of blight symptoms on Pinus spp.

Most of the pine blights not caused by parasites are caused by unfavorable weather or soil conditions. Winter kill of pine trees due to severe cold weather or to sudden fluctuations in temperature may be expressed as needle drooping, blighting of the needles on tips of branches or tops of trees, and cankers on limbs and trunks (16, 78). According to Peace (78) some pines such as P. halepensis have recovery shoots, bearing juvenile leaves, which enable them to recover from frost or drought injury.
Winter drying is a common occurrence of Pinus spp. It is believed to be caused by sudden increases in temperature, often accompanied by drying winds, after a period of cold weather (16). This causes excessive loss of water from the needles and fascicles which cannot be replaced because the soil moisture is low or frozen. The blight symptoms caused by winter drying usually begin to appear upon the advent of warm weather in the spring (16, 27). The symptoms include blighted needles and dieback of twigs.

Blights and diebacks can be caused on Pinus spp. by drought. Usually the severest drought injury occurs when there are periods of low soil moisture coupled with drying winds. Severe drought injury involves shoot and branch dieback and sometimes death of a tree (16, 78). A permanent needle droop and blight of P. resinosa was reproduced in the greenhouse by withholding water (30, 31) and by establishing artificial conditions of environment favoring rapid drying of the needles and stems (75, 76). Conditions of low soil moisture and rapid transpiration led to an absorption lag, collapsing succulent tissue near needle bases. Trees in shallow soil are often severely affected by drought (16), the trees dying from the top down and the outside inward. A condition known as "scorch" affects pines during the summer when rapid losses of water occur due to dry winds when the soil moisture is low. The predominant symptoms are bronzing and blighting of needles.
Cankers and cracks in limbs and trunks occur on conifers during periods of drought stress (16). Drought injury to trees may be increased when there is a heavy grass cover in the area in which they are growing (57).

Snell and Howard (88) report a blight of *P. strobus* caused by injury to the roots by CaCl₂ escaping from barrels stored under the trees and seeping into the soil. Symptoms included flagging, dieback and death of several trees.

Burns (19) describes a needle blight of young *P. strobus* trees due to wind. The tips of the trees were burned after several days of winds.

A dieback of Aleppo pines (*P. halepensis*) has been related to the poor development of mycorrhizial systems on trees planted in shallow soil (97). The diseased trees showed blighting, twisted needles and shoots. The needles became discolored at the base and the needles droop and shed prematurely leaving bare branches. The xylem of the branches and trunks became discolored and the trees finally dried out and died. Wahl (96) discovered that the Hartig net was thin on the roots of normal *P. halepensis* trees and thick with profuse haustorial invasions into the roots of pines affected by dieback.

Neilson-Jones recorded a condition, known as the fused needle disease of pines, on *P. halepensis* in Arizona (69). The condition is believed to be caused by a shortage
of water in the plant at the time the leaves are beginning to expand.

Several "pole blights," blights apparently related to poor root development or root death, have been reported (8, 16, 36, 78). These diseases are characterized by foliage blights and limb and trunk cankers. No pathogenic organism has been shown to be the causal agent of the root deterioration and blight symptoms.
MATERIALS AND METHODS

A. Isolation of fungi from branches and foliage

Plant material to be cultured was cut into pieces approximately two cm long, surface sterilized from 5 to 20 minutes in a 1:1 solution of 95% ethyl alcohol and 1:1000 mercuric chloride, rinsed in sterile distilled water, and placed on glucose-yeast extract agar (GYEA) or potato-dextrose agar (PDA) plates. The cultures were incubated at 20 and 24 C. Fungi growing from the stem pieces were transferred to GYEA or PDA slants in screw-cap tubes.

The media used contained the following ingredients:

(a) GYEA.

Yeast Extract (DIFCO) 2 gm
Glucose 10 gm
Agar 20 gm
Micro-element solution 2 ml
(Fe+++ 0.2 g, Zn++ 0.2 mg, Mn++ 0.1 mg/ml
Distilled water 1 liter

(b) DIFCO PDA: 39 g dehydrated material/liter

Both media were autoclaved for 15 minutes at 15 P.S.I. and poured into petri dishes.

The organisms isolated were examined microscopically, identified to genus and retained in a stock culture refrigerator at 0-4 C.
Branches, twigs, and needles from four of twenty-one 18-24" tall plants donated by Harlow Nursery, Tucson, Arizona, to be used for inoculation experiments, were cultured using previously described methods of sterilization and GYEA and PDA media. The fungi isolated from these plants were identified and compared with the fungi isolated from blighted trees.

Two samples were taken 1 inch or less apart of each sampling location from selected trees in fungicide test plots at Evergreen Cemetery, using a 1/4 inch increment borer. One of the pair was fixed in formalin-acetic acid-alcohol solution in large, corked test tubes for histological studies and the other, placed in a sterile, capped test tube. The increment borer was rinsed between each sampling with 95% ETOH from a wash bottle.

Samples were taken from different locations on the trees. Samplings were made from the bases of the tree trunks to branches at the tip of one tree. Most samples were taken from the tree trunks and lower branches and from the juncture of trunks and limbs showing symptoms. Branches from 2-7 inches in diameter were sampled, with the borer passing through smaller branches and giving a sample across the diameter of the branch.

Isolations were made by pouring 1:1 95% ETOH and 1:1000 HgCl₂ into the tubes holding the sample, sterilizing for 5 minutes, pouring out the sterilent, and replacing it.
with sterile distilled water. The surface sterilized samples were placed on GYE A medium in large petri plates. Samples too large to fit into a plate in one piece were cut into sections and arranged in sequence on the plate. The plates were incubated at 22 °C until fungus growth was observed. Fungi growing from the increment borer pieces were then isolated, identified and placed into stock culture. The position on the sample from which each isolate grew was recorded.

A branch from a 40 foot tall Aleppo pine, showing symptoms of blight, was cut off the tree with a power saw and sawed into 6-8" long sections. The branch was 14 inches in diameter at its base and was approximately twenty-five feet long. Eighteen sections were selected from the base to the top of the branch. These sections were sterilized by placing them into a bucket of 10% chlorox for 30 minutes to 1 hour. The sections were placed on cloth towels soaked in 1:400 rocal and split into pieces, from the bark inward to the stele, with a sterile chisel. Wood chips, selected to provide a sampling of the cross section from bark to center, were removed at intervals, sprayed with alcohol and flamed. The chips were placed on PDA plates and incubated at 24 °C.

