THE FUNGITOXICITY OF CAPTAN AND PHALTAN IN VITRO AND
IN VIVO AGAINST CERATOCYSTIS ULMI (BUISM.) C. MOREAU

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THE FUNGITOXICITY OF CAPTAN AND PHALTAN IN VITRO AND
IN VIVO AGAINST CHROATOCYSTIS ULMII (BUI.S.M.) C. NOBEAUS

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CHAPTER I

INTRODUCTION

The invasion of the United States by the Dutch Elm Disease in 1930 presented a potential threat to millions of valuable shade trees in this country. The disease is caused by the fungus Ceratocystis ulmi which was first classified by Buisman in 1922. The disease did not spread as rapidly as did the chestnut blight which decimated the American chestnut within a few years following its introduction in 1892 (Sill, 1963); therefore the disease offers the opportunity of time to find a suitable control.

To date, no suitable method has been found. Several methods of control have been developed and advocated by various sources, but no one method has gained recognition as ideal.

The spread of Dutch Elm Disease is variable. Originally it was thought that transmission was only by the European elm bark beetle, Scolytus multistriatus Warsham, and the American elm bark beetle, Hylurgopinus rufipes.

Eichoff, (Middleton, 1935), but it has been recently demonstrated that the fungus can be transmitted through root grafts, by wind, and contaminated tree pruning equipment.¹

Many methods have been suggested for the control of the disease and one of the more interesting and promising ones is the use of Captan (N-trichloromethylthiotetrahydrophthalimide). Elm seedlings were fed Captan at the soil surface and two months later were analyzed in vitro by extracting the leaves in acetone and applying the residues of these extracts to petri dishes containing potato dextrose agar which had been seeded with spores of Ceratocystis ulmi. Results of this investigation showed that these extracts inhibited the growth of Ceratocystis ulmi even though Captan was not chemically identified in these extracts.²

In addition to Captan another compound, similar in chemical nature, Phaltan (N-trichloromethylthiophtalimide)


²Curtis May, John G. Palmer, and Edward Hacskalo, "Inhibition of Growth of Ceratocystis ulmi in vitro by Residues from Extracts of Soils and of Plants Growing in Soils Treated with Captan or Orthocide 50W," Plant Disease Reporter, XLII (May, 1958), 696-702.
has been used, and is another commonly available fungicide.

The purpose of this investigation was to determine the fungicidal properties of Captan and Phalan in known concentrations in vitro and then to determine the effects of these fungicides in elm seedlings which were artificially infected with the fungus of Dutch Elm Disease.
The first report of Dutch Elm Disease occurred when Tubeuf reported in 1920, that the blossoms on certain elm trees appeared before the foliage in the spring, and although the green fruits temporarily acted as leaves before becoming ripe, the foliage never developed so that the branches appeared bare after the fruit dropped.\(^1\)

The first report found as to the cause of the disease appeared in 1922 when Spierenberg reported:

This disease which was seen in various parts of the Netherlands for the first time during the year 1920 and which seems to be becoming of very great importance, manifests itself by a rapid wilting and dying of the tops of the trees or of single branches, while the tree seems to lack food and water. The branches and stem in cross section show small dark spots in the rings adjoining the bark. Cultures from discolored portions of the wood have yielded a number of fungi; the work on etiology is to be continued.\(^2\)

A fungus as an agent of the disease was confirmed by Spierenberg when she was successful in 1922 of artificially infecting elm trees by transferring some of these isolated

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fungal colonies to uninfected trees.\(^1\)

According to Boyce\(^2\) the term Dutch Elm Disease does not describe the country of origin but rather is the name given because of the early and excellent investigation of it by plant pathologists in Holland. The disease was first noted in northern France, in Belgium, and in Holland. Since then it has spread north to Sweden; east to Russia, the Balkans, and Italy; south to Portugal and Spain; and west to Great Britain. Boyce further states that although the origin of the pathogen is obscure it apparently was brought to Europe from Asia during the First World War.

However, for several years there was a great deal of confusion as to the causative agent of the disease. Micrococcus ulmi was supposedly identified as the causative agent of the disease by E. Brusoff in 1927 as stated by Stapp.\(^3\) This report was discounted by the work of Wollenweber and Stapp\(^4\) in 1928 who showed that the disease was caused by the

\(^1\)Ibid., p. 58.


