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**Root rot of hydroponically grown lettuce caused by *Phytophthora
cryptogea***

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The University of Arizona, 1991

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ROOT ROT OF HYDROPONICALLY GROWN
LETTUCE CAUSED BY *PHYTOPHTHORA CRYPTOGEA*

by

Alec Robert Linde

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

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Date

This thesis is dedicated to Pat, Erik, Elliot and Alison

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ABSTRACT

In April 1989, lettuce (*Lactuca sativa* L.) plants showing severe root rot symptoms were received for diagnosis from a commercial hydroponic facility in Southern California. A species of *Phytophthora* was consistently isolated from necrotic roots. Pathogenicity trials were conducted under hydroponic conditions in a greenhouse at root temperatures of 18 and 28 C. Root necrosis, along with stem decay and plant death, occurred within 5-7 days after inoculation at both temperatures. Reisolation of the fungus from roots and stems of inoculated, but not from roots of non-inoculated plants, confirmed pathogenicity. The fungus was identified as *Phytophthora cryptogea* Pethybr. and Lafferty on the basis of cultural and morphological characteristics. However, crosses with A₁ and A₂ mating types of *Phytophthora cryptogea* and other species of *Phytophthora* were not successful. This is the first report of *Phytophthora* as a root pathogen of cultivated lettuce.

INTRODUCTION

Many different crops are now being produced using hydroponic growing systems. These are mainly vegetable crops such as lettuce, tomatoes and cucumbers, but also include some foliage and flower crops. Hydroponics is the production of a crop utilizing an aerated complete nutrient solution instead of soil. Various media such as sand, pebbles, sawdust or rockwool are sometimes used to support the crop, but often the roots are contained only in a trough in which the nutrient solution flows. Systems employing this general technique are referred to as nutrient film technique (NFT). Other types utilizing only a nutrient solution are ebb and flow, whereby plants are supported in a tank which is alternately flooded and drained to provide nutrients and oxygen to the roots, and the raceway method, in which large shallow tanks are filled with the nutrient solution, with the crop supported on the surface of the solution on polystyrene boards with the roots in the aerated nutrient solution.

There are several advantages generally attributed to the hydroponic method of growing vegetable crops. These include a higher density of the crop per unit area, efficient use of water and fertilizer, and production in areas that would otherwise be unsuitable for crop production because of temperature or soil factors. Hydroponics often provides for a

faster turnaround time for the crop, and usually allows for year-round production because most hydroponic growing is done in greenhouses. Hydroponically grown lettuce is also clean and free of field debris, grit and pesticides, which is very appealing to the consumer.

This seemingly ideal vegetable production system is not, however, without problems. It was initially thought that by growing a crop in soilless culture, disease problems caused by soil-borne plant pathogens would be reduced, if not eliminated. This has been true to some extent. However, as well as providing an ideal growing environment for the crop, the hydroponic system provides the perfect environment for some root pathogens, notably those belonging to the fungal group Oomycetes, or water molds. Several members of the Chytridiomycetes also can cause problems in hydroponics. These two classes contain the motile zoosporic fungi, which are extremely well suited to this environment. Species in the genus *Pythium* have been the most common cause of disease problems in hydroponics (Stanghellini, 1988). However, other members of the Oomycetes such as *Phytophthora* spp. (Holderness and Pegg, 1986; Skadow, et al, 1984; Van Voorst, et al, 1984), and several species of the chytrid *Olpidium* (Tomlinson and Faithfull, 1980; Tomlinson and Thomas, 1986), which are vectors of several viral diseases including big-vein of lettuce, have also caused problems in hydroponics. Also,

species outside of the zoosporic groups such as *Fusarium* (Couteaudier and Alabouvette, 1981; Evans, 1979; Staunton and Cormican, 1978) and *Colletotrichum* (Couteaudier and Alabouvette, 1981; Daughtrey and Shippers, 1980; Jenkins and Averre, 1983) have either been isolated from the roots of crops, or reported as plant pathogens.

Lettuce is one vegetable crop that is very well suited for hydroponic production and is now being grown world-wide employing hydroponic methods. And although several fungi have been reported as root pathogens of lettuce in hydroponics, no *Phytophthora* species has ever been reported as a root pathogen of lettuce, either in hydroponics or in traditional field-grown lettuce.