Fungi growing from the wood pieces were transferred to GYE A slants, identified, and stock cultures were maintained.
Two limbs about 4 inches in diameter at the base and 5 to 6 feet long were removed from the top of an Aleppo pine tree showing severe disease symptoms. About 1/2 of each limb and its side branches were dead or dying. All isolations were made from living tissues. The branches were cut into sections, 2 inches long, and pieces were selected for culturing so as to give representative sampling the length of the branches. Samples were sterilized in 10% chlorox for 15 minutes, placed on a sterile surface, and split into several pieces with a sterile chisel. The pieces were sprayed with alcohol and flamed. Splinters were taken from the surface sterilized branch pieces and placed on GYEA plates. The position in the branch of each splinter cultured was recorded. The plates were incubated at 24 C until fungus growth appeared. The fungi were transferred and maintained in screw-cap tubes of GYEA.

Isolations were made using a pine extract medium (PE) which was prepared using an extract from Aleppo pine needles as part of the nutrient source. The pine extract was prepared in the following manner:

1. 100 gm of freshly gathered new growth pine needles was added to 500 mls H2O in a Waring blender and homogenized.

2. The material was filtered through a 3 layer thickness of cheese cloth and then the filtrate was
filtered twice through Whatman No. 1 filter paper in a Büchner funnel.

3. The filtrate was filtered through a sterile "Seitz" bacteriological filter and maintained under sterile conditions.

The medium was prepared by adding ten grams of glucose, 2 grams of yeast extract and 20 grams of agar to 800 mls of distilled water. The mixture was autoclaved for 15 minutes and 200 mls of pine extract was added when it had cooled to about 50 C. The medium was immediately poured into petri dishes.

Pieces of twigs, branches, and needles were surface sterilized for 15 minutes with a 1:1 solution of 95% ETOH and 1:1000 mercuric chloride, placed on PE plates, and incubated at 24 C. Fungi growing from the pine tissues were examined and compared with the fungi previously isolated.

B. Isolations of fungi from pine roots:

Roots were collected from diseased trees in Evergreen Cemetery. Root samples were collected to a depth of 5 feet, cut into 2 cm pieces, and sterilized in 10% chlorox for 10, 15, and 20 minutes. They were rinsed in sterile distilled water and placed on plates of six different media. The media used in this experiment were water agar, corn
meal agar, GYEA, FDA, lima bean agar, and glucose-malt extract media. The media were prepared as follows:

Water Agar (WA)

Twenty grams of Bacto-agar were added to 1 liter of distilled water and autoclaved for 15 minutes at 15 P.S.I. The medium was poured into plastic petri dishes.

Corn Meal Agar (CMA)

Twenty-one grams of dehydrated corn meal agar preparation, made by the Baltimore Biological Laboratory, was placed into 1 liter of distilled water, autoclaved, and dispensed into petri dishes.

Lima Bean Agar (LBA)

Twenty-three grams of dehydrated lima bean agar manufactured by Difco Laboratories, was suspended in 1 liter of distilled water, autoclaved, and poured into petri dishes.

Glucose-Malt Extract (GME)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>5 gm</td>
</tr>
<tr>
<td>Malt extract</td>
<td>10 gm</td>
</tr>
<tr>
<td>Bacto-Agar</td>
<td>10 gm</td>
</tr>
<tr>
<td>95% ETOH.</td>
<td>10 ml</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>1 ml</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>500 mls</td>
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</tbody>
</table>
The medium was autoclaved for 15 minutes and dispensed into petri dishes, giving plates of semi-solid medium.

All plates were incubated at 23 C and the resulting fungi were transferred to plates of the medium on which they grew or to GYEA plates for identification.

Isolations were also made using green apples. With a 3/8" cork borer 3 holes about 1 inch deep were made in each apple. Root pieces from blighted pines were placed into the holes in 5 apples, and soil in holes in 4 apples. A piece of clear plastic tape was placed over each hole. After 8 days incubation at room temperature, the apples were cut open with a sterilized knife, and pieces were removed with a flamed needle from the edges of infected areas. Fungi growing from the apple tissue were isolated on GYEA, CMA, and LBA media at 24 C, identified and maintained in stock culture.

Soil, roots, and crown pieces from plants dying in the greenhouse were cultured in the above manner and the resulting fungal isolates were identified and maintained in stock culture.

Several of the young trees used for greenhouse inoculation experiments suddenly died with symptoms similar to pine blight. It was noted that the roots showed signs of damage with feeder and root hairs completely killed and larger roots showing discoloration. The vascular system
was discolored a reddish-brown to black in the crown region. Pieces of the larger, living roots and crown pieces about 2 cm long were surface sterilized with 10% chlorox for 15 minutes and 2 hours. The sterilized pieces were rinsed twice in sterile distilled water and cultured on GYEA plates. The fungi growing from the root and crown pieces were isolated and maintained in stock culture.

C. Identification of isolated fungi

All of the fungi isolated from Aleppo pine were examined microscopically and identified if fruiting structures were present. In most cases identification was to genus. The keys of Barnett, Bessey, Gäumann, Clements and Shear, and the monographs of Guba and Wehmeyer were used for all identifications of the isolated fungi (69, 23, 41, 44, 102). Fungi consistently isolated or previously reported as plant parasites were selected for inoculation experiments.

