BIOLOGY AND CONTROL OF *CONIOPHORA EREMOPHILA* ON LEMON IN ARIZONA

by

Donna Marie Bigelow

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SIGNED: Donna M Bigelow

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Robert L Gilbertson — 5/5/95

Robert L. Gilbertson
Professor of Plant Pathology

Date

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ABSTRACT

A field survey of mature lemon trees showed an average of 30% of the trees with symptoms of brown heartwood rot caused by *Coniophora* sp. The temperature range of growth in culture for *Coniophora* is 15-40C with growth optimum at 30C. Vegetative incompatibility trials from one mature orchard show isolates from different trees are incompatible. In wood block decay studies, the average weight loss over 20 weeks was 5-20%. In comparison, decay studies comparing *Coniophora* with other brown or white rotting fungi, the other fungi decayed 4-8 times more in vitro. Cultural characteristics include simple septate hyphae, moderately growing mycelium that develop crustose brown to brownish-black patches as they mature, and negative for polyphenol oxidases. In vitro fungicide trials show that only NECTEC paste was effective in reducing decay on lemon blocks inoculated 15 weeks with *Coniophora*. SEM studies show mycelial fragments, pit enlargement, in radial plates, cracking and disintegration of wood.

INTRODUCTION

In 1992 a *Coniophora* species was first reported associated with a brown heartwood rot occurring in lemon trees in Yuma, Arizona (Matheron et al. 1992). This decay had been known to occur as a serious problem in lemon for at least 30 years. It was the first report of a *Coniophora* species causing heartwood decay in living citrus or any other fruit trees. Surveyed mature orchards have a high percentage of lemon trees with visible brown heartwood rot. This decay is associated with a progressive dieback and decline and reduction of fruit production in infected trees. Growers consider this the most important pathology problem in lemon orchards in the Yuma region (Bigelow et al. 1994).

Arizona harvested lemons from 16,300 acres of orchards in the 1992-93 season, 14,800 acres of that from Yuma County and the remainder from Maricopa County (Sherman, 1993). Although the decay is known in Maricopa County, the problem is not considered to be serious in orchards there.

The only known *Coniophora* species in the Sonoran desert region was described by Lindsey and Gilbertson (1975). It was found on many desert trees, shrubs, and cacti, mainly as a saprobe on dead fallen trees and associated with brown rot. *Coniophora eremophila* has been found in the Sonoran desert and

identified from dead limbs of *Olneya tesora* Gray (ironwood), *Chilopsis linearis* (Cav.) Sweet (desert willow), *Carnegiea gigantea*, (Engelm.) Britt & Rose (saguaro), Juglans major (Torr.) Heller (Arizona black walnut), *Arctostaphylos pungens* H.B.K. (manzanita), *Fraxinus pennsylvanica* Marsh. ssp. *velutina* (Torr.) G.N. Miller (velvet ash), *Opuntia fulgida* Engelm. (jumping cholla), *Sambucus mexicana* Presl. (Mexican elder), collected from Arizona, and on *Juniperus monosperma* (Engelm.) Sarg. (one-seed juniper) from New Mexico. It was also reported on mesquite *Prosopis velutina* Woot. (velvet mesquite) (Gilbertson et al. 1976).

Since no other *Coniophora* species have ever been found in the Sonoran Desert, the heartwood rot found on lemon is probably caused by *Coniophora eremophila*. No fruiting bodies have been found associated with the disease in lemon.

The latest monograph on *Coniophora* recognizes 12 species (Ginns, 1986). Only 4 species are known to occur in Arizona (Gilbertson, 1974; Gilbertson et al., 1974, 1979). The other three species occur in conifer forest ecosystems at higher elevations in Arizona. *Coniophora eremophila* was described by Lindsey and Gilbertson (1975) from the Sonoran desert and also reported by Ginns (1982) from Chile.

In North America, most of the 12 *Coniophora* species described cause decay on dead wood. Only *C. puteana* and *C. eremophila* are reported to cause decay in live trees. Most

North American species are found on both gymnosperms and angiosperms in contrast to most brown rotting fungi which are found primarily on gymnosperms (Gilbertson, 1980, 1981; Ginns and Lefebvre, 1993).

The 1989 plant disease clinic database at The University of Arizona Department of Plant Pathology has records of diseased plants brought into the clinic from 1920 through 1989 when the disease clinic closed. Of the 467 citrus entries no disease problems where fungi directly affect heartwood were recorded. (Mihail and Nelson, 1989).

Collections listed in the database from the Arizona Mycological Herbarium at The University of Arizona, Tucson, Arizona have only 14 entries of fungi associated with citrus. The only fungus attributed to brown rot is *Amylosporus campbellii* (5 records). This is a root and buttrot fungus and is not known to cause decay in trunks and branches as does *C. eremophila*.

In the recent literature on citrus diseases, of the 26 diseases listed, no heartwood rot fungi were identified (Anonymous, 1984; Farr et al., 1989). The APS compendium of citrus diseases (Whiteside, 1988) lists six diseases associated with living wood and involving fungal pathogens. They are *Phytophthora*-induced foot rot, root rot or gummosis, *Armillaria mellea* or *Clitocybe tabescens* mushroom root rot, *Rosellinia* root rot (seen in tropical areas), heartwood rot

due to infection with *Fomes applanatus* and Rio Grande gummosis where a few causal agents have been associated, but *Physalospora rhodina* is thought to be the main causal agent. Other reported diseases in citrus include a root rot in Florida caused by *Clitocybe tabescens* (Knorr, 1980), a *Ganoderma* causing wood rot in Florida reported by Burns (1975), a root rot in California and Australia caused by *Armillaria mellea* (Munnecke, 1981; Broadbent, 1981), and a buttrot in grapefruit in Texas caused by *Ganoderma lucidum* (Skaria, 1978), (Gilbertson and Skaria, 1980). *Ganoderma lucidum* is known to occur on lemon in the Yuma area, but apparently is not an important pathogen there.

This report presents a field survey of mature trees, the morphological characteristics of the isolated fungus, temperature growth studies, decay loss studies in vitro, decay comparison studies with other wood rotting fungi, in vitro fungicide tests, and scanning electron microscope studies.

MATERIALS AND METHODS

Field survey

Orchards were surveyed in 1993 to determine the extent of heartwood rot in mature trees (29-31 yrs. old). Twenty-five trees were arbitrarily selected per orchard in 11 different orchards throughout the Yuma area. Trees were scored where symptoms of heartwood rot including brown rot, and/or an orange to brown discoloration in broken branches or trunks could be distinguished.

Cultural studies

Isolates of *Coniophora* were obtained from the following in the areas of Yuma, Lake Havasu and Maricopa from lemon heartwood rot: M-1, and M-2 and Y-1, from lemon heartwood in Yuma. LH-1 is from lemon heartwood, Lake Havasu; M-3 from lemon heartwood, Maricopa, Arizona. Later isolates were obtained from one mature orchard in Yuma (Northeast of Ave A extension and County 15th street and one isolate came from lemon heartwood rot from a tree from Coachella Valley, California (Table 1). The first five cultures were grown on 2% malt-extract agar (MEA) medium at 10C-45C, at 5 degree increments. Cultural morphology was studied as per Nobles (Nobles, 1965). Tannic (TAA) and gallic (GAA) acid agar media (Davidson et al., 1938) and gum-guaiac solution (Nobles, 1958) were used in tests for the presence of polyphenol oxidase. A

Table 1. Isolates of *Coniophora* and their collection sites.