In April of 1989, our laboratory received lettuce plants of several varieties from a large hydroponic facility in Southern California. The plants had symptoms of severe root necrosis along with shoot necrosis, chlorosis and stunting. Microscopic examination of roots led to the observation that *Phytophthora*-like sporangia were present, along with oospores identified as *Pythium uncinulatum* Plaats-Niterink & Bok. It was also determined that *Pythium dissotocum* Drechs. was present in the root samples. *P. dissotocum* has been reported as a pathogen of lettuce, and *P. uncinulatum* has been isolated from lettuce roots, both cases from hydroponic systems (Stanghellini and Kronland, 1986). Since no *Phytophthora* sp.

has been reported from lettuce roots, it raised the question of which organism was the cause of this root rot of lettuce.

Objectives

The objectives of this research were to: 1) prove the pathogenicity of this species of *Phytophthora* according to Koch's Postulates; 2) identify this fungus to species if proven pathogenic to lettuce.

MATERIALS AND METHODS

Isolation

Isolation of the fungus was attempted by two methods. First, diseased roots were thoroughly washed in running water, blotted dry on paper towels, then plated on 2% water agar (WA). Single sporangia, formed on the surface of the roots, were removed by use of a drawn out glass pipette and transferred to 10% V-8 juice agar (VJA). The second method utilized was a pear baiting technique. Symptomatic roots were thoroughly washed in running water, then placed in a 500 ml beaker filled with sterile distilled water (SDW). A non-ripe pear was suspended part way into the water by three toothpicks (Fig. 1) then incubated at room temperature for 24-36 hours. After lesions appeared, the pear was removed from the water and surface sterilized with 0.5% NaOCl for 5 minutes. Small pieces of internal tissue from lesion areas were then excised and placed on WA and incubated for 48 hours at room temperature. After incubation, the fungus was hyphal-tipped and transferred to VJA.

Pathogenicity Tests

Pathogenicity tests were conducted in the greenhouse under hydroponic conditions, under two temperature regimes using plants of two ages.

Figure 1. Pear baiting technique used for the isolation of the *Phytophthora* lettuce isolate.

Seeds of 5 lettuce varieties, Red Leas (RL), Dark Green Boston (DG), Grand Rapids (GR), Paris Island (PI) and Empire (EM) were germinated on WA plates. After 2 days the seedlings were transferred to Oasis Growing Medium Cubes (Smithers-Oasis Co., Kent, Ohio) cut into 30mm diameter cylinders. The seedlings were then transferred into holes cut into polystyrene flotation boards (32 x 27 x 1.9cm) and the boards placed into plastic tubs filled with a continuously aerated nutrient solution (Fig. 2).

The nutrient solution consisted of two stock solutions; Stock A: Hydrosol (Peter's Fertilizer Products, W.R. Grace and Co. Fogelsville, Pennsylvania), at a rate of 97.2 g/l of water along with 20.0 g/l magnesium sulfate; Stock B: Potassium nitrate at a rate of 54.4 g/l, and calcium nitrate at a rate of 87.5 g/l (Peter's fertilizer products). Each stock solution was used in equal amounts at a final dilution of 1:100 split into 2 applications: 1/2 was applied at experiment setup, the remainder was added after 2 weeks of plant growth.

Two sets of plants of different ages were used in this study and were obtained by repeating the initial germination process 14 days after the first. Different root temperature regimes were maintained by separating two sets of tubs into individual climate-controlled boxes (Fig. 3). One box was set to maintain the root nutrient solution at 18 C, the other at

Figure 2. The hydroponic system used in the study of root pathogens on lettuce.

Figure 3. Diagrammatic representation of a hydroponic cultural system employing air as a heat-exchange medium to regulate water temperature. (From Stanghellini and Kronland, 1986.)

28 C. Ambient greenhouse temperature during the experiment ranged from 28-32 C. Fresh shoot weights were taken of all living plants after 4 weeks.

In order to reisolate the fungus, roots exhibiting symptoms of browning or necrosis were randomly sampled, washed in running water for 10 minutes and plated on 2% WA.

Zoospore Production and Infestation

An 8mm diameter agar plug of *Phytophthora* was placed at the perimeter of a VJA plate and incubated at room temperature for 48 hours. From the actively growing margin of the culture, 1.5 cm² blocks were cut out and placed into petri dishes and covered with aerated SDW. The plates were incubated at room temperature for 48 hours to initiate sporangia formation, after which they were placed into 4 C for 30 minutes, then removed and allowed to return to room temperature. Zoospore emergence began within 20-30 minutes.