\(^3\)Carl Stapp, "Über die Ursache des Ulmensterbens," Mitteilungen der Deutschen Dendrologischen Gesellschaft, XL (1928), 139-46.

fungus Graphium ulmi. The latter report described the successful technique of growing the fungus on potato juice agar to which dextrose had been added. Furthermore, "The assumption that Graphium ulmi is conveyed by insects is supported by the discovery in June, 1928, of luxuriantly fructifying mycelium of the fungus in the galleries of the bark beetle, Scolytus scolytus." 

Graphium ulmi was classified by Marchal when he confirmed Miss Christine Buisman in her classification of Graphium ulmi as the agent of Dutch Elm Disease.

Wollenweber\(^1\) described the growth of Graphium ulmi fungus as white to yellow, and covered with a mass of whitish, spore bearing muscilage. The aerial mycelium was composed of septate hyphae \(0.5\) to \(0.6\) \(\mu\) in diameter. The conidiophores are branched and produce conidia which are pyriform, straight, or occasionally slightly curved, and are unicellular. There are short sterigmata upon which the spores are borne. The conidiospores are ellipsoid and measured \(5.3\) to \(5.5\) \(\mu\) by \(1.1\) to \(1.7\) \(\mu\).

\(^1\)Ibid., p. 287.
\(^3\)Wollenweber, op. cit., p. 200.
\(^4\)Ibid.
Further clarification of this classification was made by Lilly and Barnett\(^1\) (1951) when they showed that *Ceratostomella ulmi* had a non-explosive type of ascus in which the ascospores are released by the deliquescence of the ascus wall. The ascospores are embedded in muscilage and as they accumulate in the perithecium, some spores ooze out through the ostiole much like toothpaste from a tube. The spores are not well adapted to wind dissemination and thus are carried by insects or travel by some other means.

Finally in 1956, Hunt\(^2\) described *Ceratocystis ulmi* as the perfect stage of *Graphium ulmi*. The perithecia are rarely found in nature but coremia are found very commonly. The species had been classified as a *Graphium* because of the lack of observation of the perithecia.

The fungus lives in the sapwood, fruiting on the wood of dead and dying trees according to Boyce.\(^3\) The coremium consists of black stalks one millimeter high which produces vast numbers of these egg-shaped sticky spores. This is the *Graphium* stage of development.\(^4\) In vessels, spores increase


\(^3\)Boyce, *op. cit.*, p. 314.

\(^4\)Ibid.
abundantly by budding in a yeastlike manner. Zentmeyer\(^1\) quoting Banfield, states that *Ceratocystis* spores move rapidly in the spring vessels during the growing season. Spores introduced at the base of a tree were recovered sixty-two feet higher in the crown one hour after inoculation with a spore suspension.

The exact mechanism of how the fungus kills the tree is still unknown. Originally it was thought that the fungus caused a plugging of the vessels with gums and tyloses. Zentmeyer states the confusion when he mentions that this is the probable cause of the tree's destruction but mentions that the gum plugs did not appear for several days after inoculation. However, in this time, conduction of liquids was reduced as was shown experimentally when water was forced into the tree by injection and discoloration and wilting still occurred.\(^2\)

Beckman\(^3\) stated that apparently occlusion results from the hydrolysis of pectic substances in the middle lamellae and primary cell walls. The resulting molecular fragments aggregate to form vascular blocks that reduce the water-carrying capacity of the vessels. *Ceratocystis ulmi*

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\(^1\) Zentmeyer, op. cit., p. 10. \(^2\) Ibid., pp. 18-9.

\(^3\) Charles H. Beckman, "Production of Pectinase, Cellulase, and Growth Promoting Substances by *Ceratocystis ulmi*," *Phytopathology*, XLVI (June, 1956), 605-9.
produces a substance similar to indolacetic acid and could be important in the disease mechanism because residues of this reaction act as agents to produce the plugging of vessels.

Zentmeyer\textsuperscript{1} went further by filtering the fungal mats of *Ceratocystis ulmi* grown on potato dextrose agar in a Berkefeld filter and then injected the filtrate into American elm trees. He discovered that typical symptoms occurred: young leaves wilted, died, and fell from the trees and that older leaves curled. In addition the walls of the xylem vessels became discolored and gum plugs formed in some vessels only three days after inoculation. From these results he concluded that wilting, resulting from introduction of *Ceratocystis ulmi*, was not due to the fungus directly but rather to some unnamed toxic substance.