D. Inoculation experiments

1. Inoculation of 1½-2 year old seedlings

Twenty-one seedlings, obtained from Harlow Nursery and grown in Arizona from seed, were inoculated with six fungal organisms isolated from diseased Aleppo pine trees. Eighteen trees were inoculated and 3 trees were maintained as controls.
The six fungi used were a *Pyrenophora* sp., *Gelasinospora* sp., 2 isolates of *Macrophoma* sp. and 2 isolates of *Phoma* spp. The fungi were grown on GYEA medium for 10 days. The trees were inoculated by making a 1½ cm slit with a sterile scalpel in the bark of a branch near the trunk union and placing a 1 cm\(^2\) piece of inoculum over the wound with the side having fungus growth next to the wound. The wound and inoculum piece were covered with a water-soaked wad of cotton. The cotton was held in place over the wound with a piece of plastic tape. The wound was made with a scalpel which was flame sterilized between each inoculation. The control trees were wounded with the sterile scalpel, a 1 cm\(^2\) piece of sterile GYEA medium was placed over each wound, and the wounds were covered with water-soaked cotton pads taped into place. Four branches on three trees were inoculated with each fungus and four branches on three trees were prepared and maintained as controls. The cotton pads were removed from the wounds after 5 days and the trees were observed over an 18 month period for disease symptoms expression. After approximately 18 months branches were removed from the inoculated trees and controls and cultured on PDA and GYEA media. One tree was selected from each inoculation treatment and the 4 inoculated branches were removed. One branch was cut into 2 cm pieces, surface sterilized for 10 minutes in a 1:1 solution of 95% ETOH and 1:1000 HgCl\(_2\), rinsed with sterile
distilled water, and placed on GYEA plates in a series so that the pieces were in their original order; i.e., as they were on the uncut branch. Two branches were cut into pieces and surface sterilized in the same manner for 15 minutes. The branch pieces were serially arranged on PDA and GYEA plates, one branch to each medium. The fourth branch was cut into sections, surface sterilized for 20 minutes, and serially placed onto GYEA plates. The plates were incubated at 21-22°C and the fungi growing from the plates were isolated and identified. The positions on the branch, in centimeters from the site of inoculation, from which the fungi grew were noted and recorded. Needles from one of the branches from each inoculation were removed, surface sterilized for 5 minutes, and cultured on GYEA plates at 21-22°C. The fungi growing from the needles and needle bases were isolated and identified.

2. Branch, needle and fascicle inoculations on 3 year old Aleppo pines

Inoculations using three different techniques were made on 40 three year old Aleppo pine trees which had been imported from a California wholesale nursery. The 40 trees were maintained in the greenhouse during the inoculation experiments. The trees used ranged from 30-48 inches in height and were grown in 1 gallon tin cans.
(a) Branch inoculations

Five branches on 5 different trees were inoculated with each fungus. The fungi used in this experiment were:

1. *Alternaria* sp. (2 isolates)
2. *Helminthosporium* sp.
3. *Gelasinospora* sp.
4. *Phoma* sp. (b)
5. *Fusarium* sp.
6. *Coniothecium*, or *Piricauda* sp.
7. *Macrophoma* sp. (3 isolates)
8. *Coniothyrium* sp.
10. *Pullularia pullulans* (white variant and normal isolate) (2 isolates)
11. *Sphaeropsis* sp.

The fungi were grown on GYEA plates for 10 days. One cm$^2$ pieces were cut from the plates with a flamed needle. The branches were wounded by cutting down to the wood with a sterile razor blade, cutting parallel with the branch for a 3-4 cm, and lifting the resulting flap of bark and sapwood. The flap was left attached at one end. The inoculum was placed under the bark flap with the side having fungus growth against the wood, the flap was pushed down to hold the inoculum in place, and the wound was wrapped with watersoaked cotton. The cotton pad was secured in place with
plastic tape. The cotton was left in place over the wound for a minimum of 20 days.

Two fungi were inoculated by the above method into each set of 5 trees, using 2 branches/tree. Each branch inoculation was used as a replicate, giving 5 reps; one replicate on each of 5 different trees. A control was established by placing sterile GYEA blocks into 5 branches selected at random among the 40 trees. The inoculated branches were observed for symptom expression and after 3 months, were harvested, surface sterilized, and fungi were cultured from the branches and identified.

(b) Needle inoculations

The fungi used in this experiment were:

1. Alternaria sp.
2. Helminthosporium sp.
3. Gelatinospora sp.
4. Phoma sp. (a)
5. Phoma sp. (b)
6. Fusarium sp.
7. Coniothecium or Piricauda sp.
8. Macrophoma sp. (3 isolates)
9. Coniothyrium sp.
10. Peyronella sp.
11. Pullularia pullulans (white variant and normal isolate) (2 isolates)
12. *Sphaeropsis* sp.

A spore suspension was prepared by growing the fungi on GYEA plates until mature spores or fruiting bodies were present, blending one plate of each fungus with 100 mls of sterile distilled water using a Waring blender, and filtering the solutions through several layers of glass wool. The number of spores/ml in each spore suspension was determined using a Levy counting chamber (Table 1). One-half ml of each spore suspension was pipetted into each of 2 GYEA plates. The plates were incubated at 24 C for 5 days to check the viability of the spores. Ninety to ninety-five mls of spore suspension was collected from each fungus. This material was divided into 2 equal parts, one-half was used for the needle inoculations and one-half was used in the fascicle inoculation experiment.

Five branches on 5 different trees were used for needle inoculations with each fungus. Each fungus was inoculated onto needles of the same trees into which the stem inoculations with the fungus had been made. The inoculations were made by painting approximately 5 mls of spore suspension, to which carborundium had been added, onto the needles of a branch. One gram of carborundium had been added to each 45 mls of spore suspension. A small, disposable brush was used for the inoculations with each fungus. After the needles of a branch were inoculated a plastic bag containing a water-soaked cotton pad was placed
<table>
<thead>
<tr>
<th>Fungus</th>
<th>Spores/ml of suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria sp. (a)</td>
<td>$8.0 \times 10^5$</td>
</tr>
<tr>
<td>Alternaria sp. (b)</td>
<td>$4.0 \times 10^4$</td>
</tr>
<tr>
<td>Coniothyrium sp.</td>
<td>$4.8 \times 10^6$</td>
</tr>
<tr>
<td>Coniothecium sp.</td>
<td>$3.0 \times 10^5$</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>$2.8 \times 10^6$</td>
</tr>
<tr>
<td>Gelasinospora sp.</td>
<td>$5.0 \times 10^5$</td>
</tr>
<tr>
<td>Helminthesporium sp.</td>
<td>$2.0 \times 10^5$</td>
</tr>
<tr>
<td>Macrophoma sp. (a)</td>
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</tr>
<tr>
<td>Macrophoma sp. (b)</td>
<td>$1.6 \times 10^6$</td>
</tr>
<tr>
<td>Macrophoma sp. (c)</td>
<td>$4.0 \times 10^5$</td>
</tr>
<tr>
<td>Phoma sp. (a)</td>
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</tr>
<tr>
<td>Phoma sp. (b)</td>
<td>$9.2 \times 10^6$</td>
</tr>
<tr>
<td>Peryronellae sp.</td>
<td>$8.7 \times 10^6$</td>
</tr>
<tr>
<td>P. Pullulans</td>
<td>$17.6 \times 10^6$</td>
</tr>
<tr>
<td>P. pullulans (white variant)</td>
<td>$16.1 \times 10^6$</td>
</tr>
<tr>
<td>Sphaeropsis sp.</td>
<td>$4.8 \times 10^6$</td>
</tr>
</tbody>
</table>
over the branch and secured with a rubber band. The plastic bags and cotton pads were removed after 72 hours. The control was established by blending the medium from a GYEA plate in 100 mls of sterile distilled water, filtering the solution through glass wool, and inoculating in the above manner with the solution onto the needles of 5 branches selected at random among the 40 trees. Because no spores were produced by AP-42, the blended solution was not filtered through glass wool, but left unfiltered for the inoculations.