Two lettuce plants of each variety were selected and their roots placed into the zoospore suspension for 3 hours. The plants were removed and one plant of each variety was placed into a tub containing 4 healthy plants, two each of the two ages, and all of the same variety. These "mother plants" served as the inoculum source for the remainder of the plants in that tub. Control plants were handled in a similar manner except that they were placed into SDW for 3 hours.

Cultural and Morphological Characteristics

Few species of *Phytophthora* can be identified by a single characteristic, although several species have one or two unique characteristics. One example would be the compound sporangial form exhibited by *Phytophthora infestans*. Generally, however, *Phytophthora* species are identified by gathering information on many characteristics such as cardinal temperature, sporangial dimensions, antheridial type and oogonium size, and presence of chlamydospores. Different features carry more or less weight depending on its constancy. Several cultural and morphological characteristics for this lettuce isolate were examined in order to help identify it to species.

Cardinal growth temperatures

From the actively growing margin of a 48 hour old culture, 8 mm diameter plugs were transferred to the perimeter of 9 cm diameter petri plates containing corn meal agar (CMA). The plates were incubated at temperatures of 10, 15, 20, 27, 32 and 37 C. Following a 24 hour base period, radial growth was measured to the nearest mm every 24 hours for 96 hours and recorded. The growth distances for each 24 hour period were averaged to obtain a mean daily growth rate. The experiment was replicated two times and repeated twice.

Sporangia Size Determination

From the actively growing margin of a culture, 8 mm diameter plugs were placed on clarified VJA prepared as follows: 100 ml of V-8 juice was added to 500 ml of water and boiled for 3 minutes, then filtered first through cheesecloth, then Whatman no. 1 filter papers. To the filtrate was added 1 g CaCO₃ and 20 g agar. The volume was brought to 1000 ml with distilled water and autoclaved. After the mycelium had covered the plate, random field measurements were made of the length and width of 25 sporangia and recorded.

Mating tests

Mating tests were carried out with several known species of *Phytophthora*, including both A₁ and A₂ mating types of *P. cryptogea* (Table 1).

Eight millimeter diameter plugs from each of two actively growing cultures of the species or isolate to be tested were placed opposite each other at the perimeter of 9 cm diameter petri plates containing clarified VJA. Plates were inspected weekly for 4 weeks and the presence or absence of oogonia development noted. When oogonia were present, the isolate from which the oogonia originated from was determined.

Table 1. Species and isolates of *Phytophthora* mated with the *Phytophthora* lettuce isolate.

A₁ mating type

A₂ mating type

P. cambivora
P. capsici
P. cryptogea
P. drechsleri
P. parasitica
P. palmnivora

P. capsici
P. cinnamomi
P. drechsleri
P. parasitica
P. palmnivora
P. cryptogea

RESULTS

Isolation

Pure cultures of *Phytophthora* were successfully obtained using both the single sporangia method and the pear-baiting technique.

Pathogenicity Tests

By day 7 after inoculation, all of the mother plants exhibited extensive root browning and stunting. By day 14, the fungus had spread to most of the plants in the inoculated tubs. Figure 4 shows the high degree of stunting and root necrosis resulting from infection by this pathogen compared to the control. These plants are from the group grown at 28 C. The symptoms on plants grown at 18 C were similar.

The fresh shoot weights of the test plants and control plants grown at 18 C and 28 C are shown in Figure 5. These weights were taken only from the oldest plants in the treatments. Most of the young plants died within the experimental period.

The *Phytophthora* lettuce isolate was successfully isolated from all diseased roots sampled.

Figure 4. Lettuce plants from pathogenicity trials. Healthy plant (right) and infected (left) showing severe stunting and chlorosis.

SHOOT WEIGHTS 18 C

SHOOT WEIGHTS 28 C

Figure 5. Fresh shoot weights of the 5 varieties of lettuce used in pathogenicity trials. A. 18 C root temperature. B. 28 C root temperature. GR=Grand Rapids, PI=Paris Island, DG=Dark Green Boston, RL=Red Leas, EM=Empire.

Figure 6. Mycelial growth rate of *Phytophthora* lettuce isolate on CMA.

Cultural and Morphological Characteristics

Cardinal growth temperatures

The mycelial growth rate of the lettuce isolate over a range of temperatures is shown in Figure 6. The optimum temperature is around 27 C, but this species grew well in the range from 15-30 C. The fungus was able to grow at 10 C, the minimum temperature tested, but growth at 34 C was completely inhibited.