Isolate #	Place collected
M-1†	Yuma, AZ
M-2†	Yuma, AZ
M-3‡	Maricopa, AZ
LH-1‡	Lake Havasu, AZ
Y-1‡	Yuma, AZ
A-1	‡Northeast orchard, Ext. Ave A and County 15th
A-2*	Northeast orchard, Ext. Ave A and County 15th
G	Northeast orchard, Ext. Ave A and County 15th
J	Northeast orchard, Ext. Ave A and County 15th
K-1	Northeast orchard, Ext. Ave A and County 15th
K-2*	Northeast orchard, Ext. Ave A and County 15th
MD	Northeast orchard, Ext. Ave A and County 15th
N	Northeast orchard, Ext. Ave A and County 15th
P	Northeast orchard, Ext. Ave A and County 15th
CA-1	Coachella Valley, CA

†M-1 and M-2 were collected in Yuma and studies started in September, 1992.
‡M-3, LH-1, and Y-1 were collected and studies began in May, 1993.
‡All Northeast orchard collections were from one orchard in Yuma, AZ collected on the same day, January, 1994.
*A-2 and K-2 came from the same tree as A-1 and K-1 respectively.
All cultures were isolated from brown heartwood rot in lemon trees.

1 cm mycelial plug of actively growing *Coniophora* was placed in the middle of gallic or tannic acid plates and actively growing cultures of *Coniophora* were tested by placing a drop of gum guaiac solution on the mycelium.

Temperature studies

To determine growth rate and optimum growth rate for *Coniophora* isolates, a 1 cm plug of actively growing mycelium from each isolate was placed on the edge of Petri dishes, and growth was measured and recorded daily. Plates were incubated at 5 degree increments in the dark from 10-45C. Each isolate was grown on 3 different plates at each temperature and the experiment was repeated twice.

Decay study

Decay loss for the five isolates M-1, M-2, M-3, LH-1 , and Y-1 was tested at optimum temperatures using lemon wood blocks in a vermiculite block test following the American Society for Testing Materials protocol (American Society for Testing Materials, 1993). Lemon wood was sawn into approximately 2½ cm^3 blocks, dried for 3 days at 93C. Blocks were weighed to the nearest .001g, soaked in water ½ hr, placed in French square chambers on vermiculite, 50 ml. of water were added, and the assembled chambers were autoclaved for 1hr. The blocks were inoculated with the fungus from an actively growing

culture. A 1 cm plug was aseptically placed within each chamber, or in later experiments a 1×2×3cm lemon wood feeder strip was inoculated 2 weeks prior to adding the lemon wood block to insure that there would be actively growing inoculum and infection by the fungus. At least five chambers were prepared for each fungal isolate at each temperature, and at least three controls (uninoculated blocks) were added. After incubation for 20 weeks, chambers were disassembled, blocks brushed free of mycelium, oven dried for 3 days and weighed. Percent weight losses were calculated (oven dry weight before decay minus the oven dry weight after decay/oven dry weight before decay X 100).

Comparison study

To compare the relative amounts of decay by *Coniophora* isolates versus other brown rotting or white rotting fungi, a block decay test using 5 *Coniophora* isolates, plus 5 other brown rotting and 4 white rotting fungi was done. The brown rotting fungi were *Paxillus panuoides*, *Heliocybe sulcata*, *Gleophyllum trabeum*, *Fomitopsis pinicola*, and *Fomitopsis palustris*. The white rotting fungi were *Trametes versicolor*, *Ganoderma lucidum*, *Spongipellis unicolor*, and *Perenniporia fraxinophila*. Chambers were assembled in the vermiculite block test procedure described above (decay study); feeder strips inoculated with the different fungi actively growing at least two weeks prior to addition of autoclaved lemon blocks. The

chambers were incubated at optimum temperatures for fungal growth, which in the case of *Coniophora* was 30C, but for all other fungi was 25C. After 15 weeks the chambers were disassembled, blocks brushed free of mycelium, dried at 93C for 2 days and weights recorded. Percent weight loss was calculated as described above.

Pairings in culture

Presumptive heterokaryotic isolates obtained from one mature orchard with a high percentage of symptoms were cultured and paired in all possible combinations on MEA by taking 2 cores from actively growing cultures and placing them approximately 1 cm apart in 60×15 mm Petri dishes. Nine isolates designated A-1, A-2 (isolated from the same branch of one tree), G, J, K-1, K-2 (K-1 and K-2 are from the same branch), MD, N, and P, were initially pure cultured from trees with heartwood rot symptoms. Isolates paired against themselves served as controls and all pairings were replicated at least two times. Hyphal interactions of opposing cultures were noted 2 wk after inoculation.

Fungicide study

First in vitro fungicide trials

Laboratory trials to test fungicide effect for four fungicides at concentrations .01-100 PPM showed that the fungus was greatly inhibited on potato dextrose agar plated with as little as 10 PPM (Gilbertson, et al. 1995). The

fungicides used were Fosetyl-Al (Aliette), CGA-173506 (an unreleased experimental compound from Ciba-Geigy corporation), Imazalil and Propiconazole (Tilt). To test fungicidal effects in vitro, the four fungicides and a wound dressing treatment, NECTEC paste (formulation: 1% Propiconazole, 2% Imazalil in a paint-like consistency) were applied to lemon blocks and placed in chambers with *Coniophora* isolate M-2 or Y-1. The lemon blocks were prepared following ASTM procedure for wood preservatives (ASTM, 1993). They were oven dried at 93C for 3 days, then weighed and recorded for oven dry weight. The NECTEC paste covered blocks were painted, dried overnight, and weighed to determine additional paste weight which was averaged and subtracted at the end of the trials. Blocks were submerged in three concentrations of each of the four fungicides, 10 PPM, 50 PPM, or 100 PPM. The submerged blocks underwent vacuum treatment for approximately 10 minutes at 10 psi to fully infiltrate the blocks with the fungicide. Blocks were then air dried in room conditions and weighed. The blocks were steam sterilized at 20 lbs. pressure for 20 minutes, aseptically transferred to chambers containing actively growing cultures of either M-2 or Y-1 isolates of *Coniophora*. The chambers were incubated at 30C for 15 weeks. They were then disassembled, blocks brushed free of mycelium, oven dried over 2 days at 93C, and weighed. Percent weight loss was calculated as described above accounting for

additional weight of NECTEC paste treatment blocks or fungicides (subtracting average NECTEC paste weight for calculations or using conditioned block weights for all fungicides rather than oven dried weights for final calculations).

