Biology and Control of *Coniophora* Causing Decay and Decline in Arizona Citrus[1]

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Abstract

A field survey of mature lemon trees showed an average of 30% of trees with symptoms of brown heartwood rot caused by Coniophora sp. In vivo growth of Coniophora inoculated into branches of different types of citrus (Valencia orange, Marsh grapefruit, Orlando tangelo or Lisbon lemon) on rough lemon rootstock was significantly higher in lemon while Coniophora inoculated into Lisbon lemon wood branches on trees established on rough lemon, volkameriana, macrophylla, Cleopatra mandarin, sour orange or Troyer citrange rootstocks showed no significant differences in growth. Vegetative incompatibility trials from one mature orchard demonstrated that isolates from different trees are incompatible. In vitro fungicide trials showed that only NECTEC paste effectively reduced decay on lemon blocks 15 weeks after inoculation with Coniophora. Field fungicide trials showed that NECTEC P paste as well as the blank paste without fungicides, propiconazole at 10,000 µg/ml, imazalil at 20,000 µg/ml or propiconazole plus imazalil in combination at 10,000 and 20,000 µg/ml, respectively, significantly inhibited the advance of fungus 7 mo. after inoculation. A second fungus isolated from brown rot in branches in younger orchards was identified as Antrodia sinuosa, a native decay fungus on conifers in Arizona.

Introduction

In 1992 a *Coniophora* species was first reported to be associated with a brown heartwood rot occurring in lemon trees in Yuma, Arizona (19). 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 (3). Growers consider this to be the most important pathology problem in lemon orchards in the Yuma region.

In Arizona, lemons were harvested from 6,520 ha. in the 1993-94 season, 5,880 ha. of that from Yuma County and the remainder from Maricopa County (22). Although the brown rot decay occurs in Maricopa County, the problem is not considered to be serious in orchards in this region.

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The only known *Coniophora* species in the Sonoran desert region, *Coniophora eremophila* Lindsey & Gilb., was described in 1975 (18). It has been found fruiting on a number of species of desert trees, shrubs, and cacti, mainly as a saprobe on dead fallen wood and associated with a brown rot (11,12,13). Sonoran desert substrates on which *Coniophora eremophila* have been recorded include *Olneya tesota* 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. (point-leaf manzanita), *Fraxinus velutina* Torr. (velvet ash), *Opuntia fulgida* Engelm. (jumping cholla), *Sambucus mexicana* Presl (Mexican elder), *Prosopis velutina* Woot. (velvet mesquite) and on *Juniperus monosperma* (Engelm.) Sarg. (one-seed juniper) from New Mexico. An isolate from brown rot in a recently fallen saguaro also has the characteristics of a *Coniophora* and is identical to isolates from lemon. The cultural morphology of *Coniophora* species is unique at the genus level but it is not possible to differentiate *Coniophora* species in culture (14,26).

Since no other *Coniophora* species have ever been found in the Sonoran Desert, we conclude that the *Coniophora* causing the common brown heartrot in lemon is *Coniophora eremophila.* No fruiting bodies have been found associated with the disease in lemon.

The latest monograph on *Coniophora* recognizes 12 species (14). Only 5 species are known to occur in Arizona (8,9,12,13,15). The four species other than *C. eremophila* occur in conifer forest ecosystems at higher elevations in Arizona. The only other record for *Coniophora eremophila* was reported by Ginns (14) from Chile occurring on dead wood.

Most of the 12 *Coniophora* species in North America are not known to cause decay on living trees. Four species other than *C. eremophila, C. arida* (Fr.) P. Karst., *C. puteana* (Fr.) P. Karst., *C. olivacea* (Pers.) P. Karst., and *C. fusispora* (Cooke & Ellis) Sacc. are reported to cause decay in living trees (10,14,15). These four species mainly cause root and butt rots and are not known to cause decay in upper trunks and branches.

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 clinic closed. Of the 467 citrus entries listed there were no disease problems recorded where fungi directly affect heartwood (20).

Collections listed in the database from the Arizona Mycological Herbarium at The University of Arizona, Tucson, Arizona have only 14 entries of wood decay fungi associated with citrus. The only brown rot fungus (5 records) reported on citrus is *Amylosporus campbellii* (Berk.) Ryvarden (13, as *Tyromyces graminicola* Murrill). This is a root and buttrot fungus and is not known to cause decay in trunks and branches as does *C. eremophila.*

In the literature on citrus diseases, no fungi that decay heartwood in trunks or branches were identified (2,7,6,16,17,23,27). The APS compendium of citrus diseases (27) lists six diseases associated with living wood and involving fungal pathogens. The only wood decay fungi included are butt and root rot pathogens and cause white rots. Other reported diseases in citrus include a root rot in Florida caused by *Clitocybe tabescens* Bres. (17,28), a butt and root rot caused by *Ganoderma* in Florida (5), a root rot in California and Australia caused by *Armillaria mellea* (Vahl:Fr.) P. Kumm. (4,21), and a root and butt rot in grapefruit in Texas caused by *Ganoderma lucidum* (Curtis:Fr.) P. Karst. (24,25). *Ganoderma lucidum* is known to occur on lemon in the Yuma area, but apparently is not an important pathogen there.