More recent investigations by Kurtzman, as quoted by Sylvan,\textsuperscript{2} showed that xylem vessels only rarely are occluded, and no interference with capillary action occurs, but rather that active absorption is stopped. Using an extract of the fungus, he caused wilting in trees but could

\textsuperscript{1}Zentmeyer, op. cit., p. 7.

prevent this wilting by mixing adenosine-tri-phosphate with this fungal extract. He believed that adenosine-tri-phosphate supplies the energy necessary to push the water through the membrane but that **Ceratocystis ulmi** produces an anti-adenosine-tri-phosphate compound which interferes with active absorption.

The control of Dutch Elm Disease has followed several patterns since its discovery in this country. Curtis May\(^1\) discussed the rapid spread of the disease and mentioned that the first diagnosed case of the disease appeared in Cleveland, Ohio in 1930. He further stated that **Scolytus multistriatus** Marsham, had been imported into Boston in 1925. Dr. Christine Buisman identified the Cleveland disease and later found hundreds of diseased trees in New York and New Jersey. On the basis of this report a huge campaign was launched to eliminate the disease by removal of diseased trees but it was soon apparent that the disease was too well established to be controlled in this manner.

The rate at which the disease spreads can be seen from the reports of Neely and Campana\(^2\) who studied the


progress of the disease in Illinois. The first reported case was found in southeastern Illinois in 1950 and by 1960 it had been reported in every county of the state. In some locations, such as the Champaign-Urbana area, over 67 per cent of the trees had died by 1960.

Chemotherapy as a means of control of Dutch Elm Disease was first attempted by Zentmeyer and Wallace\(^1\) who worked with 8-hydroxyquinoline sulphate and disodium ethylene bis-dithiocarbamate (Dithane) applied to soil at the rate of two grams per liter. These authors concluded the substances did some good in controlling the disease.

The next work reported was the tremendous effort by Zentmeyer, Horsfall, and Wallace\(^2\)(1946), who worked on the disease transmission, toxicity, rate of spread, and its chemotherapy. Using over thirty-seven chemical compounds in various concentrations, and methods of application, they attempted to find a control for this disease. At the end of their experimentation they concluded that of all the compounds tested, 8-hydroxyquinoline sulphate worked the

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\(^{2}\)May, Palmer, and Hacskalo, *op. cit.*, pp. 44-57.
best in disease control even though a tree showed symptoms of the disease after receiving forty-three gallons of a one gram per liter, 8-hydroxyquinoline sulphate solution, over a one year period of time.

Diamond\(^1\) quoting Went mentioned that in her work with 8-hydroxyquinoline sulphate applied on the surface of the ground and in sub-surface application, to infected trees, that negative results occurred.

Potter\(^2\) (1954), reported that 8-hydroxyquinoline benzoate was partially successful in controlling Dutch Elm Disease in artificially infected trees and in addition H.D. 109 (2-carboxymethyl mercaptobenzothiazole) was somewhat more effective in control. Additional research by Potter and May\(^3\) again demonstrated the effectiveness of H.D. 109 and the lesser phytotoxicity of 8-hydroxyquinoline benzoate.

Beckman\(^4\) (1958) reported a variation in Dutch Elm Disease in artificially infected trees and in addition H.D. 109 (2-carboxymethyl mercaptobenzothiazole) was somewhat more effective in control. Additional research by Potter and May\(^3\) again demonstrated the effectiveness of H.D. 109 and the lesser phytotoxicity of 8-hydroxyquinoline benzoate.

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chemotherapy. Using M.T.M.A. (4,5-dimethyl, 1,2-thiazole-ethylmercaptoacetate), he found an inhibition in the sapwood formation in *Ulmus americana*. Inhibition of the spread of *Ceratocystis ulmi* in treated trees was reported since the fungus will grow only in new sapwood and the formation of this sapwood was retarded by the action of M.T.M.A.

Using another approach, Beckman\(^1\) (1959) used a 500 parts per million solution of 2,3,5,6 tetrachlorobenzoic acid in oil and painted two inch strips on trees during bud break. He treated another group of trees later in a similar manner when the trees were at full leaf. All of the trees were inoculated with conidiospores and analyzed one month later. Beckman found that 52 per cent of the trees treated at full leaf developed the disease but only 16 per cent of those treated at bud break developed the disease.