Second in vitro fungicide trials

Since no significant effect but that of the NECTEC paste was achieved with initial fungicide concentrations, a second series of trials were run using higher concentrations of the fungicides. Aliette and CG-173506 were dropped from the study but higher concentrations of Propiconazole and Imazalil were tried. The effectiveness of NECTEC paste was tested using only the paint type paste with no active fungicide ingredients, while each concentration of Propiconazole (1%) and Imazalil (2%) was tested separately to determine which ingredient had the fungicidal or fungistatic effect. Blocks were set up similarly to the first trials following ASTM procedures for wood preservative screening. The lemon blocks were oven dried at 93C for 3 days, then weighed and recorded for oven dry weight. The NECTEC paste formulation with no fungicides was painted on the blocks, dried overnight, and weighed to determine additional paste weight which was averaged and subtracted at the end of the trials. Blocks were submerged in one concentration of each of the two fungicides,

10,000 PPM Propiconazole, or 20,000 PPM of Imazalil. The submerged blocks underwent vacuum treatment for approximately 10 minutes at 10 psi to fully infiltrate the blocks with the fungicide. Blocks were then air dried in room conditions and weighed. The blocks were steam sterilized at 20 lbs. pressure for 20 minutes, aseptically transferred to chambers containing actively growing cultures of either M-2 or Y-1 isolates of *Coniophora*. The chambers were incubated at 30C for 15 weeks. They were then disassembled, blocks brushed free of mycelium, oven dried over 2 days at 93C, and weighed. Percent weight loss was calculated as described above in the first fungicide trials.

Electron microscopy

Scanning electron microscopy on decayed and healthy lemon wood was done on tangential sections of wood using the following protocol:

Pieces of lemon wood were sawn into approximately 3X3X15mm blocks.

Fixation step in 3% glutaraldehyde, 0.1M PO_4 buffer pH 7.4 for 45 minutes

Rinsed in .01M PO_4 buffer pH 7.4 3 times for 10 minutes.

Postfixation in 1% osmium tetroxide in PO_4 buffer pH 7.4 for 45 minutes. Rinsed in distilled water 3 times for 10 minutes each rinse.

Dehydration through 30, 50, 70, and 95% ethanol for 10 minutes each percentage.

100% ethanol for 3 times for 10 minutes each change.

50% ethanol:50% Freon TF for 15 minutes.

100% Freon TF for 15 minutes.

Critical point dried.

Wood pieces were then immersed in liquid Nitrogen and scraped with cold razor blade.

Wood pieces were mounted and sputter coated with 60 nm gold and attached to stud with silver paint for SEM viewing.

RESULTS

Field survey

Trees were scored for no symptoms (0), dead tree (D), unable to ascertain symptoms (9), or symptomatic (1). Of the 11 orchards surveyed there was a range of decay occurring from 4-100% in mature lemon trees. The average decay percentage was 30% (Table 2).

Cultural studies

Key code following Nobles classification: 1.5.32.36.44.54 (Nobles, 1965). Hyphae at advancing zone hyaline, thin-walled, simple septate, double or multiple clamps rare on larger hyphae, 2-5 μm in diam, no asexual spores produced. Mycelia softens the agar. Growth rate moderate, dish covered in 3-4 weeks. Mycelial mat white to pallid pale buff with some brown crustose patches, turning brown to blackish brown in four weeks. Tests on gallic and tannic acid media negative for polyphenol oxidases. Growth on gallic acid medium 1.5 cm in two weeks (Figure 2). Growth on tannic acid medium 1.5 cm in two weeks. Negative reactions occurred with gum guaiac test (no color change).

Temperature studies

All cultures grew between 15 and 40C, but not at 10 or 45C (Figure 3). Optimum temperature for growth

Table 2. Orchard survey: Yuma Arizona, 1993.

Grove #	0†	1	9	D
1	46	42	13	0
2	8	29	38	5
3	79	4	13	0
4	79	4	13	0
5	54	17	33	0
6	17	83	8	0
7	0	100	0	0
8	92	4	4	0
9	42	25	38	0
10	79	4	17	0
11	33	21	38	0
Average	48	30	19	.45

Percent symptomatic lemon trees for brown rot where 25 trees (average age=29 years) were arbitrarily selected from each of 11 orchards from Yuma, Arizona, scored and average percent calculated for each category.

†0=no symptoms 1=symptoms 9=questionable for symptoms D=dead.

Figure 1. Wood decay symptoms in a mature lemon branch.

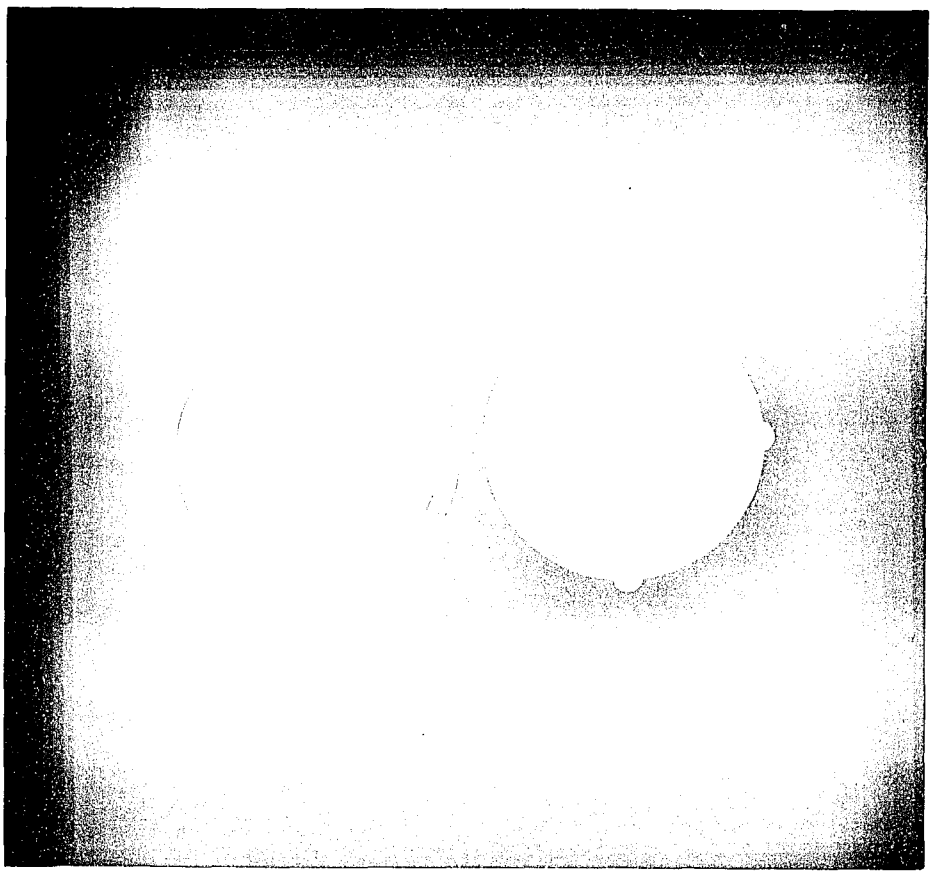

Figure 2. Cultures of *Coniophora* isolate A-1 grown on gallic and tannic media at 30C.

Left. gallic acid at 2 wk.
Right. tannic acid at 2 wk.