The objectives of this research were to determine the extent of brown heartwood rot in mature lemon orchards in Arizona; to determine the relative susceptibility of various types of citrus to *Coniophora* brown heartwood rot; to evaluate the possible effect of different rootstocks on brown heartwood rot development in Lisbon lemon; and to explore the potential role of chemical control as a disease management tool.

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. In 1994 younger orchards (age= 10 yrs.) were surveyed and sampled where symptoms occurred.

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 studies. 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 1x2x3cm 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 studies. To compare the relative amounts of decay by *Coniophora* and *Antrodia* isolates versus other brown rotting or white rotting isolates, a block decay test using 5 *Coniophora* isolates, plus 5 other brown rotting and 4 white rotting fungi was done. The brown rot fungi were *Paxillus panuoides*, *Heliocybe sulcata*, *Gleophyllum trabeum*, *Fomitopsis pinicola*, and *Fomitopsis palustris*. The white rot 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.

Effect of citrus type and rootstock on disease development. Branches ranging from 6-10 cm in diameter on 20-25 yr old Valencia sweet orange (*Citrus sinensis* (L.) Osbeck), Marsh grapefruit (*C. paradisi* Macf.), Orlando tangelo (*C. reticulata* Blanco X *C. paradisi* Macf.), and Lisbon lemon (*C. limon* (L.) Burm.) trees established on rough lemon (*C. jambhiri* Lush.) were used in studies to determine the relative susceptibility of different types of citrus to *Coniophora* brown wood rot. To prepare inoculum, autoclaved pieces of wood dowel 8 mm in diameter X 13 mm long were placed on mycelia of an isolate (Y-1) of *C. eremophila* growing in 100 X 15 mm plastic Petri dishes containing potato dextrose agar (PDA) and incubated for one month in the dark at 28 C. This isolate of the pathogen was originally recovered from a diseased lemon tree in Yuma County, Arizona. Test trees were inoculated by placing one dowel segment containing *C. eremophila* into a 9-mm-diameter X 26-mm-long hole in a series of branches on several trees. The dowel segment containing the pathogen was positioned and retained in the bottom of each inoculation hole by driving another dowel piece not containing *C. eremophila* into each wound. This longer dowel piece was cut off flush with the surface of the branch and the wound was sealed with melted paraffin. Disease development was assessed 10 mo later by removing infected branches, splitting them in half and measuring the length of resultant brown wood rot columns. For each type of citrus, one branch on each of eight different trees was inoculated for the 1993 and 1994 trial. Control trees received dowel pieces that did not contain *C. eremophila*.

To evaluate the effect of rootstock on development of Coniophora brown wood rot, branches ranging from 6-10 cm in diameter were inoculated on 20-yr-old Lisbon lemon trees established on rough lemon, Volkamer lemon (commonly referred to as Volkameriana, *C. volkameriana* (Pasq.) Tan.), alemow (commonly referred to as Macrophylla, *C. macrophylla* Wester), Cleopatra mandarin (*C. reshni* Hort. ex Tan.), sour orange (*C. aurantium* L.), or Troyer citrange (*C. sinensis* X *Poncirus trifoliata* (L.) Raf.). Inoculation procedures for this study were identical to those described above. One branch on each of eight different trees established on each tested rootstock was inoculated for each of two runs of this experiment. Values obtained from each execution of an experiment

were analyzed by analysis of variance (ANOVA) using the SigmaStat statistical software package (Jandel Scientific, San Rafael, CA). The Duncan-Waller-K-Ratio (LSD) test was used to compare treatment means.

Pairings in culture. Presumptive heterokaryotic isolates obtained from decayed branches in one mature orchard with a high percentage of symptoms were cultured and paired in all possible combinations on malt extract agar (MEA) (2% malt, 1.5% agar) by taking two cores from actively growing cultures and placing them approximately 1 cm apart in 60 X 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.

***In vitro* fungicide studies.** To assess the effects of fungicides on mycelial growth, the following materials were examined: fosetyl-Al (Aliette 80WDG, Rhone-Poulenc Ag Co., Research Triangle Park, NC); CGA-173506 50WP (Ciba-Geigy Corp., Greensboro, NC); imazalil (97.5% technical grade, Janssen Pharmaceutica, Inc., Piscataway, NJ); and propiconazole (91% technical grade, Janssen Pharmaceutica, Inc., Piscataway, NJ). Various concentrations of fosetyl-Al and CGA-173506 were prepared in sterile distilled water, while imazalil and propiconazole were prepared in 95% ethanol, then added into autoclaved Difco corn meal agar (CMA) cooled to 50-55 C. The medium was thoroughly mixed after addition of the fungicide to insure uniform distribution within the agar, then 10 ml was dispensed into a series of 60 X 15 mm plastic Petri dishes. Final concentrations of each fungicide in the agar medium were 0.1, 0.5, 1, 5, 10, 50, and 100 μg/ml. Control petri dishes contained CMA alone.