The inoculated needles were observed and after the second and third months the percentage of dead needles on each inoculated branch was estimated. Needles from inoculated branches were harvested, surface sterilized, the needles cultured, and the fungi obtained in culture were identified.

(c) Fascicle inoculations

The spore suspensions used in this experiment were prepared as for the needle inoculations except that carborundium was not added. A sterile, disposable plastic syringe, with a 2361 detachable needle, was used to inoculate fascicles with the same test organisms used in the needle inoculation experiments. A small puncture was made at the base of each fascicle, in areas marked off on each branch used, and a drop of spore suspension was
injected into the wound. Twenty to 100 fascicles were inoculated with each organism on one branch on each of 5 trees. Approximately 350 fascicles were inoculated with each fungus. A control was established using fascicles on 5 branches scattered at random among the 40 test trees. The needles were observed for symptom expression and after 2 and 3 months fascicles were removed and cultured to recover the test fungi. Each month, beginning 2 months after inoculation, the percent of dead fascicles on each inoculated branch was estimated.

3. Inoculation of seedlings and fascicles in a sterile system

In an effort to determine the pathogenicity in vitro of several fungus isolates to Aleppo pine, an experiment was initiated using fascicles and small seedlings growing on White's medium (105). The medium was prepared and placed in 250 ml flasks, 100 mls of medium per flask. The fascicles were collected from the new growth on a 30 foot pine tree, surface sterilized in 10% chlorox, with 1 ml ETOL per 100 mls chlorox solution, rinsed in sterile distilled water, and placed into the medium so that the fascicles were completely submerged and the needles were above the surface. The seedlings were obtained by surface sterilizing seed, obtained from Herbst Brothers Inc., New York, for 2 hours in 20% chlorox solution, germinating the seed in water agar plates, surface sterilizing the
seedlings with 10% chlorox for 15 minutes, and placing the seedlings on the medium in the flasks.

Five flasks, each containing 3 fascicles and needles, were inoculated with the Sphaeropsis sp. and two flasks with fascicles were maintained as uninoculated controls. The needles were observed for 1 month for symptoms or other evidences of invasion of the tissues by the fungus. After a month the fascicles and needles were collected, sectioned, and the tissues examined microscopically.

The seedlings were inoculated, 3 flasks each containing one seedling, with 3 different Fusarium isolates, one Phoma sp., and a Pestalotia sp. isolated from the roots of blighted Aleppo pines. After 2 weeks of growth the plants were examined for evidences of infection by the test fungi.

E. Histological examination of blighted tissue

Tissues from trees showing blight symptoms were prepared for microscopic examination to determine the extent of fungus penetration into living cells.

1. Microtome sections of twigs, needles, and buds

Small branches, twigs, buds, and needles were killed and fixed in a formalin-aceto-alcohol (FAA) solution (52). The plant tissues to be killed and fixed were cut
into pieces 1-2 cm long and placed in vials of FAA containing a volume of FAA at least 5 times that of the tissues. The tissue pieces were dehydrated with tertiary butyl alcohol using a modification of Johansen's dehydration schedule and the plant tissues were embedded in parowax using Johansen's embedding procedures (52). The pieces of plant material were sectioned with a sliding microtome and pieces of wax ribbon containing sectioned plant material were affixed to slides with Haupts' adhesive (52). The plant sections were stained with safranin and fast green or crystal violet (52). The sections were stained for 15 minutes with safranin and counterstained with fast green or crystal violet for 10-20 seconds, then mounted in balsam.

2. Sectioning and examination of dead pine needles

Needles which had shown blight symptoms for approximately one month were removed from trees, soaked overnight in distilled water, sectioned with a freezing microtome or by hand and stained with orseillin BB in modified Sartory's solution (1).

3. Examination of increment borer samples for fungus mycelium

Increment borer samples, described in the section on culturing of plant tissues, were cut into 1/2 cm long pieces, soaked overnight in water to which several drops
of liquid detergent had been added to remove the FAA and soften the tissues, and the pieces were macerated using Jeffrey's method (52). The pieces were maintained serially and stained with safranin. Tracheids were examined for the presence of fungus cells and the results of this examination were compared with results of fungus isolations from the second of the paired increment borer samples.

F. Attempted control of Aleppo pine blight with fungicide sprays

Histological evidence of a fungus in the tissues of blighted Aleppo pines combined with control attempts by commercial treemen indicated that an experimental evaluation of fungicides for the control of blight symptoms should be attempted. An experiment was initiated in Evergreen Cemetery (Fig. 13), in October of 1963 to determine the effect of the application of two fungicides, Bordeaux and cycloheximide (Acti-dione PM), on the control of blight in a large planting of Aleppo pines. Bordeaux was one of the fungicides recommended by commercial treemen in Tucson, Arizona. The concentration (4-4-50) of Bordeaux used was one recommended for the control of many plant diseases caused by fungi (72, 80). This fungicide has low penetrating power, but gives a lasting, protective covering (93). Cycloheximide (Acti-dione PM) was selected because of its past use in the control of pine diseases and because of its systemic nature (34, 20, 25, 33, 35, 40, 56,
Fig. 13. A view of Evergreen Cemetery, site of fungicide experiments.
64, 66, 67, 77, 79, 90, 94, 95, 104). The cycloheximide was applied at the concentration (100 ppm) recommended by the manufacturer.

One hundred and forty-four trees of varying age were used in the experiment. The trees occurred in three size groups, 28 ranging from 4-8 ft., 96 from 20-25 ft., and 20 trees approximately 40 ft. in height. For identification purposes the trees were classified as small, medium and large.