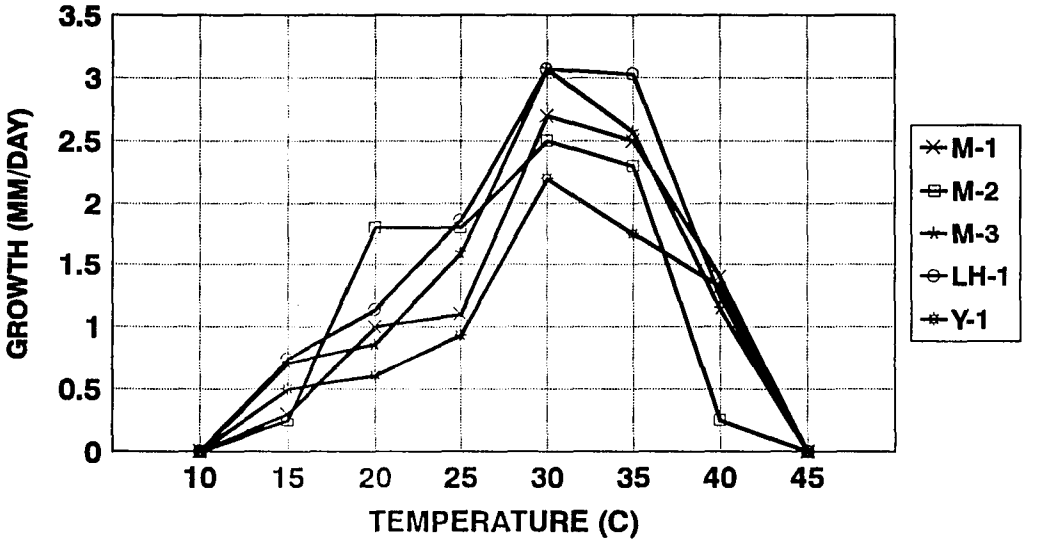

Figure 3. Growth rates of 5 *Coniophora* isolates at temperatures 10-45C.

was 30C averaging 2.3-3.1 mm per day (Figure 4).

Decay study

Average weight loss for blocks decayed by the five isolates of *Coniophora* was between 5-20%. Percent weight increased with temperature for all isolates (Figure 5). Significant differences were observed between M-1 and M-2 at 25C (Table 3) and between M-3, and Y-1 at 25 and 30C (Table 4).

Comparison study

Average weight loss for *Coniophora* isolates compared with all but one brown and all white rot species was far less in vitro at optimum temperatures (Table 5). 3-8 times as much weight loss occurred with the other fungi except for *Spongipellis unicolor* in blocks tested (Figure 6).

Pairings in culture

Sharing of genotypes would indicate vegetative spread of the fungus from tree to tree. Presence of different genotypes in adjacent trees would indicate infection by airborne basidiospores. Decay by *Coniophora* is typically located in trunks and branches and not in roots. Basidiospores were retrieved by spore traps in citrus orchards although not in large numbers. Presumptive heterokaryon crosses for vegetative incompatibility showed that all crosses were incompatible (evident by dark zones of interaction

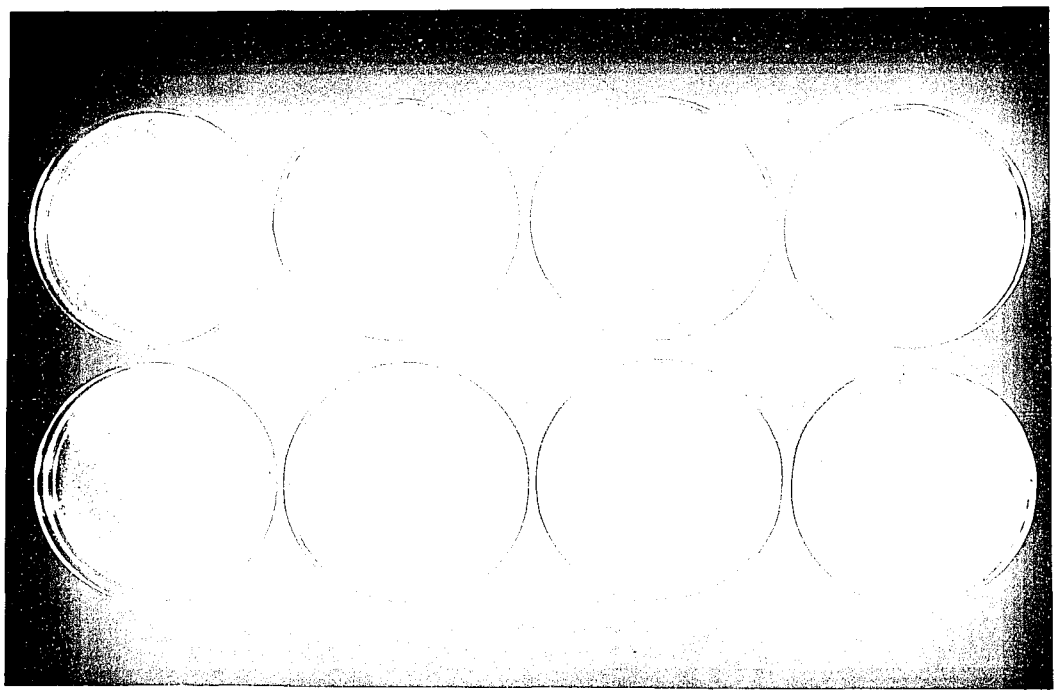

Figure 4. Growth rates for *Coniophora* isolate M-1 at temperatures 10-45C.

Top row. left to right: 10, 15, 20, 25C.
Bottom row. left to right: 30, 35, 40 and 45C.

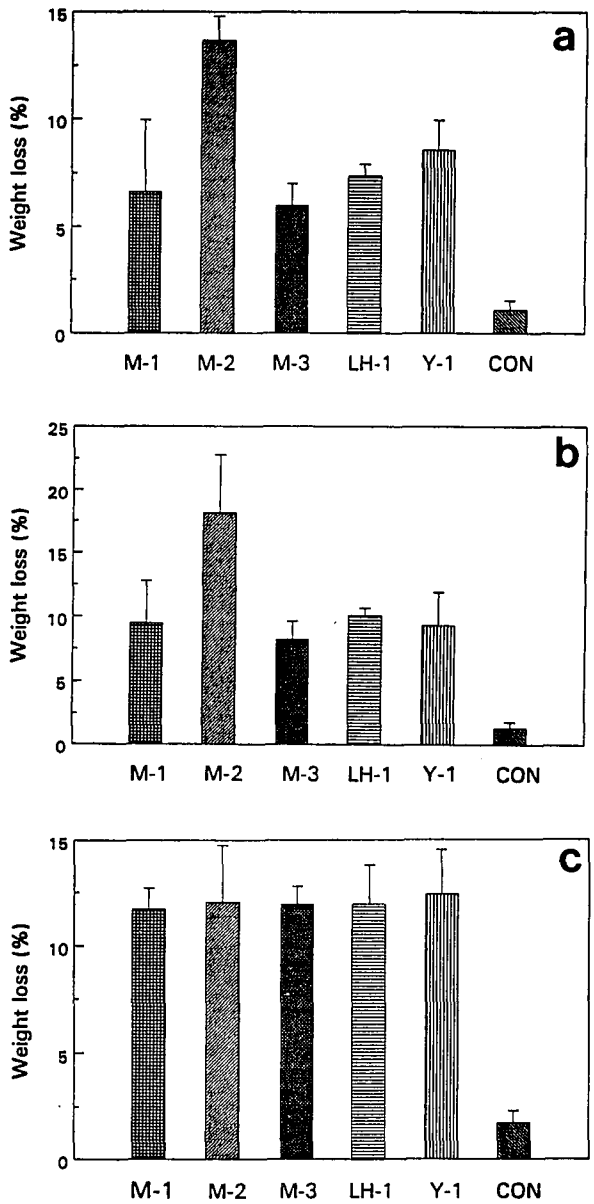

Figure 5a-c. Lemon wood decay block tests with 5 *Coniophora* isolates decayed for 20 weeks.