One isolate of *C. eremophila* from Yuma County (Y-1) and one isolate from Maricopa County (M-1) were used in these studies. To assess the effect of each fungicide on mycelial growth of the pathogen, a 6-mm-diameter agar disk from the periphery of an actively growing colony of isolate Y-1 or M-1 was placed on the edge of a series of Petri dishes containing CMA alone or amended with fungicides. Inoculated dishes were incubated for 5 days at 34 C, after which the radial growth of the fungus was measured. Each treatment contained 10 replicates, five with isolate Y-1 and five with isolate M-1. This test was performed twice.

To test fungicidal effects of these materials on lemon wood in vitro, the four fungicides and a wound dressing treatment, NECTEC P paste (formulation: 10,000 μg/ml propiconazole, 20,000 μg/ml imazalil in a paint-like paste) were applied to lemon wood blocks and placed in chambers with one of two different isolates of *Coniophora*. The lemon blocks were prepared following ASTM procedure for evaluating wood preservatives (1). They were oven dried at 93 C for 3 days, then weighed and the oven dry weight recorded. The wood blocks treated with NECTEC P paste were coated with the material, dried overnight, and weighed to determine additional weight due to the treatment which was averaged and subtracted at the end of the trials. Blocks were submerged in three concentrations of each of the four fungicides, at 10, 50, or 100 μg/ml. The submerged blocks underwent vacuum treatment for approximately 10 min at 0.7 kg/ cm^2 to infiltrate the blocks with the fungicide. Blocks were then air dried in room conditions and weighed. The blocks were steam sterilized at 3.6 kg/ cm^2 pressure for 20 min, aseptically transferred to chambers containing actively growing cultures of either M-2 or Y-1 isolates of *Coniophora*. The chambers were incubated at 30 C for 15 wk, then disassembled, and the wood blocks brushed free of mycelium, oven dried over 2 days at 93 C, and weighed. Percent weight loss was calculated accounting for additional weight of NECTEC P paste treatment blocks or fungicides (subtracting average NECTEC P paste weight for calculations or using room conditioned block weights for all fungicides rather than oven dried weights for final calculations).

Since no significant effect (Fig. 4) but that of the NECTEC P paste was achieved with initial fungicide concentrations, a second series of trials were run using higher concentrations of the fungicides. Aliette and CGA-173506 were dropped from the study but higher concentrations of propiconazole and imazalil were tried. The effectiveness of NECTEC P paste was tested using only the paste with no active fungicide ingredients. Each concentration of propiconazole (10,000 μg/ml) and imazalil (20,000 μg/ml) 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. Percent weight loss was determined as described in the first in vitro fungicide trials.

Field fungicide trials. The following materials were used in the first two field studies: fosetyl-Al (Aliette 80WDG), CGA-173506 50WP, imazalil (97.5% technical), propiconazole (91% technical), and Nectec P paste (2% imazalil, 1% propiconazole; Janssen Pharmaceutica, Piscataway, NJ). Branches ranging from 6-10 cm in diameter on 25-yr-old Lisbon lemon trees and 20-yr-old Orlando tangelo trees were used in these trials. To prepare inoculum, autoclaved pieces of wood dowel 8 mm in diameter X 13 mm long were placed on mycelia of the Y-1 isolate of *C. eremophila* growing in 100 X 15 mm plastic Petri dishes containing PDA, then incubated for one month in the dark at 28 C. Prior to inoculation of tree branches, aqueous mixtures containing 100 μg/ml of imazalil or propiconazole were prepared by initially dissolving each fungicide in 95% ethanol, then adding 500 ml of distilled water. Aqueous mixtures of fosetyl-Al or CGA-173506 were prepared by suspending the appropriate amount of each material in 500 ml of water. The Nectec P paste was used as supplied. A 9-mm-diameter X 26-mm-long hole was drilled into each branch that was to be used in the study. For branches to be treated with a fungicide, the hole in the branch was filled with the test material, then the dowel segment containing the *C. eremophila* was coated with the same fungicide and placed into the hole in the branch. The dowel segment containing the pathogen was positioned and retained in the bottom of each inoculation hole by driving another dowel piece, also coated with the test fungicide but not containing *C. eremophila*, into each wound. This longer dowel piece was cut off flush with the surface of the branch and the wound was sealed with melted paraffin. Disease severity was assessed 6 mo later by removing infected branches, splitting them in half, and measuring the length of resultant brown wood rot columns. Inoculated control branches received dowel pieces containing *C. eremophila* but no fungicide was applied to the dowel piece or in the inoculation hole. Noninoculated control branches received dowel pieces that did not contain the pathogen. One branch on each of seven different Lisbon lemon trees and one branch on each of nine different Orlando tangelo trees were used for each treatment.
In a subsequent series of two field trials, the following treatments were used: 10,000 μg/ml of propiconazole, 20,000 μg/ml of imazalil, 10,000 μg/ml of propiconazole + 20,000 μg/ml of imazalil, Nectec P paste, and a blank paste in which the fungicides were omitted. For each treatment, one branch on each of seven 2-yr-old Lisbon lemon or 21-yr-old Orlando tangelo trees was inoculated and treated as described earlier. Disease severity was assessed 7 mo later. All treatments in the field fungicide trials were arranged in a randomized complete block design. Values obtained from each trial were analyzed by analysis of variance and the Duncan-Waller-K-Ratio (LSD) test was used to compare treatment means.