Epstein and Smalley\(^2\) (1962) used sodium hypochloride on one year old Elm seedlings as a soil drench, in dilutions ranging from one part to 5000 parts sodium hypochlorite per million of water. They reported that there was no control in the ranges of one to 1000 parts per million and that

\(^1\)Carl H. Beckman, "Dutch Elm Disease Control with Polychlorobenzoic Acid," *Phytopathology*, XLIX (April, 1959), 227.

higher concentrations of sodium hypochlorite killed the trees.

In another experiment, Smalley (1962) found that 2,3,6-trichlorophenolacetic acid resulted in a high level of protection in nursery elms in the Milwaukee Municipal Nursery. Injection of the compound proved to be the best technique and resulted in no foliar toxicity. Histological examination of treated trees which had been infected with *Ceratocystis ulmi* showed that 2,3,6-trichlorophenolacetic acid had induced heavy tyloses in the large xylem vessels suggesting that vessel occlusion limited the spread of the disease.

Many commercial compounds have been advocated in the control of Dutch Elm Disease. Schreiber and Harrison (1962) used zinc chloride on fifty trees of 16.6 inches diameter and found that there was more wilting in the treated trees than those that had been untreated. They also tested "Tree Saver," a compound produced by the Sondel

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1Eugene B. Smalley, "Prevention of Dutch Elm Disease by Treatments with 2,3,6-trichlorophenol Acetic Acid," *Phytopathology*, LII (December, 1962), 1080-91.

2Laurence R. Schreiber, and James M. Harrison, "Results of Three Treatments for the Control of Dutch Elm Disease," *Plant Disease Reporter*, XIV (June, 1962), 401-3.
Company of Mt. Vernon, Ohio. In using this compound on infected trees they found that 81.3 per cent of the treated trees developed the disease while 82.8 per cent of the control trees developed the disease. A compound called "Soil Life" also was tested but these results were also negative.

Another form of control is in the area of genetics. Went\(^1\) first stated that *Ceratocystis ulmi* could be controlled by careful selective breeding of resistant forms of the tree.

May, Palmer, and Hacskaio\(^2\) (1958) tested theories of inheritance by using acetone extracts of leaves of various trees and tested these extracts in vitro against *Ceratocystis ulmi*. They found that none of the species of elms, including *Ulmus carpinifolia* var. Christine Buissman, exhibited any resistance to the disease although the Christine Buissman elm shows resistance in nature.

Spraying of trees as a method of control is the one most advocated today because of cost and speed of application. Hafstead and Reynolds\(^3\) (1961), reported that this


\(^2\)Curtis May, James Palmer and Edward Hacskaio, "In vitro Inhibition of *Ceratocystis ulmi* by Acetone Extracts of Leaves and Stems of Some Species of Higher Plants," *Plant Disease Reporter*, XLIV (March, 1958), 399-401.

treatment is 95 per cent effective in the control of the elm bark beetles if a solution of D D T is used as a dormant spray.

More recent methods of chemotherapy entail the use of systemic insecticides. One of the first compounds used was Chipman R-6199 (O,O-diethyl S-(2-diethylamino)-ethyl phosphorothioate) by Al-Azawi and Morris\(^1\) (1959). In their investigation they were able to reduce the number of beetle feeding niches by over 95 per cent when they injected this compound into the sapstream of trees and then applied beetles carrying the spores of *Ceratocystis ulmi* to both treated and untreated trees.

More recently, Bidrin has been used and in field tests 100 per cent protection against the elm bark beetles has been demonstrated. This compound, manufactured by the Wauget Company of South El Monte, California, has been field tested for several years and is currently being used in several cities.\(^2\)

Captan, which first appeared under the trivial name

\(^1\)A. F. Al-Azawi, and Dale M. Norris, Jr., "Experimental Prevention of Bark Beetle Transmission of *Ceratocystis ulmi* with the Systemic Insecticide Chipman R-6199," *Journal of Economic Entomology*, LII (October, 1959), 902-4.

of "Kittleson's Killer" in 1951,\(^1\) and Phaltan are commonly available fungicides that have the ability to penetrate the membrane of a fungus. Captan and Phaltan are both highly insoluble in water. Both Captan and Phaltan reach a maximum solubility of one part to one million of water.\(^2\) These compounds do not rely on the penetration of the adsorbed water on a cell membrane, but rather penetrate the cell membrane by being highly soluble in the lipid layer of the membrane.\(^3\)