Sporangial Size Determination

Table 2 shows the results of the mean length and width measurements of sporangia of the lettuce isolate, compared with those of two other *P. cryptogea* species. There was no significant difference between the measurements of these three isolates.

Mating tests

Mating attempts between the *Phytophthora* lettuce isolate and the tester strains were not successful. No sexual structures that were found in crosses involving the lettuce isolate could be traced back and determined to originate from the *Phytophthora* lettuce species.

Table 2. The mean sporangial measurements of the lettuce isolate and two known *Phytophthora cryptogea* isolates.

<i>P. cryptogea</i>	<i>P. cryptogea</i>	Lettuce isolate
37.9 ^a	35.0	35.0
55.3	50.5	50.3

^a average of 25 sporangia/isolate (microns)

DISCUSSION

Diseases of hydroponically grown crops, specifically those caused by *Pythium* and *Phytophthora* species can be very devastating. The extremely high inoculum potential, growth rate and motility of these organisms enable them to spread very rapidly in these systems often causing total crop loss (Bates and Stanghellini, 1984; Stanghellini and Russell, 1971). For these same reasons, prediction and prevention are also difficult since the whole system becomes infested with the organism within days after introduction. Another difficulty faced by hydroponic growers is eliminating these root pathogens from both the crop and the cultural system. No fungicides are registered for use in hydroponics, therefore, they cannot be used as either preventive or curative measures. Other methods of control have been evaluated and have been successful for use in some applications. Successful work has been done using surfactants to eliminate zoospores from the nutrient solution (Stanghellini and Tomlinson, 1987; Tomlinson and Faithfull, 1980). This methodology is currently being used in other countries. However, the use of surfactants in hydroponic vegetable production is not allowed in the United States. Another method is the recirculation of the infested nutrient solution through an ultraviolet light chamber which sterilizes the solution. Stanghellini, et al. (1984) found

this method works very well for the elimination of *P. aphanidermatum* inoculum, but only at the relatively small flow rate of 124 l/min. If the flow rate was increased to 300 l/min., sterilization was not accomplished. This excludes large commercial systems from utilizing this technology without large capital expenditures. Filtration is also an option. Goldberg (1990) demonstrated that zoospores of *P. aphanidermatum* can be effectively filtered from the solution by using a 7 μ m filter. But again, this technology is limited by flow capacity and the large expenditures required for installation and maintenance of these systems.

Based on morphological and cultural characteristics, the lettuce isolate has been identified as *Phytophthora cryptogea*. This species is ubiquitous in distribution and causes root diseases of many cultivated crops. However, this is the first reported case of any *Phytophthora* spp. as a root pathogen of lettuce, although a stem rot of lettuce caused by *Phytophthora porri* has been reported in Australia by Sitepu and Bumbieris (1981). This research has also shown that all of the lettuce varieties tested are susceptible to root rot by *Phytophthora cryptogea* even though this disease has never been reported in the field. There are many reasons that could account for this. Simple explanations include: lettuce simply is not susceptible in the field to *Phytophthora* infection; or susceptible plants and the pathogen are not present together in the lettuce

growing regions, separated either spatially or temporally. A more complex explanation could be that changes occur in the virulence of the pathogen or susceptibility of the host due to the hydroponic environment. In addition, the difficulty in isolating and separating this organism from these roots in the presence of much faster growing *Pythium* spp. may provide an additional reason as to why *Phytophthora* has not before been isolated from either field or hydroponically grown lettuce. Upon plating on WA, other organisms such as *Pythium* may grow out more rapidly causing any *Phytophthora* to be overlooked. In my particular case, the pear-baiting method used for isolation is effective because *Pythium* spp. are not, to my knowledge, pathogenic to unripe pears as are many *Phytophthora* spp.

Pathogenicity tests demonstrated no measureable differences in disease severity between plants grown at 18 C root temperature and those grown at 28 C, even though there is a mycelial growth rate difference between these temperatures.

This is the only reported case of *Phytophthora* root rot of lettuce. Based on cultural and morphological characteristics, this isolate from hydroponically grown lettuce has been identified as *Phytophthora cryptogea*. Pathogenicity trials carried out under hydroponic conditions, confirmed that this isolate was highly pathogenic to all lettuce cultivars tested.

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