Results

In August 1994, a Burkard spore trap was positioned at the Yuma Agricultural station. Seven day tapes were evaluated weekly for four months and showed small numbers of *Coniophora* spores present. Apparently during this period conditions were not favorable for spore production.

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 percentage of decayed trees in all orchards was 30% (Table 1).
Further collection and testing of isolates from younger plantings of lemon in Yuma (average orchard age=10 yrs.) showed that a very low percentage of trees sampled were infected with *Coniophora*. Twenty-seven samples were taken from 4 orchard locations where symptoms or branch breakage were evident. Pure cultures were obtained from 6 of these samples, and of those only one is characteristic of *Coniophora*. The others are also apparently from brown rots but are another wood-rotting basidiomycete with different cultural morphology. These cultures are currently producing fruiting bodies in culture and we have identified this fungus as *Antrodia sinuosa* (Fr.) Karst. In laboratory decay tests this fungus is a much more aggressive than the *Coniophora* isolates.

Temperature studies. All *Coniophora* cultures grew between 15 and 40C, but not at 10 or 45C. Optimum temperature for growth was 30-35C averaging 2.7-3.1 mm per day as seen for isolate M-1.
The temperature optimum for *Antrodia* isolates is 30-35C, similar to that of *Coniophora*. The *Antrodia* isolates grow much faster, 7.3-7.5 mm per day, than the *Coniophora* isolates (Fig. 2 and 3).

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

Comparison studies. 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. *Antrodia* isolates caused weight loss intermediate between that of *Coniophora* and other isolates tested. 3-8 times as much weight loss occurred with the other fungi except for *Spongipellis unicolor* in blocks tested (Table 4).

Effect of citrus type and rootstock on disease development.
The length of brown wood rot decay columns that developed in Lisbon lemon branches inoculated with *C. eremophila* was significantly greater than that occurring in branches of Orlando tangelo, Marsh grapefruit, or Valencia orange inoculated with the same pathogen (Table 5). Also, the relative development of wood decay columns on branches of Orlando tangelo, Marsh grapefruit, and Valencia orange did not differ significantly from each other in both the 1993 and 1994 trials.
There was no significant difference between the length of brown wood rot decay columns that developed on branches of Lisbon lemon trees established on rough lemon, Volkameriana, Macrophylla, Cleopatra mandarin, sour orange, or Troyer citrange rootstocks (Table 6).

Pairings in culture. Sharing of genotypes would indicate probable vegetative spread of the fungus from tree to tree. Presence of different genotypes in adjacent trees or in different rot columns in the same tree would indicate probable 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 between isolates). 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 areas of the rot column in one branch (see A-1 X A-2 and K-1 X K-2, Table 7).

***In vitro* fungicide studies.** Growth of *C. eremophila* was completely inhibited by CGA-173506 at a concentration of 10 μg/ml and propiconazole at a concentration of 50 μg/ml (Figure 1). At a concentration of 100 μg/ml, the highest rate tested in this study, imazalil reduced mycelial growth by over 80% compared to growth in the absence of the material. Fosetyl-Al at all tested concentrations had no appreciable effect on mycelial growth of *C. eremophila*.
Decayed blocks treated with 10, 50 or 100 μg/ml of fosetyl-Al, CGA-173506, imazalil, or propiconazole showed no significant difference in weight loss compared to inoculated control blocks. On the other hand NECTEC P paste was the only treatment that had a significant effect against weight loss due to fungal wood decay. In a subsequent series of trials, NECTEC blank paste without fungicides was the only treatment that inhibited the fungus. The higher concentrations of fungicides compared to earlier studies did not inhibit the fungus compared with inoculated controls (Fig. 4 and 5).

Field fungicide trials. In the initial series of field fungicide trials, the length of brown wood rot decay columns in branches treated with fosetyl-AL, CGA-173506, imazalil, or propiconazole at a concentration of 100 μg/ml did not differ significantly from the extent of disease recorded on branches receiving no fungicide treatment (Tables 8 and 9). On the other hand, the length of wood decay columns in branches treated with Nectec P paste was significantly smaller than that recorded on branches receiving no fungicide as well as all other fungicide treatments. In the subsequent series of field trials, all tested materials on Lisbon lemon and Orlando tangelo trees significantly reduced the development of brown wood rot decay columns compared to disease development in the absence of a fungicide (Tables 10 and 11). Equivalent development of decay columns were observed for both Lisbon lemon and Orlando tangelo trees in branches treated with Nectec P paste (containing 2% imazalil + 1% propiconazole) or the blank paste without fungicides.