Sixty trees were used in testing the effectiveness of Bordeaux. Sixteen interspersed through the planting were not sprayed and maintained as controls. Fifteen were sprayed once in November 1963, 14 twice, in November and December of 1963-64 and 15 were sprayed three times, in November, December, and January of 1963-64. All trees were sprayed to runoff with 4-4-50 Bordeaux at a rate of approximately 1-2 gallons/small tree, 4-5 gallons/medium tree and 8-10 gallons/large tree.

Eighty-four trees were used in the cycloheximide spray test. Sixteen were not sprayed and were maintained as controls. Nineteen trees were sprayed in November 1963, 30 in November and December 1963, 7 in November, December and January 1963-65. Five trees were sprayed four times; in November, December, January, and February 1963-64 and 7 five times in November, December, January, February, and March of 1963-64. All trees in this group were sprayed
with Bordeaux. The spraying was done with a 50 gallon capacity Bean sprayer.

The trees were rated, at two month intervals for 18 months, on a scale of 0-10, 0 representing an apparently dead tree and 10 a tree free of blight symptoms. Numbers between 0 and 10 stood for varying degrees of symptom expression as estimated by the two persons doing the rating.

G. Testing the effects of physiological drought on small Aleppo pine trees

The sudden blighting symptoms on Aleppo pine may be caused by some factor affecting water uptake by the roots. Several tests were run to determine the effect of physiological drought on young trees.

Six 1-2 year old trees having over one-half of their needles in the mature fascicled form were used in this experiment. Three trees were watered every other day with a 1.5 molar sucrose solution and three trees were watered normally with tap water. At regular intervals the trees were examined for needle blighting or other symptoms similar to those of Aleppo pine blight.

Six 1-2 year old trees were selected for a test in which 2 molar CaCl₂ was used to create a physiological drought. Six hundred and sixty-six mls of 2 molar CaCl₂ was added to each of three trees. The trees were then
watered with tap water at 2-3 day intervals. Three trees, not treated, were maintained as controls.

Three 3 year old trees which were from 4-5 feet tall were used to determine the effect, on larger trees, of watering with CaCl₂ solutions. Two of the trees were given an initial drench with 2 liters each of 2 molar CaCl₂. The trees were then watered normally. Soil samples were collected from the cans of treated and control plants and analyzed for total soluble salts.

Soil samples were collected from the area surrounding two trees showing blight symptoms under natural conditions. Five samples were taken from the area around each tree with samples being taken at depths of 1-5 feet. The samples were analyzed by the Department of Agricultural Chemistry and Soils for total soluble salts and for ppm of NO₃⁻ and PO₄³⁻.
RESULTS AND DISCUSSION

A. Fungi isolated from blighted Aleppo pines

The fungi routinely isolated from the branches and foliage of blighted Aleppo pine trees, using the techniques described in part A of materials and methods were as follows:

1. Alternaria spp.
2. Aspergillus spp.
3. Coniothecium or Piricauda sp.
4. Coniothyrium sp.
5. Fusarium spp.
6. Gelasinospora sp.
8. Macrophoma sp.
10. Peyronella sp.
11. Phoma spp.
12. Pullularia pullulans
13. Pyrenophora sp.
14. Sphaeropsis sp.
15. Unidentified species of Mycelia Sterilia

Alternaria spp. accounted for over one-half of the total isolations and Helminthosporium spp. accounted for
almost one-fourth of the rest. The other fungi were isolated 10 times or more from the pine tissues. Isolation of some of the fungi depended on variations in the sterilization techniques. *Pullularia pullulans* could be isolated consistently by picking pieces of the fungus from pine tissues which were not surface sterilized. Surface sterilization appeared to kill the *Pullularia* under most conditions. The *Sphaeropsis* sp. could be isolated consistently on White's medium from needle fascicles, necrotic spots on needles, and dead needles. *Sphaeropsis* was occasionally isolated on other media but it was usually overgrown by other fungi. The *Phoma* spp. were isolated from all of the foliage and branch tissues cultured and they made up the majority of isolates that were not *Alternaria* or *Helminthesporium* spp.

The genera represented among isolates from the small trees maintained in the greenhouse for inoculation experiments were identical to those of trees in the field except for the absence of the *Sphaeropsis* sp., *Pullularia pullulans*, *Pyrenophora* sp., *Macrophoma* sp., and *Conitheciun or Piricouda* sp.

Several fungi were selected from this group of isolates for inoculation experiments. The isolates were selected because they did not appear to be associated with the small greenhouse plants which were not blighted or because they were reported as being parasitic on plants.
One isolate, a Pyrenophora sp., an Ascomycete with muriform ascospores (Fig. 14-I), was selected for stem inoculation experiments even though it was only isolated one time. The isolates selected from this group were the Sphaeropsis sp., Pullularia pullulans (dark spored), Coniothrium sp., unidentified mycelin sterilia, Coniothyrium sp., Phoma sp. (a), Phoma sp. (b), Peyronella sp., Macrophoma sp. (3 isolates), Alternaria spp. (2 isolates), and a Helminthosporium sp. These isolates were used in experiments where they were inoculated into stems, fascicles, and needles. The Pyrenophora sp. was selected because several Ascomycetes in this group have been reported as plant pathogens (9, 102).

Representatives of four genera of fungi were isolated from the increment borer samples. These were species of Alternaria, Aspergillus, Coniothyrium and Phoma sp. (a). All of the fungi grew from the bark and cambium region. No fungi were isolated from the active xylem, wood, or stele. Three of the fungi: Alternaria, Coniothyrium, and Phoma sp. (a), had already been selected for inoculation experiments. The Aspergillus sp. very obviously grew from the bark and was considered to be a saprophyte.

The fungi isolated from a 30 foot long branch, which was cut into small pieces and sectioned, were Alternaria sp., Helminthosporium sp., Hormiscicum sp., Phoma sp., and a Sphaeropsis sp. The limb had a
longitudinal red discoloration which ran nearly the length of the underside of the branch. *Sphaeropsis*, *Coniothyrium*, and *Hormiscicum* were isolated from the discolored region. These three fungi were the only ones isolated from tissue more than 1/2 inch under the bark. The *Alternaria*, *Helminthosporium*, and *Phoma* isolates grew from the bark or the underside of the bark possibly indicating their saprophytic nature.