This ability of a highly water insoluble substance's being effective in the control of Dutch Elm Disease is in sharp contrast to Stoddard's contention who maintains that *Ceratocystis ulmi* is an internal parasite and therefore it must be combated internally by a water soluble substance of high fungicidal action and a low toxicity to the elms.\(^4\)

Horsfall\(^5\) suggested that the action of Phaltan and Captan is due to their tremendous ability to penetrate the cell membrane of the fungus while still carrying a sufficient amount of toxicity to disrupt the cell's metabolism. Captan

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\(^2\)Ibid., p. 61. \(^3\)Ibid.


\(^5\)Horsfall, op. cit., pp. 77-8.
and Phaltan are metabolic inhibitors of oxidative enzymes, enzymes associated with phosphorus metabolism, and citrate synthesis. In citrate synthesis Captan and Phaltan inactivate coenzyme A. In addition the trichloromethyl group, when in the presence of sulfhydryl groups, forms thiophosgene which disrupts additional metabolic processes.

While both Captan and Phaltan are only slightly soluble in water, they persist for long periods of time in the soil and therefore are ideally suited to be used as a soil drench. Munnecke recorded that these compounds were found to be very stable 150 days after application as a soil drench.

Curtis May, Palmer, and Hacskalo reported that Ceratozystis ulmi was very sensitive to Captan in vitro. In their work, residues of leaves or stems were tested by bioassay on potato dextrose agar plates needed with Ceratozystis ulmi. In their report they never identified Captan in any extract, but inferred that Captan, or a closely allied chemical, was present in extracts of plants grown in treated soil.

1 Ibid., p. 117. 2 Ibid., p. 113. 3 Ibid., p. 119.
5 May, Horsfall and Hacskalo, op. cit., p. 696.
CHAPTER III

METHODS AND MATERIALS

The organism used in this investigation was a culture of Ceratocystis ulmi obtained from Roy McFall, then associated with the Dutch Elm Diagnostic Laboratory at Des Moines, Iowa, who had received the original sample from a diseased Elm tree.

The isolated mold was maintained by transferring it at maximum intervals of one month to petri plates containing sterile potato dextrose agar (Difco). The agar had been prepared from 3.9 per cent potato dextrose agar and distilled water, autoclaved at fifteen pounds of pressure for fifteen minutes in a Presto Canner Cooker (National Presto Industries, Inc.) and poured into plastic petri dishes nine centimeters in diameter.

The investigation was divided into two distinct parts. The first part was to test the action of Captan (Orthocide 50W, California Chemical Company) and Phaltan (Phaltan 75 per cent, California Chemical Company) in 

vitro. Both Captan and Phaltan are highly insoluble in water (one part Captan or Phaltan to 1,000,000 of water) but are prepared in a wettable base. A series of experimental petri plates of potato dextrose agar (Difco) plus
Captan or Phaltan in varying concentrations were prepared. The concentrations of the fungicides used were 1:10,000, 1:5000, 1:2000, 1:1000, and 1:500 prepared by adding the appropriate dry weight of fungicide to the distilled water. The fungicides were added before sterilization. Each plate was then inoculated by a stab technique five times in a regular pattern from a ten day old stock culture of Ceratocystis ulmi, on the culture plate. The plates were incubated at 22°C Centigrade in an oven (LaPine, Model 317-86-A) and examined daily for growth. No growth was apparent in any of the plates containing the 1:500 or 1:1000 concentrations of Captan or Phaltan, after seven days of incubation, but growth did occur in plates containing lesser concentrations. Further experimentation showed that a concentration of one part Captan or Phaltan to 1500 parts of water was the minimal concentration of fungicide necessary to completely inhibit the growth of Ceratocystis ulmi. To further ascertain that this was the minimal concentration for growth inhibition, wood chips from trees known to be infected with Dutch Elm Disease were placed on five plates containing one part Captan to 1500 parts of water and five plates containing one part Phaltan to 1500 parts of water and no growth was noted in any of the plates used after seven days' incubation at 22°C Centigrade.
The second part of this investigation was an *in vivo* test to ascertain that these concentrations of fungicides were actually effective in controlling the disease in living trees. Two hundred Elm seedlings (*Ulmus americana*) were collected in a yard in the Village of Brown Deer, Wisconsin during July, 1963. The soil was gently loosened around the roots which then had any remaining soil removed by gentle rinsing in tap water. The plants were then transferred to six centimeter clay pots filled with garden loam soil from the same location as the trees had been obtained. The soil had been heated in a gas oven at 100°C Centigrade for two hours to eliminate most soil nematodes and fungi.¹

The elm seedlings obtained were from four to six centimeters in height measured from the ground line to the terminal portion of the main stem. This measurement was made with a fifteen centimeter plastic ruler, (General Biological Supply House).