Discussion

Coniophora eremophila is the only known *Coniophora* species to grow in the low desert areas of Arizona. It is a major cause of heartwood rot in lemon trees in the Yuma area, causing decay and decline of mature 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 40 C. Lemon trees are grown in the hotter regions of Arizona, where daily temperatures commonly reach over 40 C in summer. Decay apparently originates from airborne basidiospores germinating on pruning wounds or broken or split branches and spreads through the heartwood and encroaches on the sapwood.

Symptoms of rot include dieback of branches, 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. Longitudinal splits often occur on branches with heavy fruit loads and apparently provide another potential infection court.

Native trees and shrubs occur in close proximity to the Arizona citrus orchards, especially in washes. 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 large distances without desiccation. They then could infect open wounds or exposed heartwood.

The fungicidal action of the compounds tested was much more pronounced in growth tests on agar media as compared to growth tests in inoculated trees. This may be due to an even diffusion of the fungicide in the agar medium and a relative immobility in the inoculated branches. Once the fungus grows beyond the impregnated zone it is able to spread more rapidly through the untreated wood. The NECTEC paste alone was equally effective in inhibiting decay as compared to NECTEC paste with added fungicides. Apparently NECTEC paste contains some fungistatic compounds. Inoculation tests showed that *Coniophora eremophila* is able to cause decay in orange, grapefruit, and tangelo although significantly less than in lemon. This presumably is due to structural and chemical characters of lemon wood which are unknown at this time. We have not been able to find any data relating to this subject. Decay in lemon trees may be intensified by more intensive pruning and creation of infection courts. Also lemon may be more subject to cracking and breaking under heavy fruit loads than other types of citrus. This could also result in a greater incidence of infection courts in lemon with a consequent increase in incidence of decay.

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. Lemon trees grow rapidly and are subjected to heavy pruning annually. 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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Table 1. Orchard survey: Yuma Arizona, 1993.

	Percentage of lemon trees in each rating category[a]			
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

[a]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.

Table 2. 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 3. 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 4. 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 TXA21	42.4±4.6bc
Perenniporia fraxinophila	35.9±17.2cd
Antrodia sinuosa ANT2	28.0±4.7
Antrodia sinuosa ANT6	29.1±3.9
Antrodia sinuosa ANT7	25.5±3.6
Antrodia sinuosa ANT14	32.5±2.2
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.

Table 5. Development of brown wood rot on various types of citrus trees established on rough lemon rootstock.

Type of Citrus	Mean length of rot column (mm)[a]	
	1992	1993
Valencia orange	51.0±12.0a[b]	28.0±5.0a
Orlando tangelo	69.0±17.0a	35.0±9.0a
Marsh Grapefruit	74.0±25.0a	31.0±10.0a
Lisbon lemon	123.0±74.0b	104.0±21.0b

[a]Means were determined from eight inoculated trees.
[b]Means followed by the same letter are not significantly different at P=0.05 using Duncan-Waller K-Ratio (LSD) test of the data.

Table 6. Development of brown wood rot in branches of Lisbon lemon trees established on various rootstocks.

Rootstock	Mean length of rot column(mm)[a]	
	1992	1993
Cleopatra mandarin	187.0±73.0a[b]	86.0±19.0a
Troyer citrange	152.0±93.0a	86.0±18.0a
Volkameriana	171.0±91.0a	86.9±28.0a
Rough lemon	113.0±97.0a	104.0±21.0a
Sour orange	179.0±70.0a	106.0±26.0a
Macrophylla	130.0±100.0a	109.0±26.0a

[a]Means were determined from eight inoculated trees.
[b]Means followed by the same letter are not significantly different at P=0.05 using Duncan-Waller K-Ratio (LSD) test of the data.

Table 7. Vegetative incompatibility tests for 9 isolates of *Coniophora* from one mature orchard.[a]

	Coniophora isolate								
Coniophora isolate	**A-1**	**A-2**	**G**	**J**	**K-1**	**K-2**	**MD**	**N**	**P**
A-1 -	-	-[b]	++	++	++	++	++	++	++
A-2		-	++	++	++	++	++	++	++
G			-	++	++	++	++	++	++
J				-	++	++	++	++	++
K-1				-	-[b]	++	++	++	
K-2					-	-	++	++	
MD							-	++	++
N								-	++
P									-

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

Table 8. Field fungicide trials on Lisbon lemon.

Fungicide[a]	Mean length of wood rot column[b]
NECTEC P paste	1.0±0.0a[c]
Imazalil	29.6±15.3b
Propiconazole	40.9±29.7b
No fungicide	42.0±18.8b
CGA-173506	51.3±18.7b
Aliette	83.1±42.1c

[a]Fungicide formulations were 100 mg/ml for each fungicide treatment except NECTEC P paste which has 2% imazalil plus 1% propiconazole.
[b]Means were determined from eight Lisbon lemon tree branches.
[c]Means followed by the same letter are not significantly different at P=0.05 using Duncan-Waller K-Ratio (LSD)$_t$ test of the data.

Table 9. Field fungicide trials on Orlando tangelo.