Results of isolations, made from two limbs which were dying back after the needles had been blighted several years in a row, indicated that fungi isolated represented 3 genera. *Pullularia pullulans* was isolated from the wood of a piece of branch form near the branch tip. The piece of branch was about one-half dead with the cambium and phloem on one side dead and the other side alive. The *Pullularia* isolate grew from the area between dead and live tissues. *P. pullulans* was also isolated from a discolored area in a branch piece which was 1 1/2 inch in diameter. The fungus grew from an area 3/4 of an inch below the bark, well into the wood. The fungus was isolated from a branch piece 3 inches in diameter from an area about 1/2 inch below the bark. A white variant of *P. pullulans* was isolated from several places along the dying branches 1 inch or less below the bark. An *Alternaria* sp. was isolated twice from the dying branches, both times from an area immediately under the bark. *Phoma* sp. (a) was isolated
once, from the initiation point of a small side branch, 2 inches into a branch piece 4 inches in diameter. The side branch was dead almost to the center of the larger branch. All of the fungi were isolated from discolored, dying or dead tissues. These fungi were not isolated from healthy wood covered by bark with an active cambium layer. The fungi were probably saprophytic or weakly parasitic on tissue dying from some other cause.

Fungi consistently isolated from cultured crown and root pieces from small trees dying in the greenhouse were identified as species of Alternaria, Phoma, Pestalotia, Cunninghamella, Fusarium, and Trichoderma sp. The Pestalotia and one Fusarium sp. were isolated from crown pieces, root pieces, and soil placed into apples. The Phoma spp., Pestalotia sp., and Fusarium spp. were isolated from cultured surface sterilized root and crown pieces. Three different Fusarium spp. and two Phoma spp. were consistently isolated from the pine tissues. The Fusarium spp., Cunninghamella sp., Alternaria sp. and Phoma spp. were isolated on all of the media used. The Pestalotia sp. and Trichoderma sp. were isolated on GYEA medium.

When cultured through apple, roots from blighted trees yielded only a Fusarium sp. Fungi from surface sterilized root pieces cultured on agar media included 3 Fusarium spp., Pestalotia sp., and Phoma spp. as well as Alternaria, Aspergillus, Rhizopus, and Cunninghamella spp.
From this group of isolates 3 *Fusarium* spp., the *Pestalotia* sp., the *Cunninghamella* sp., and a *Phoma* sp. were selected for inoculation experiments because they were consistently isolated and the *Pestalotia* sp. has been reported as a pathogen on *Pinus* sp. (51). Photomicrographs of the characteristic fruiting structures of the fungi used in the inoculation experiments are presented in Fig. 14 to Fig. 17.

B. The effect of inoculations with fungi into Aleppo pines

After 18 months no blight symptoms were observed on the branches of plants inoculated with *Pyremophora* sp., *Gelasinospora* sp., *Macrophoma* sp., and 2 isolates of *Phoma* sp. (a). None of the cankers appeared to have enlarged and they were callused over completely. *Phoma* sp. (a) was the only test fungus reisolated from the inoculated branches and it was isolated only from the inoculation point. This *Phoma* sp. evidently colonized the wounded tissue of the inoculation wound but was not able to parasitize the healthy tissues. The other fungi used in this experiment failed to colonize the wounded tissue or infect healthy tissues.

Three and one-half months after the stem inoculations were made on 3 year old trees in the greenhouse, the following fungi were reisolated:

*Alternaria* sp. (b)

*Gelasinospora* sp.
Fig. 16. Characteristic fruiting structures of \textit{Sphaeropsis, Pestalotia, Coniothyrium, Phoma} (a), and \textit{Coniothecium} spp. isolated from blighted Aleppo pines. A and B) Pycnidium and conidia of the \textit{Sphaeropsis} sp. C) Conidia of \textit{Pestalotia} sp. D and E) Pycnidium and conidia of \textit{Coniothyrium} sp. F and G) Pycnidia and conidia of \textit{Phoma} sp. (a). H) Muriform conidia of the \textit{Coniothecium} sp. I) Conidiophore of the \textit{Coniothecium} sp.
Macrophoma sp. (a) and (c)

Peyronella sp.

Sphaeropsis sp.

All of the fungi reisolated grew from the inoculation wound. None of the fungi had infected the pine tissues at more than one-half centimeters distance from the inoculation point.

An average of less than 1% of the needles on any of the inoculated branches were killed or showed necrotic lesions. None of the wounds had spread along the branches but there were differences in the rate of callusing of the wounds. Two months after inoculation none of the wounds were completely callused over, however, the wounds of the control inoculations averaged approximately $\frac{3}{4}$ of the wound covered with callus tissue. Only the wounds on branches inoculated with the 3 isolates of Macrophoma sp. were in a similar condition. The wounds on the branches inoculated with Sphaeropsis sp., Alternaria sp. (a), Phoma sp. (b), Pullularia pullulans, and the white variant of P. pullulans showed little or no formation of callus tissue. The wounds of the branches inoculated with the other fungi were approximately $\frac{1}{2}$ callused over. Three months after the inoculations were made all of the wounds were $\frac{3}{4}$ to completely callused over except the wounds inoculated with Phoma sp. (b) and both isolates of Pullularia pullulans. The wounds inoculated with Sphaeropsis sp. were callused.
over, but they were discolored and black pycnidia were formed at the edges of the wound. The pycnidia were not embedded in the pine tissues but they appeared to be slightly embedded in the resin at the edges of the wounds. None of the cankers made by wounding the branches while inoculating them with the test fungi appeared to have enlarged during the 3 month period and no blight symptoms appeared on the needles of inoculated branches.

Two months after the needles were inoculated an average of less than 1% of the needles on the inoculated branches were dead. Most of the inoculated branches, including the control branches, had a few small necrotic lesions along some of the needles or had needles with dead tips. This was apparently caused by the carborundium used in the spore suspension. When the plastic bags were removed from the inoculated branches some of the dead tips and small lesions had fungus growth or fruiting bodies on them. After the branches had dried out for several days the fungus growth was no longer visible.

When needles were cultured 2 months after the inoculations, 5 of the fungi were reisolated, all of them from dead or yellowed lesions or needle tips. Alternaria sp. (a), Phoma sp. (a), Phoma sp. (b), Macrophoma isolate (b), and the Sphaeropsis sp. were recovered.