5a. Weight loss at 25C.
5b. Weight loss at 30C.
5c. Weight loss at 35C

Table 3. Percent weight loss for M-1 and M-2 at three different temperatures in lemon wood blocks*

	Temperature (degrees Celsius)		
	25	30	35
M-1	6.64±3.31a[1]	9.49±3.31a	11.72±0.97a
M-2	13.66±1.14b	18.13±4.63b	12.03±2.73a
Control	1.09±0.42c	1.34±0.45c	1.72±0.59b

*Percentages followed by the same letter within column were not significantly different at P=.05 by Duncan test within each temperature.

[1]Average percent weight loss determined from replications after 20 weeks.

By analysis of variance there was an interaction between temperature and isolates. This means that percent weight loss depended on both isolate and temperature.

Table 4. Percent weight loss for M-3, LH-1, and Y-1 at three temperatures in lemon wood blocks.†

	Temperature (degrees Celsius)		
	25	30	35
M-3	6.00±1.01a[1]	8.20±1.46a	11.93±0.84a
LH-1	7.35±0.56ab	10.09±0.59a	11.90±1.90a
Y-1	8.56±1.38b	9.31±2.60a	12.39±2.12a

† Percentages followed by the same letter within each column were not significantly different at P=.05 by Duncan test within each replicate.

[1]Average percent weight loss determined from replications after 20 weeks.

By analysis of variance there was no interaction between temperature and isolates.

Table 5. Comparison weight loss in vitro decayed lemon wood*

Isolate	Percent weight loss†
Heliocybe sulcata	75.0**
Gleophyllum trabeum	74.1**
Trametes versicolor	52.3±7.4a
Fomitopsis pinicola	45.5±4.9ab
F. palustris	43.4±2.9b
Ganoderma lucidum	42.4±4.6bc
Perenniporia fraxinophila	35.9±17.2cd
Paxillus panuoides	30.7±2.2d
Coniophora M-2	9.1±3.3e
Coniophora Y-1	8.7±1.5e
Spongipellis unicolor	8.6±1.9e
Coniophora M-1	8.3±1.8e
Coniophora LH-1	7.9±0.8e
Coniophora M-3	5.5±1.2e
Control	1.0±0.2f

*Average percent weight loss determined from replications after 20 weeks at optimum temperatures.
**Five replicates were weighed together and averaged to determine final weights.
†Percent weight losses followed by the same letter are not significantly different at P=0.05 (Duncan analysis) using an arcsin transformation of the data.

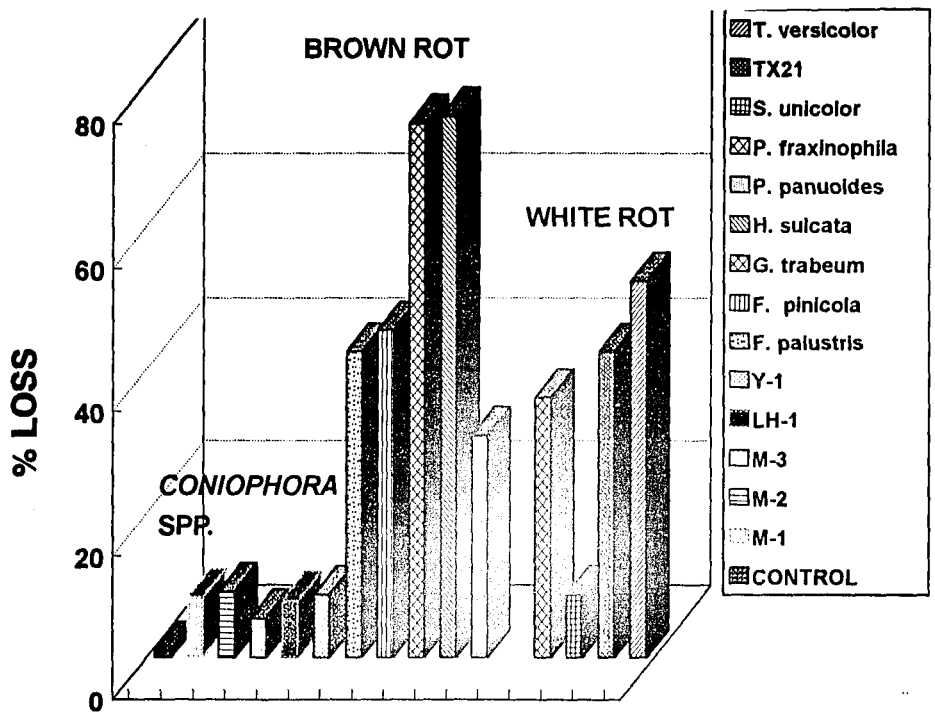

Figure 6. Comparison weight loss studies for 5 *Coniophora* isolates with 5 brown wood rotting and 4 white rotting species.

between isolates) (Figure 7). Homozygous crosses (selfs) showed no interaction zone. Interaction zones were seen in all crosses from different trees (incompatible reaction) while all selfs were compatible as were two isolates recovered from different parts of the same branch (see A-1 X A-2 and K-1 X K-1, Table 6).

Fungicide study

First in vitro fungicide trials

Growth of *Coniophora* was strongly inhibited with low concentrations of the fungicides in plates. Inoculated blocks at 10, 50 or 100 PPM fungicide concentrations showed little inhibition of decay compared to control blocks. The NECTEC paste was the only treatment that had a significant effect against fungal wood decay loss (Figure 8).

Second in vitro fungicide trials

The NECTEC paste without fungicides was the only treatment that inhibited the fungus. Higher concentrations of the fungicides did not inhibit the fungus compared with inoculated controls (Figure 9).

Electron microscopy

In scanning electron micrographs mycelial fragments can be seen in vessels and through diseased wood (Figure 11 and 12, Mf arrow). Shrinking and cracking were apparent in ray parenchyma cell walls (Figure 12). Decayed wood has enlarged

pits in vessels and parenchyma (Figure 12, P arrow), degradation of wood cell walls, and general structural disintegration compared to healthy wood sections (Figure 10 and 11). Total structural collapse in advanced rot is seen in Figure 13.