Fungicide[a]	Mean length of wood rot column (cm)[b]
NECTEC P paste	1.1±0.3a[c]
Propiconazole	15.7±8.9b
Imazalil	17.1±4.8bc
Aliette	18.8±9.2bc
CGA-173506	19.8±5.4bc
No fungicide	22.8±7.4c

[a]Fungicide formulations were 100 mg/ml for each fungicide treatment except NECTEC P paste which has 1% imazalil plus 2% propiconazole.
[b]Means were determined from nine inoculated tangelo branches.
[c]Means followed by the same letter are not significantly different at P=0.05 using Duncan-Waller K-Ratio (LSD) test of the data.

Table 10. Field fungicide trials on Lisbon lemon trees using higher concentrations of fungicides.

Fungicide[a]	Mean length of wood rot column (cm)[b]
Uninoculated control	0.0±0.0a[c]
Propiconazole	0.3±0.7ab
Imazalil + Propiconazole	0.4±0.7ab
NECTEC blank paste	1.3±1.7ab
Imazalil	1.4±0.9ab
NECTEC P paste	3.6±3.2b
Inoculated control	7.7±6.4c

[a]Concentrations of the fungicides used were 10,000 mg/ml propiconazole or 20,000 mg/ml imazalil. NECTEC Paste contains 1% propiconazole plus 2% imazalil. NECTEC blank paste has no fungicides.
[b]Means were determined from seven inoculated lemon trees.
[c]Means followed by the same letter are not significantly different (P=0.05) using Duncan-Waller K-Ratio (LSD) test of the data.

Table 11. Field trials using higher concentrations of fungicides on Orlando tangelo.

Fungicide[a]	Mean length of wood rot column (cm)[b]
Propiconazole	0.6±0.9a[c]
Uninoculated control	2.9±2.6ab
NECTEC blank paste	2.9±1.0ab
Imazalil	4.1±3.1ab
NECTEC P paste	4.4±7.7ab
Imazalil + propiconazole	5.1±3.7b
Inoculated control	15.8±6.4c

[a]Concentrations of the fungicides used were 10,000 mg/ml propiconazole or 20,000 mg/ml imazalil. NECTEC P paste contains 1% propiconazole plus 2% imazalil. NECTEC blank contains no fungicides.
[b]Means were determined from seven inoculated tangelo tree branches.
[c]Means followed by the same letter are not significantly different (P=0.05) using the Duncan-Waller K-Ratio (LSD) test of the data.

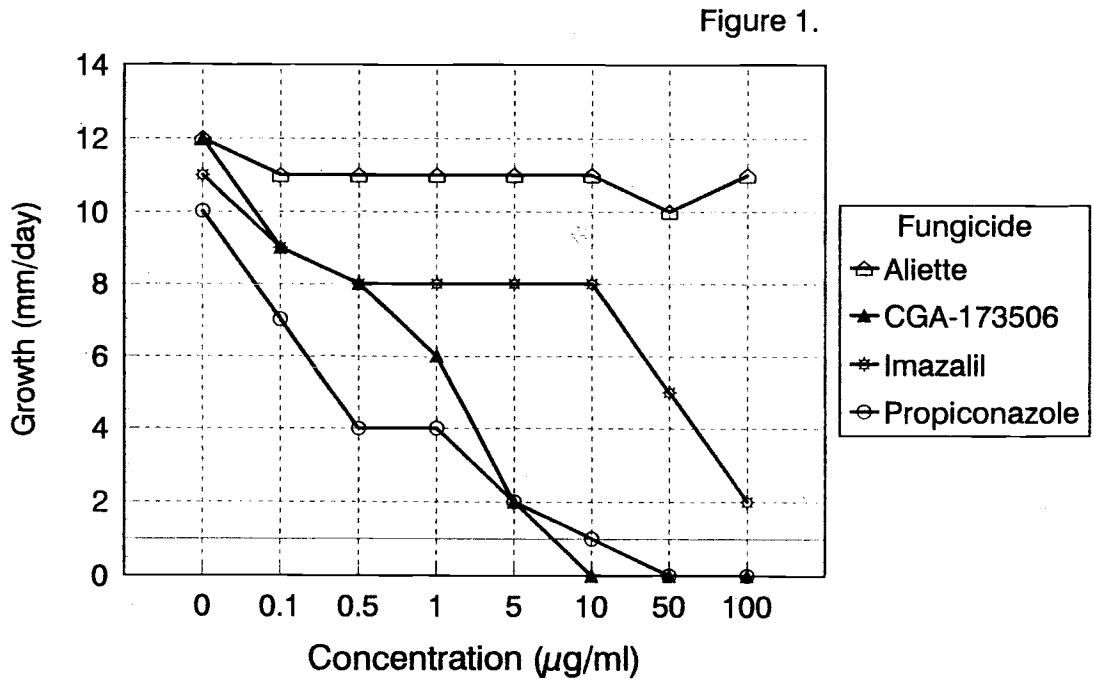

Figure 1. Growth of *Coniophora eremophila* on corn meal agar plates amended with different concentrations of active ingredient after five days at 34 C.

Figure 2.