The seedlings were transferred to wooden flats each containing thirty-six pots and moved to the Whitefish Bay High School greenhouse in Milwaukee, Wisconsin during September, 1963. On about November 1, 1963 active growth

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ceased and the leaves dropped. This normal dormancy was broken during the early part of April, 1964 as evidenced by the growth of new leaves and stem elongation. By April 10, 1964 all of the trees used in the in vivo test had produced new leaves and were showing stem elongation. Twenty seedlings were used in a preliminary test designed to find out if Captan or Phaltan had any deleterious effect when used as a soil drench. Watering of these twenty plants was done in two groups of ten each. One group received water with one part of Captan to 100 parts of water and the other group of ten trees received water with one part of Phaltan to 100 parts of water. No adverse effects on growth were noted after one month of this treatment. This group of tests terminated the preliminary tests.

The remaining trees were watered daily with tap water and once every two weeks were watered with a solution of Hyponex (Hydroponic Chemical Company, Copley, Ohio) a soluble fertilizer, following the manufacturer's recommendations. The trees reached an average height of from six to nine centimeters, measured from ground level to the top of the main stem, by June 1, 1964.

For testing the effects of the fungicides in vivo the trees were divided at random into ten groups each consisting of ten trees. Fifty trees were treated with Captan,
fifty trees with Phaltan, and ten trees were inoculated with Ceratocystis ulmi but untreated with fungicides and were to serve as control trees.

The fifty trees in each category were divided into five groupings based on the number of fungicide treatments they were to receive. The application of both Captan and Phaltan to the trees was made in the concentration of one part fungicide to 1500 parts of water. The applications were made every two days, following the inoculation of the trees with Ceratocystis ulmi, by applying these concentrations to the soil surface of the potted trees. A thirty milliliter quantity was used as this amount of fluid just saturated the soil as determined by the soil's appearance. Using this method of application of Captan and Phaltan, ten trees received one thirty milliliter application of Captan and ten trees received one thirty milliliter application of Phaltan. Similarly, ten trees received three applications of either Captan or Phaltan; ten trees received five applications of either Captan or Phaltan; ten trees received eight applications of either Captan or Phaltan; and ten trees received ten applications of either Captan or Phaltan. The trees were grown on the north side of Harvey Ingham Hall in room 112. There was no attempt to control the temperature
which ranged from 22° Centigrade to 30° Centigrade during the period of investigation. At these higher temperatures rapid drying of the soil occurred and additional tap water was added to prevent dessication.

The method of inoculation of the experimental trees with Ceratocystis ulmi was the same method as that employed by Zentmeyer who succeeded in infecting over 95 per cent of the trees employed in his tests, by introducing the fungus into the sapstream by cutting to the xylem vessels. The technique employed in this study was to select trees in active growth, take a sharp microtechnique scalpel (Turtox, Model 308 A 16) and cut a vertical slit one centimeter long through the bark. The fungus was introduced by scraping two ten day old fungal mats of Ceratocystis ulmi from a potato dextrose agar (Difco) plate, diluting these mats in ten milliliters of distilled water, and then pipet one drop of this suspension from a seven centimeter pipet into this cut.

The trees were examined daily for any of the symptoms of the disease such as drooping of the leaves, discoloration of the leaves, curling of the leaf margins or leaf drop. No attempt was made to grade the results. If a tree showed any one of the symptoms, it was considered to be diseased.

The inoculation of the trees with Ceratocystis ulmi

1Zentmeyer, op. cit., p. 9.
was done on June 13, 1964. This date in June was chosen as it had been shown by Zentmeyer\textsuperscript{1} that almost 100 percent infection occurred in his trees at this time of the year. Preliminary results of ten trees inoculated in the same manner on May 15, 1964 showed typical symptoms of the disease by June 8, 1964 for a total of twenty-five days from the time of inoculation with Ceratocystis ulmi to the time when symptoms of the disease were detected.