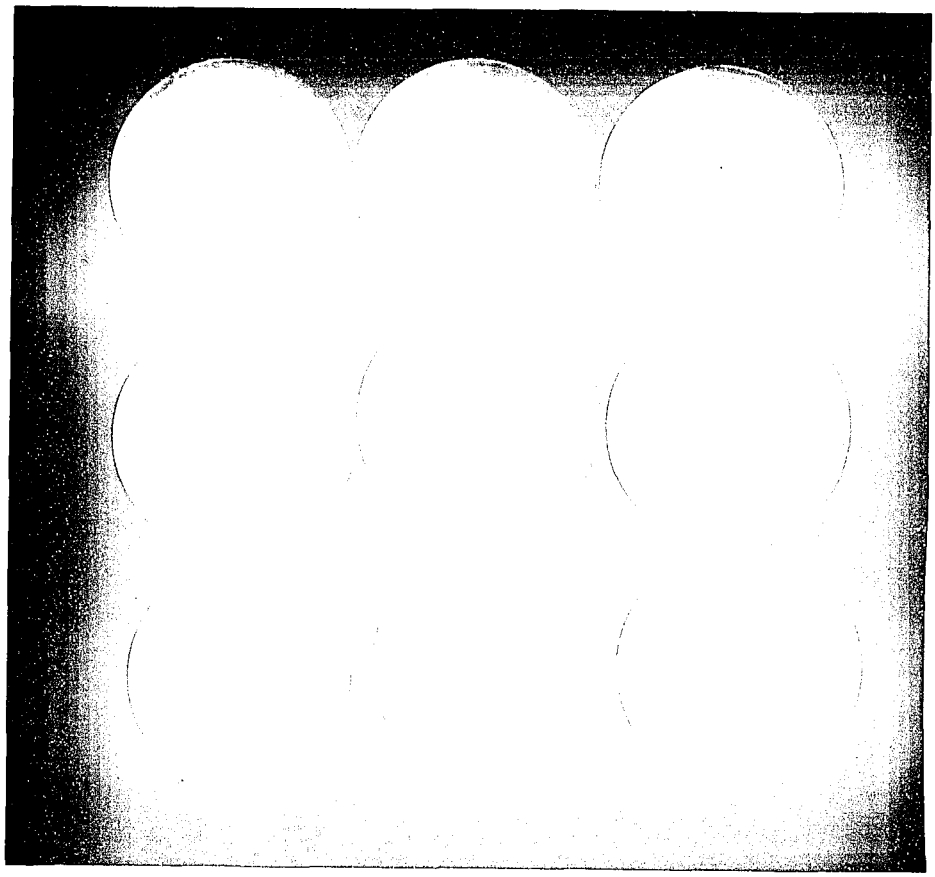

Figure 7. Vegetative incompatibility tests for 9 isolates from one mature orchard.

Top row. A-1 X A-1, A-1 X A-2, A-1 X G.
2nd row. A-1 X J, A-1 X K-1, A-1 X K-2.
3rd row. A-1 X MD, A-1 X N, and A-1 X P.

Table 6. Vegetative incompatibility tests for 9 isolates of *Coniophora* from one mature orchard.

	A-1	A-2	G	J	K-1	K-2	MD	N	P
A-1	-[a]	-*	++	++	++	++	++	++	++
A-2		-	++	++	++	++	++	++	++
G			-	++	++	++	++	++	++
J				-	++	++	++	++	++
K-1					-	-*	++	++	++
K-2						-	++	++	++
MD							-	++	++
N								-	++
P									-

[a]-: no interaction zone; +: weak to moderate interaction zone; ++: strong interaction zone.
*A-1 and A-2, K-1 and K-2 were taken from different branches on the same tree in two trees.

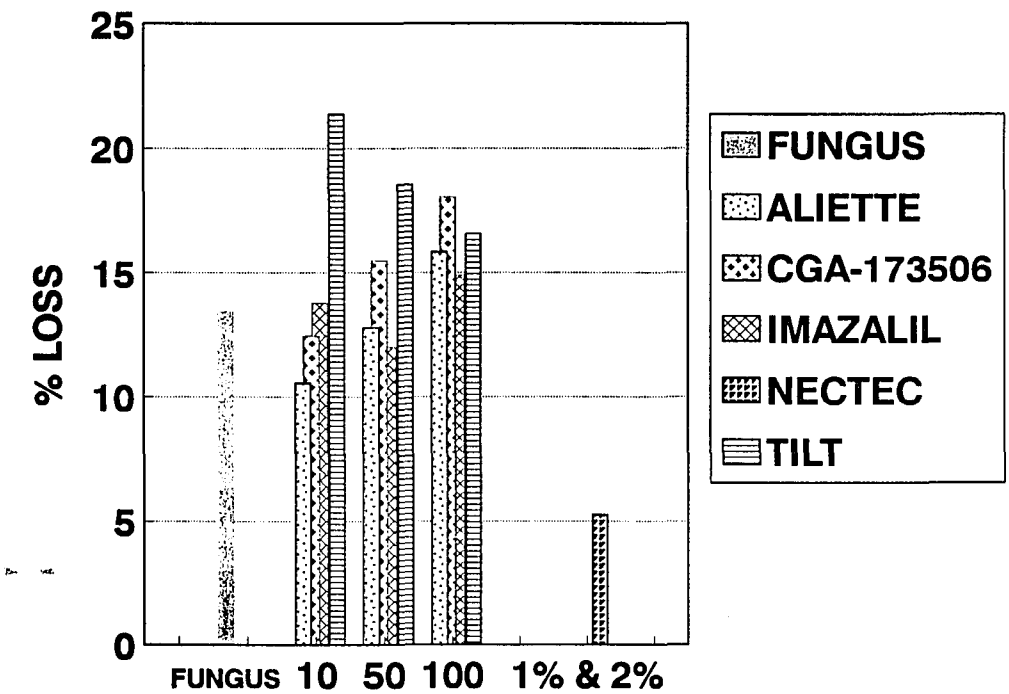

Figure 8. First study in vitro fungicide tests on lemon wood.

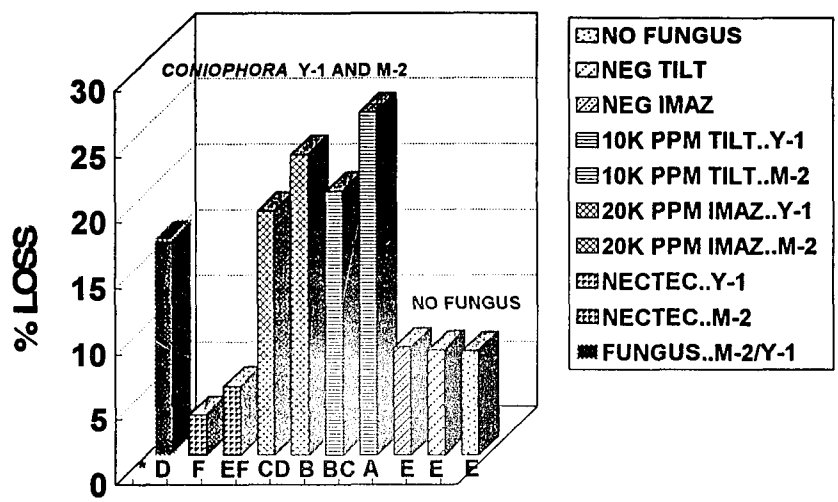

Figure 9. Second study in vitro fungicide tests on lemon wood.