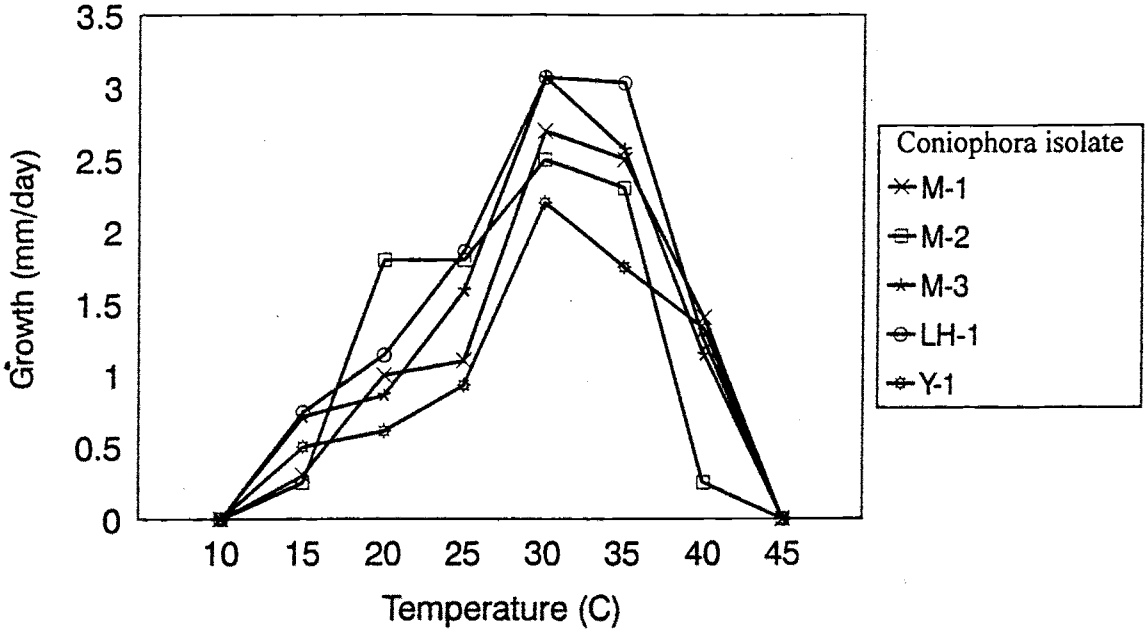

Figure 2. Average growth (mm/day) for *Coniophora* on malt extract agar media after 14 days at 10-45C.

Figure 3.

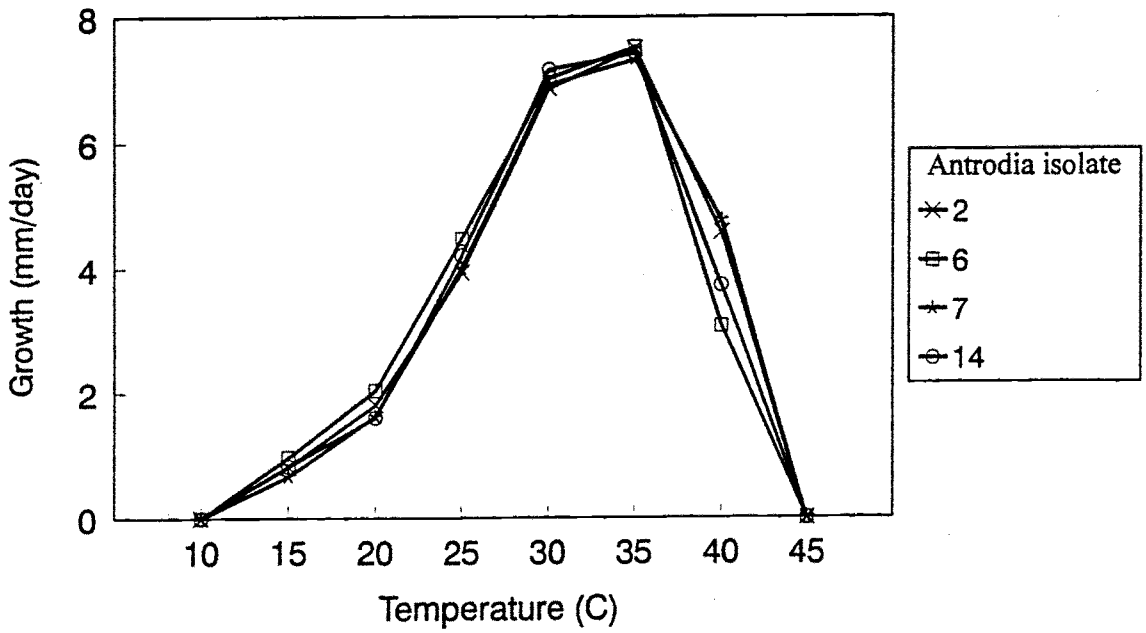

Figure 3. Average growth (mm/day) for *Antrodia* on malt extract agar media after 14 days at 10-45C.

Figure 4.