Based on these results, a twenty-five day period of time was allowed for this investigation, and the investigation was terminated July 8, 1964.

\textsuperscript{1}Ibid., p. 12.
CHAPTER IV

RESULTS AND INTERPRETATION OF DATA

Ceratocystis ulmi is highly susceptible to the action of Captan and Phaltan in vitro. Colonies observed on potato dextrose agar (Difco) plates containing Captan or Phaltan completely inhibited the growth of Ceratocystis ulmi in concentrations of one part fungicide to 1500 parts of water. Lesser concentrations of Captan and Phaltan retarded the growth of Ceratocystis ulmi greatly but failed to completely inhibit growth.

Captan and Phaltan, in concentrations of one part fungicide to 5000 parts of water or greater, caused changes in the appearance of the mold when grown on potato dextrose agar. The changes observed were: (1) a color change in the mold from a normal white color to a dark brown-grey color as the concentration of Captan or Phaltan was increased, and (2) a decreased rate of growth.

Horsfall\(^1\) mentioned that Captan and Phaltan are inhibitors of fungal enzyme systems, particularly enzymes associated with the respiratory cycle. The decreased rate

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\(^1\) Horsfall, op. cit., p. 117.
of growth or the complete inhibition of growth of the fungus grown in association with Captan or Phaltan apparently results from the inability of the fungus to metabolize foods properly and release the necessary energy to maintain vital activities.

Preliminary tests showed that Captan and Phaltan exhibited no deleterious effects in the growth of two year old Elm seedlings (Ulmus americana), in vitro when used at the rate of one part fungicide to one-hundred parts of water by weight. May, Palmer and Hacskalo\(^1\) reported that three year old Elm seedlings developed stiff and brittle leaves when treated with Captan equal to 1 per cent Captan to the weight of air dried soil.

The results of the in vivo test using varied numbers of applications of either Captan or Phaltan showed that a total of 46 per cent of those trees treated with Captan died while a total of 56 per cent of those trees treated with Phaltan died. It is, however, interesting to note that of the groups receiving eight and ten applications of Captan or Phaltan, there was an average survival of 85 per cent of those treated with Phaltan and a 75 per cent survival of those trees treated with Captan. It appears from these

\(^1\)May, Palmer, and Hacskalo, op. cit., p. 696.
TABLE I

RESULTS OF IN VIVO TESTS USING A SUSPENSION OF ONE PART CAPTAN TO 1500 PARTS OF WATER ON TREES (ULMUS AMERICANA), ARTIFICIALLY INOCULATED WITH CERATOCYSTIS ULMI TWENTY-FIVE DAYS EARLIER

<table>
<thead>
<tr>
<th>Number of 20 ml. applications</th>
<th>Number of trees in test</th>
<th>Per cent of trees showing symptoms of Dutch Elm Disease</th>
<th>Per cent of trees surviving</th>
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<td>0</td>
<td>10</td>
<td>80</td>
<td>20</td>
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<tr>
<td>10</td>
<td>10</td>
<td>30</td>
<td>70</td>
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</tbody>
</table>
### Table II

**Results of In Vivo Tests Using a Suspension of One Part Phaltan to 1500 Parts of Water on Trees (Ulmus Americana), Artificially Inoculated with Ceratocystis Ulmi Twenty-Five Days Earlier**

<table>
<thead>
<tr>
<th>Number of 30 ml. applications</th>
<th>Number of trees in test</th>
<th>Per cent of trees showing symptoms of Dutch Elm Disease</th>
<th>Per cent of trees surviving</th>
</tr>
</thead>
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<tr>
<td>0</td>
<td>10</td>
<td>80</td>
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</tr>
<tr>
<td>1</td>
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<td>60</td>
<td>40</td>
</tr>
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<td>90</td>
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</tr>
<tr>
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<td>90</td>
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<tr>
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<td>10</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>
data that trees receiving a greater number of Phaltan applications showed a higher survival rate than those treated with Captan.

In the trees receiving three to five applications there was an average survival of 50 per cent of those trees treated with Captan but only a 10 per cent survival of those treated with Phaltan. Apparently at a lower number of treatments, Captan was the more successful substance in the reduction of the disease.