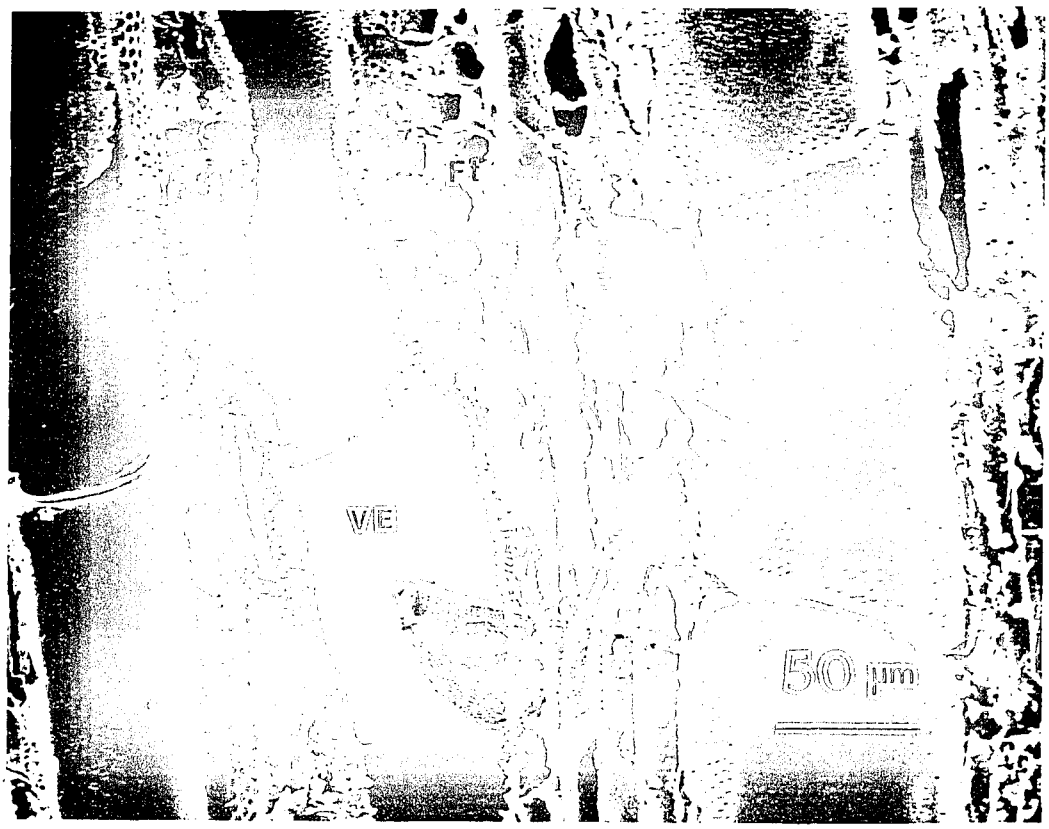

Figure 10. Scanning electron micrograph of healthy lemon wood tangential section.

VE= vessel element, Ft= fiber tracheid.

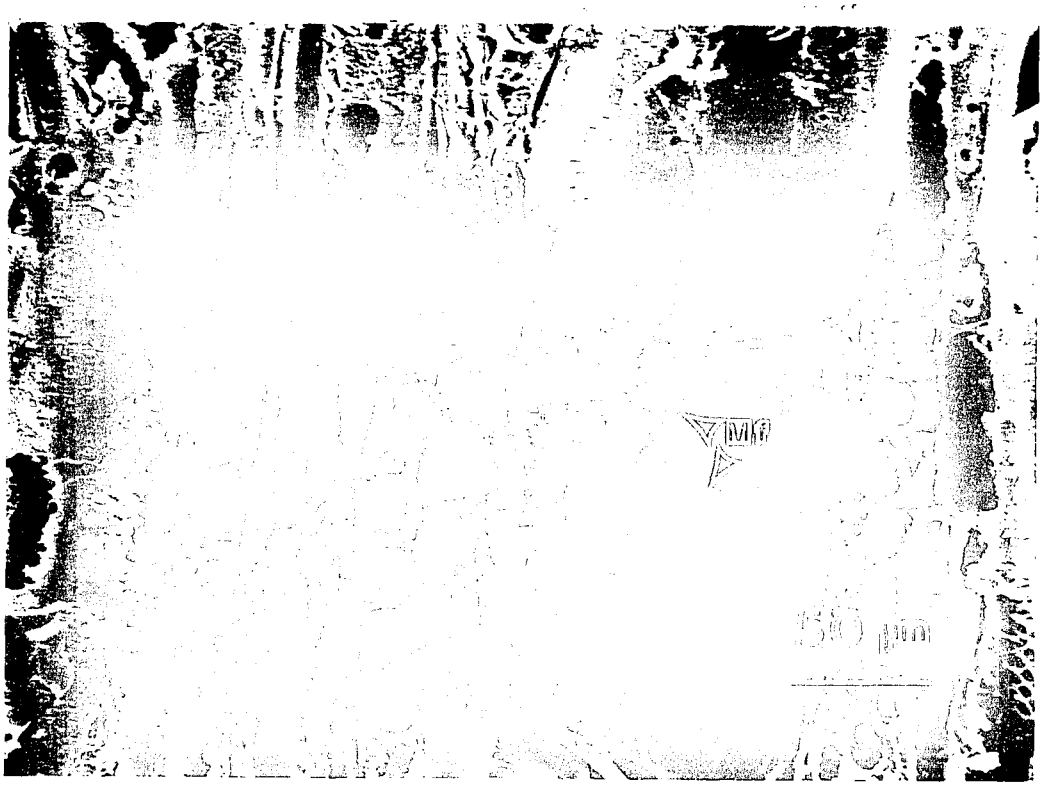

Figure 11. Scanning electron micrograph of *Coniophora* decayed lemon wood tangential section.

Mf arrow= mycelial fragments.

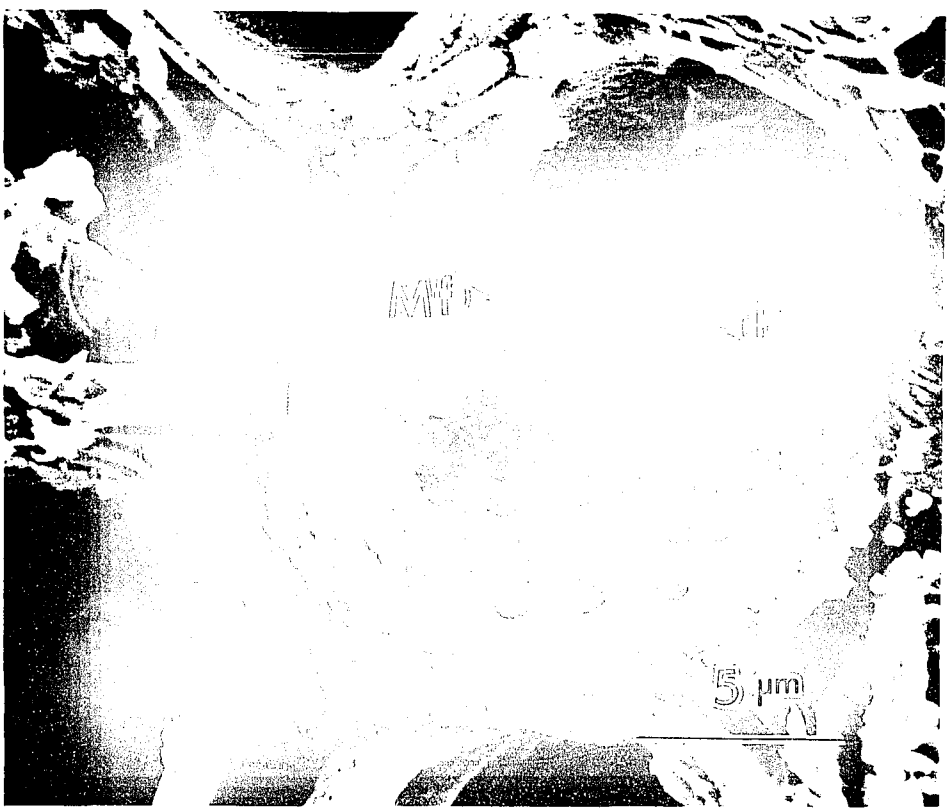

Figure 12. Scanning electron micrograph magnification of Figure 11.