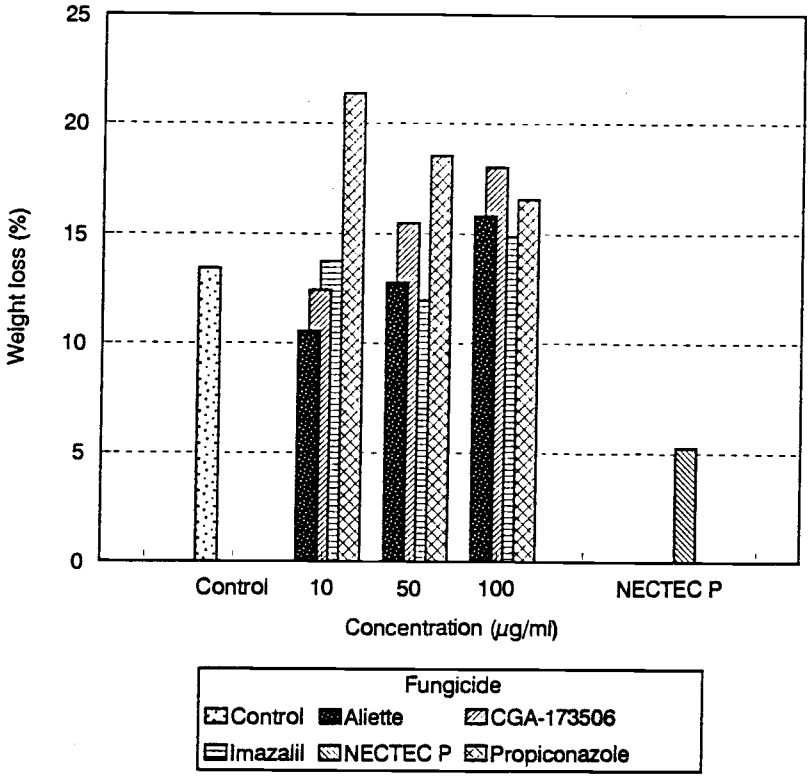

Figure 4. Fungicide tests on lemon blocks inoculated with *Coniophora* isolate M-2. The treated and control blocks were incubated and weight loss calculated after 15 weeks *in vitro*. The mean weight loss of five replicates for each treatment and control was calculated for each concentration of the fungicides tested.

Figure 5.

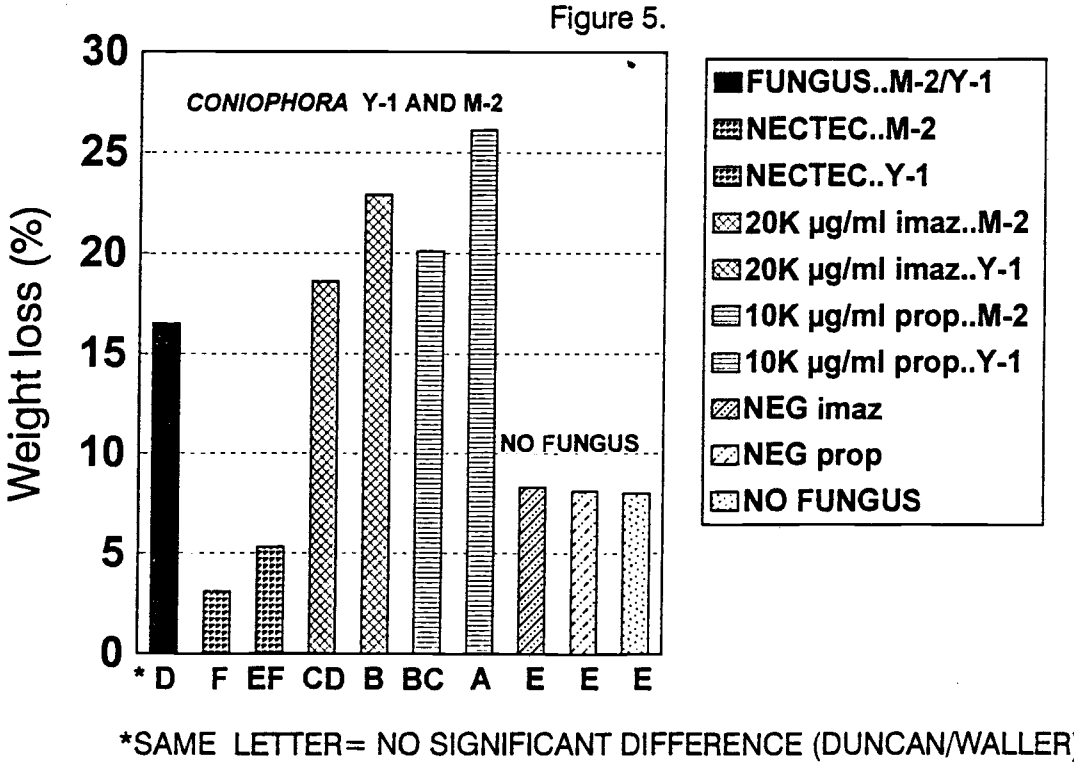

Figure 5. Second *in vitro* fungicide trials on lemon blocks inoculated with *Coniophora*. Higher concentrations of fungicides imazalil, propiconazole and NECTEC with no fungicides were tested. The mean weight loss of five replicates *in vitro* was calculated for each concentration of fungicide.