The control plants exhibited an 80 per cent rate of infection. Most investigations have shown that artificially infected plants also are infected at a rate of less than 100 per cent. It had been decided to use the month of June for this investigation, because Zentmeyer had recorded an almost 100 per cent rate of infection in trees inoculated with Ceratocystis ulmi at this time of the year. It is believed that the percentage figure for these control plants might have been higher if a greater number of trees had been used in this investigation.

Those trees receiving one application of Captan showed a 10 per cent lower rate of survival than those trees receiving one application of Phaltan. However, it is believed that again the small number of trees used in this investigation could have influenced these results.
There appears to be a trend in the reduction of the number of diseased trees as the number of applications of fungicides were increased. In the review of previous work in the area of chemotherapy, particularly that of Zentmeyer, there are reports which also indicate this trend of reduction of the incidence of the disease by increasing the number of applications of chemotherapeutic agents.

The erratic points in these data are possibly due to several factors. One of these might be the selection of a date to terminate the experiment. This date was selected based on a preliminary investigation which showed that a twenty-five day period of time was a sufficient period of time to allow for the development of the symptoms of Dutch Elm Disease. These preliminary tests were conducted almost one month earlier and the virility of the disease varies as to the time of the year infection occurs.

Another factor which may have played a role in these data were the temperature extremes in the room where the experiment was conducted. One of the criteria used in the determination of diseased trees was whether the leaves showed signs of loss of turgor and on the day that the results were read, the room temperature was 30° Centigrade.

The application of Captan and Phaltan did show a reduction in the incidence of the disease in the greater
numbers of treatments of one part fungicide to 1500 parts of water. There was a marked increase with both compounds between five applications and eight applications, in the reduction of the incidence of the disease. Both compounds did not exhibit any deleterious effects on the growth of elm trees used in this test.

Both compounds were found to have the ability to completely inhibit growth of *Ceratocystis ulmi* in *vitro* at a concentration of one part Captan or Phaltan to 1500 parts of water in potato dextrose agar.

Further research with Dutch Elm Disease could be carried on in other areas. One of these areas is genetic control with the breeding of disease resistant varieties of elms. Another area would be in the production of agents effective in the control of the elm bark beetles. Work in this area is promising but more research is indicated.

Both preventative and curative chemotherapy should also be continued as method of internal control of the disease. Captan and Phaltan showed definite control in this investigation and continued research is recommended.
CHAPTER V
SUMMARY

The literature shows that there exists a great deal of interest in the Dutch Elm Disease. Much work has been done in this country and in Europe in the area of chemotherapy but there still is very little agreement on the substances that should be used in the control of the disease.

It was interesting to note that although acetone extracts of Captan, from Captan treated plants, showed an inhibition of Ceratocystis ulmi in vitro there was no follow up work done in this area. There appear to be few studies on the effects of Phaltan in the area of Dutch Elm Disease control.

In this investigation both Captan and Phaltan were analyzed experimentally by testing their effects by incorporating them in potato dextrose agar petri plates. The fungicides were tested in various concentrations based on the amount of water used in the making of the potato dextrose agar. It was found in this investigation that a concentration of one part fungicide to 1500 parts of water would completely inhibit the growth of Ceratocystis ulmi in vitro.
The second part of the investigation was the application of this concentration of fungicide to elm trees that had been artificially inoculated with Dutch Elm Disease. The fungicide suspension was added to the soil beginning the day of the inoculation of the tree with the fungus. This part of the investigation required twenty-five days in order to evaluate the effectiveness of Captan and Phaltan in vivo. Additional experimentation showed that Captan and Phaltan exhibited no deleterious effects on the growth of the elm trees used in this test.

In vivo evaluation of Captan and Phaltan showed that as the number of applications of the one part fungicide to 1500 parts of water were increased there was a considerable reduction in the incidence of the disease in trees that had been artificially inoculated with Ceratocystis ulmi. In using the two compounds in ranges of one to ten applications, Captan reduced incidence of the disease by 54 per cent while Phaltan reduced the incidence by 46 per cent. In the range of eight to ten applications of these compounds, Phaltan was 10 per cent more effective in disease control.

Both compounds show the possibility for use as chemotherapeutic agents in the control of Dutch Elm Disease. Both compounds apparently were compatible with elm trees when used in the manner of this investigation.
On the basis of this study the use of Captan and Phalan as chemothrapeutic agents in the control of Dutch Elm Disease is recommended and it would be profitable to study these agents further.
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