Three months after the inoculations only the needles on branches inoculated with one fungus showed more
than 2% infection. The needle inoculated with *Sphaeropsis* sp. had 5.5% infection. The symptoms included dead fascicles, needles, needle tips, and chlorotic or necrotic lesions on the needles. When the needles were cultured to reisolate the test fungi, the species of the genera *Alternaria* (a) and (b), *Gelasinospora*, *Fusarium*, *Phoma* (a), *Coniothyrium*, *Sphaeropsis*, and *Pullularia pullulans* were recovered. Most of the fungi were definitely recovered from dead needle tips or yellow lesions on the needles. The *Sphaeropsis* sp. was recovered several times from the yellow or necrotic lesions on needles as well as from dead needle tips.

Two months after inoculation the branches with fascicles inoculated showed an average of 2% needle death on control branches and 4 fungi causing between 2 and 4% needle death. The fungi were *Alternaria* sp. (a), *Gelasinospora* sp., *Fusarium* sp., and the *Peyronella* sp. Four per cent of the fascicles inoculated with *Gelasinospora* and *Fusarium* spp. died. The dead needles were scattered along the branches and none of the fungi appeared to spread up or down branches from the fascicle inoculations. When fascicles and needles were cultured to reisolate the test fungi, 5 were recovered. The species of *Coniothyrium*, *Gelasinospora*, *Peyronella*, *Phoma* (a), and *Pullularia pullulans* were recovered, all of them from fascicles.
Three months after the fascicles were inoculated there was no increase in the number of dead fascicles on inoculated branches. When fascicles and needles were cultured to recover the test organisms three of the test fungi were reisolated. The *Gelasinospora* sp. and *Peyronella* sp. were recovered from fascicles and the *Coniothyrium* sp. was recovered from a dead needle tip.

With the exception of inoculations made with *Sphaeropsis*, *Fusarium*, *Gelasinospora*, and *Peyronella* there was little correlation between symptom expression and the fungi reisolated.

*Sphaeropsis* killed needles growing in White's medium within 2 weeks. The needles did not grow well in White's medium and although callus tissue formed at the bases of fascicles the needles appeared stressed and began to die after 1 month. *Sphaeropsis* killed all of the needles in flasks into which it was inoculated and fruiting bodies were formed on the needles at the surface of the medium.

All of the fungus isolates from pine roots killed the small pine seedlings on White's medium except the *Phoma* sp., *Trichodema* sp. and *Cunninghamella* sp. The *Fusarium* spp. appeared to be most active against the seedlings while the *Pestalotia* sp. was slower. The pine seedlings were weakened by the surface sterilization techniques and were slow to grow into the White's medium. The *Phoma* sp. may have been beneficial to growth of the seedlings as those
inoculated with *Phoma* grew better than the uninoculated controls.

C. Observations of fungi on or in pine tissues

1. Microtome sections

Fungus tissue was observed only one time in the microtome sections of pine tissues. Stem sections from a tree which was severely blighted had fungus hyphae or chlamydospores in the bark, in leaf scar tissue, and in canker tissue (Fig. 18). This was the only instance when fungus tissue was observed, in microtomed sections, below the bark.

2. Sections of dead needles

Hand or freezing microtome sections of dead needles often had fungus hyphae below the needle epidermis. Photomicrographs were prepared showing *Alternaria* and *Pullularia* hyphae in dead pine needles (Fig. 19). The *Pullularia* formed dense clusters of chlamydospores on the surface of the needles (Fig. 19), and produced filamentous hypha inside of the dead needles. Clusters of chlamydospores and masses of hyphae of *Pullularia* were often observed on healthy and blighted pine with the fungus concentrating around needle scars (Fig. 20), but the fungus was superficial on healthy pine tissues and did not penetrate below the epidermal layer of branches or needles.
Fig. 18. Fungus chlamydospores in the tissues of a needle scar.
Fig. 20. Mycelium, chlamydospores, and fruiting structures of Pullularia pullulans on a needle scar.
Resin cysts were occasionally observed in freshly blighted pine needles (Fig. 19). These cysts are believed to be associated with fungus infection under certain circumstances (78).

3. Increment borer samples

Tracheids and other cells from macerated increment borer samples were large and easily observed for the presence of fungus tissue (Fig. 19). Fungus hyphae was observed only one time and this was in a cell from the bark area (Fig. 19). None of the tracheids examined which were from below the bark contained fungus hyphae.

D. Results of the fungicide spray program

The ratings given trees in each treatment category were averaged and listed for Bordeaux in Table 3 and cycoheximide in Table 4. The effects of tree size on reaction to spray treatments were shown in Tables 5 and 6. In Table 6 the trees sprayed 3 or more times were averaged together.

The trees sprayed with Bordeaux showed no persistent benefit traceable to the fungicide. In only one case did a sprayed tree differ by more than one rating point (Table 5). This was the large tree sprayed one time. This tree was a poor tree and often showed more symptom expression than the other large trees.
Table 2. Total soluble salts in soil samples collected from soil around blighted pine trees

<table>
<thead>
<tr>
<th>Sample</th>
<th>PH Soil Paste</th>
<th>Soluble Salts in Saturation Est.</th>
<th>ppm</th>
<th>ppm NO₃</th>
<th>ppm PO₄</th>
<th>ml EDTA</th>
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<td>Soil from 4 feet</td>
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<td>406</td>
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<td>Soil from 1 foot depth 10 feet from trunk of 20-30 foot tall tree</td>
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<td>0.52</td>
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<td>Soil from Greenhouse plants treated with 2 M CaCl₂ solution</td>
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Table 3. The average rating per spray treatment for all trees sprayed with Bordeaux mixture

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Table 4. The average rating per treatment for all trees sprayed with cycloheximide

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Table 5. The average rating per treatment of trees sprayed with Bordeaux mixture and listed according to size

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Table 6. The average rating per treatment of trees sprayed with cycloheximide and listed according to size

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These trees received 3-5 spray treatments.
The average ratings for the cycloheximide spray treatments showed little deviation from the control (Table 4). No changes in symptom expression due to the number of spray applications were observed. The low average ratings given the trees sprayed 5 times were caused by the presence of one badly affected tree which consistently received a low rating. It was noted, however, that small trees sprayed 3 or more times had values consistently lower than the unsprayed control trees (Table 6).

The results of these spray tests indicate that little or no control was given by either of the two fungicides. Bordeaux was not phytotoxic to Aleppo pine at the concentration used, but cycloheximide may have caused some browning of needles on small trees, however, phytotoxicity if present was impossible to distinguish from blight symptoms.