P arrow= pit, Mf arrow= Mycelial fragment.

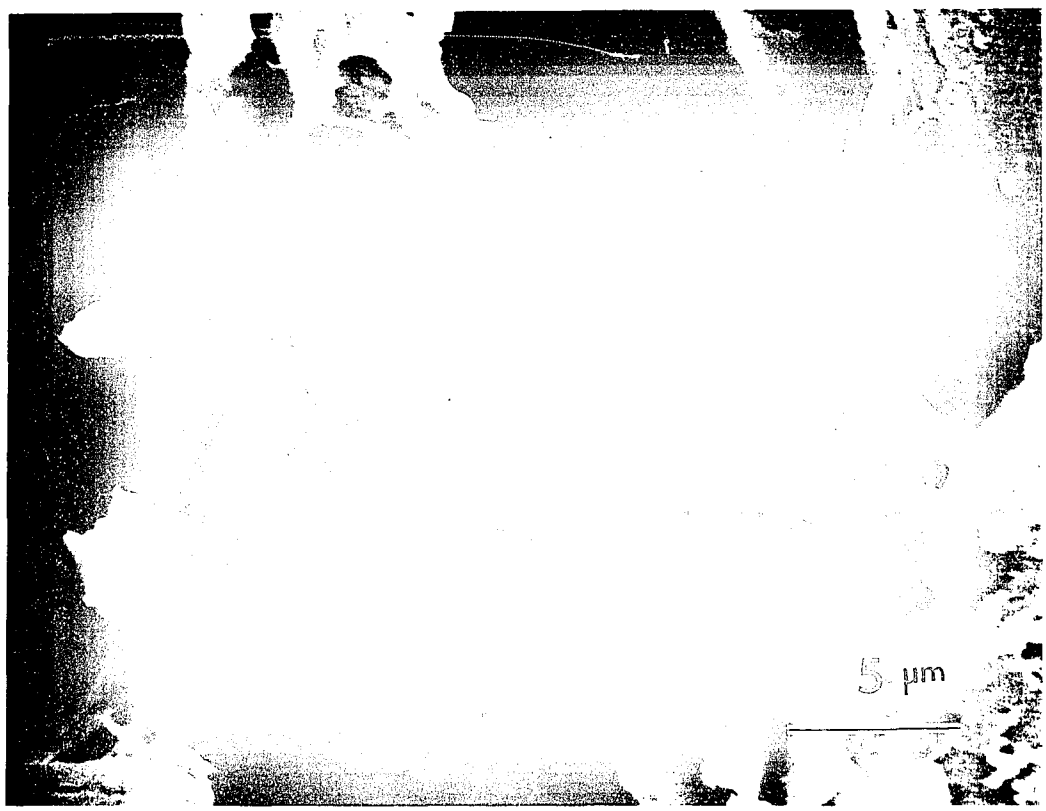

Figure 13. Scanning electron micrograph of severe decay in lemon wood tangential section.

DISCUSSION

Coniophora eremophila is the only known *Coniophora* species to grow in the low desert areas in Arizona. It is a major cause of heartwood rot in lemon in trees in the Yuma area, causing decay and decline of mature lemon trees. The fungus was isolated from typical brown rot decay columns in living trees. These isolates were used to inoculate healthy lemon trees and the typical brown rot decay columns developed as a result of these inoculations. The fungus was reisolated from these decay columns and shown to be the same as originally isolated. Therefore Koch's postulates were fulfilled. Growth in vitro proves that this fungus is adapted to high temperatures and can survive up to 40C. Lemon is grown in the hotter regions especially Yuma, Arizona, where daily temperatures commonly reach over 40C in summer. Decay apparently originates and develops through pruning wounds or broken or split branches and spreads through the heartwood.

Wood is made of approximately 75 percent cellulose and hemicellulose and 25 percent lignin by weight. Brown rot fungi selectively remove the cellulose and hemicellulose from wood leaving a residue of slightly modified lignin, an amorphous compound. As little as 10 percent removal of cellulose and hemicellulose critically affects the strength of the wood (Wood Handbook, Anonymous, 1974). Symptoms of rot

include breaking of branches and cracking of large tree limbs due to the weakening from brown rot disease. Heavy fruit bearing trees like lemon commonly show these symptoms especially when stressed by wind, heavy rain or other climatic factors.

Native trees and shrubs occur in close proximity to the Arizona citrus orchards. Basidiocarps of *Coniophora eremophila* on these hosts could provide basidiospores that are aerially dispersed to citrus trees. No fruiting bodies of *Coniophora* have been found on citrus. Basidiospores of *Coniophora* are thick-walled and presumably could travel distances without desiccation. They then could inoculate open wounds or exposed heartwood.

Genera of *Coniophora* are typically brown rotting fungi. They are often found on fallen dead branches, logs, and stumps as saprophytes. A few are associated with heartwood rot in living conifers. In northern Europe, *Coniophora* has been associated with structural damage as it rots housing timbers (Jülich and Stalpers, 1980).

Brown rot fungi secrete cellulases that penetrate into the wood cells. Non-enzymatic degradation processes may be involved. The polysaccharide components after breakdown then diffuse into the fungus. This action predominantly occurs from the mycelial hyphal tips (Liese, 1970). The hyphae do not have to have direct contact with the wood cell surface to

degrade as the chemical reactions occur. In early stages of brown rot decay, the extracellular enzymes produced by the fungi initially cause breakdown and enlargement of naturally occurring pits (Figure 12, arrow P). Later the hyphae penetrate making bore holes through the wood cell or vessel walls. The progression of brown rot fungal hyphae starts with enzymatic breakdown and absorption from the S2 cellulose layer of the secondary cell wall, making oblong cavities parallel to the wood cell fibrils. The rot progresses at an irregular rate through wood cells and ray parenchyma. The irregular pattern of removal of polysaccharides leads to shrinkage and consequent cracking across the grain of the wood. Finally as the rot advances to later stages, the structure of the wood cells is so undermined, that the wood collapses (Wilcox, 1970) (Figure 13). The decay residue in the final stages is largely slightly modified (demethylated) lignin. After celluloses and hemicelluloses are removed, lignin remains in an empty weak skeletal framework; in nature this is then returned as humus to the soil profile.

With the limited information currently available on the biology of the fungus and the decay in relation to cultural practices, it is difficult to make specific recommendations for control. Reduction in the incidence of susceptible infection courts would presumably be a primary measure. Pruning wounds theoretically provide abundant infection

courts. However other injuries such as splitting as a result of heavy fruit load, equipment damage, wind damage, frost cracks, and insect injuries could also provide suitable infection courts. Treatment of all of these potential infection sites with fungicides is probably impractical as well as economically unfeasible. Active sanitation programs that involve frequent removal of decayed branches with treatment of pruning wounds in younger orchards are probably the most effective control measures that can be recommended at the present time.

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