Comparison of trees before spraying, October 1963, and one year later, October 1964, showed a general decrease in symptom expression in all trees. This was attributed to variability in symptom expression due to some factor other than the chemicals used in this experiment. Variability has been observed in past years by the investigators working on this problem and was not unexpected.
E. The effect of physiological drought on P. halepensis

The 1-2 year old pine trees watered with 1.5M sucrose solution began to show symptoms after one week and were completely blighted after 2 weeks. The trees were killed by the treatment approximately 3 weeks after the experiment was begun. The needles of the trees first began to droop at the fascicles and turn a pale yellow. As the needles died they turned bright yellow and then reddish-yellow. The symptoms were very close to those exhibited by naturally blighted trees. The control trees remained normal throughout the experiment.

The trees treated with CaCl₂ began showing symptoms 3 days after the experiment was begun. The needles dropped at the fascicle, turned grey-green, then yellow-green, and finally bright yellow-red. The trees were completely blighted after 1½ weeks and were dead after 2 weeks. The trees were compared with branches from naturally blighted trees and the symptoms were very similar. The control trees remained normal throughout the experiment. The 2 larger trees treated with CaCl₂ showed the same symptoms and were dead after 3 weeks, while the control tree remained healthy.

The results of the analysis of soil samples collected from trees treated with CaCl₂ and from the ground near blighted trees are shown in Table 2. The ppm total soluble salts was very high in the soil around the trees treated
with CaCl\(_2\), but at the time of sampling the ppm total soluble salts in the soil from samples around naturally blighted trees were well below the ppm soluble salts of the soil in the control tree in the greenhouse. The control tree showed no blight symptoms so it would appear that the salt concentrations in the soil around naturally blighted trees are not high enough to cause the blight symptoms. From the analysis data (Table 2) it appears unlikely that a nitrogen or phosphorus deficiency causes the blight on pines.

While collecting the soil samples from around blighted trees it was observed that a shallow caliche hard pan was under the area where the trees were growing. The hard pan ranged from 2-5 feet or more in depth and was extremely hard. Trees planted in an area where the hard pan was approximately 2 feet deep remained about the size they were when they were planted, 3-4 feet tall, while trees planted at the same time in deeper soil were 10-15 feet tall. The small trees were observed over 2 years time and it was noted that they often blighted severely and then recovered by putting out new growth. The blighting appeared to be connected with cold weather or drying winds. It is possible that the shallow soil did not hold soil moisture effectively and moisture was unavailable to the needles during periods of increased transpiration.
Numerous fungi were found to be associated with blighted Aleppo pines but only *Sphaeropsis* sp. showed any indications of being pathogenic. Fungi representing several genera could be reisolated after 2 or more months from inoculated pine trees but none appeared to be more than saprophytes or weak pathogens. *Pullularia pullulans* reported by Patton and Riker and others (68, 74, 75) as a parasite associated with blight or needle droop of *P. resinosa* and as a saprophyte or weak parasite it was observed in the same role on *P. halepensis*. *Sphaeropsis ellisii* has been reported as pathogenic on *Pinus* spp. or as weakly pathogenic on pines growing under poor climatic or soil conditions (78, 99). A *Sphaeropsis* sp. was found to be associated with blighted Aleppo pines but was only weakly parasitic on inoculated trees grown in the greenhouse. It seems unlikely that any of the fungi associated with blighted *P. halepensis* trees is the primary cause of the blight. However, it was observed many times that these associated fungi can quickly colonize dead or dying pine tissues.

The cause of the disease is more likely to be a physiological phenomenon than a fungus parasite. Several reports have been given of blights on *Pinus* spp. caused
by drought or unavailable soil moisture under conditions of rapid transpiration (16, 74, 75, 78). When this occurs plasmolysis or dessication of cells in the fascicles cause the needles to droop or become blighted, making them susceptible to invasion by weak parasites or saprophytic fungi. The possibility of these conditions existing in Southern Arizona are excellent. Much of the soil in which Aleppo pine trees grow is shallow and poorly drained because of an underlying caleche hard pan. At the same time Arizona is well known for its rapid temperature changes, its low rainfall, and its low humidity. Fall and spring winds coupled with low humidity could create a condition causing rapid transpiration from the pine trees when soil moisture is low because of the lack of winter rains and decreased irrigation during the winter months. The poor root systems observed on blighted Aleppo pines along with the noticeable lack of feeder roots and mycorrhizal development would indicate that the blight symptoms might be caused by a disturbance of the roots by parasitic microorganisms, insects, or poor water and soil relationships. It is strongly indicated that further investigations of Aleppo pine blight should be directed towards a study of the root systems of blighted trees.
SUMMARY

A survey was made of the fungi associated with a blight of Aleppo pine, *Pinus halepensis* Miller and into the conditions favoring the development and persistence of the associated fungi. Representatives of over 20 genera of fungi were isolated from foliage, branches, and from roots of blighted Aleppo pine. Fungi consistently isolated or previously reported as pathogenic on *Pinus* spp. or other plants were screened for pathogenicity to *P. halepensis* in greenhouse and laboratory experiments. Representatives of the genera *Alternaria*, *Coniothyrium*, *Fusarium*, *Gelasinospora*, *Phoma*, *Peyronella*, *Pullularia*, and *Sphaeropsis* were able to maintain themselves on inoculated plants for 2 or more months but apparently were saprophytic or only weakly pathogenic. These fungi did not cause blight symptoms on inoculated plants but they were found to quickly colonize pine tissues dying or dead from some other cause.

Histological examination of blighted tissue revealed fungus hyphae in the wood only once in living tissue. *Alternaria* sp. and *Pullularia pullulans* were shown to penetrate and colonize the tissues of dead pine needles.

A fungicide spray program was initiated in an effort to control blight symptoms. Two fungicides Bordeaux mixture (8-8-100) and Actidione PM (cycloheximide) 100 ppm
were used in the experiment. The fungicides appeared to have no effect on blight symptoms indicating the possibility that a parasitic fungus attacking the pine foliage was not the primary cause of the blight.

A study was made of the effects of physiological drought on P. halepensis trees in the greenhouse. Blight symptoms were induced on plants treated with sucrose or CaCl₂ solutions added to soil in which they were growing. An analysis of soil from near the roots of blighted greenhouse trees and naturally blighted trees indicated that an excess of total soluble salts in the soil near roots was not the cause of Aleppo pine blight.

The cause of blight symptoms on the foliage appears to be a physiological problem. Drought, excessive transpiration, or poor development of the root system probably are among the major causes.


