ROOT DISEASE TREATMENT METHODS
FOR COMMERCIAL PRODUCTION OF HYDROPONIC SPINACH

Final Report

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**ABSTRACT**

*Pythium aphanadermatum* is a devastating root disease organism to which spinach is particularly susceptible. This disease has prevented successful hydroponic spinach production in the United States. In this project, several conventional and one novel nutrient solution cleansing methods (ultraviolet radiation, sonication, filtration, and electrochemical treatment) were evaluated to determine their efficacies in suppressing disease in continuous production for at least as long as required for baby-spinach to reach harvest (approximately two weeks after germination). Additionally, aeroponics production was contrasted to deep-pond production. No conventional method worked in the deep-flow system. The method that did work was to reduce nutrient solution temperature to 20°C (68°F) and produce commercial-quality crops within 14 days. A surer method was to create sequential production ponds where plants are moved from one to a second part-way through the production cycle. The method is believed to work by taking advantage of the disease reproduction period, which appears to be approximately 15 days at 20°C. This method requires limited refrigeration capacity in an insulated deep-pond system of commercial size but does absolutely require supplemental lighting and daily light integral control to achieve sufficient productivity within the allowable production period before disease strikes, as it will.

**KEY WORDS**

hydroponics, *Pythium aphanadermatum*, spinach, root disease, temperature control, water treatment
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SUMMARY

Consumer demand for fresh, high-quality spinach has increased dramatically during recent years. Outbreaks of *E. coli* in field-grown spinach (as well as disease outbreaks associated with other fresh produce), coupled with increased transportation and energy costs, make clear the need for commercial-scale, local production of fresh hydroponic vegetables in New York State. The biggest obstacle to hydroponic spinach production is *Pythium aphanidermatum (Pa)*, a water mold that spreads through the nutrient solution and devastates the roots of a crop.

Objectives of this project were twofold; first, to investigate new treatment methods to eliminate *Pa* zoospores from nutrient solution and, second, to compare efficacies of two production systems, aeroponics and a shallow-flow version of deep-flow systems, in terms of suppressing development and spread of *Pa* infections.

The first experiments examined several treatment methods on a bench scale. Bench scale tests allowed various operational parameters to be tested quickly and easily and allowed identification of treatment methods that should be investigated further, on a larger scale. The treatment methods initially tested included the more traditional methods of pasteurization and ultraviolet irradiation, along with sonication and electrochemical disease control. Although considerable research has been published on pasteurization and ultraviolet irradiation, these methods were selected to verify the recommendations and to compare, directly, their efficacies to the newer techniques. To evaluate and quantify the efficacies of all of the methods, a simple spinach seedling bioassay was developed.

Concurrently with evaluations of the bench top units, experiments were completed to determine the best growing system to produce baby-leaf spinach. The systems examined were aeroponics, and a modified floating pond (deep-trough) system. An aeroponics bench was constructed and several methods of developing the nutrient mist were investigated. The second system examined was a modified pond system. Shallow benches were constructed to reduce the nutrient solution volume. The shallow flow benches were constructed with a solution depth of 7 cm. Aeroponics and the shallow flow systems were used to grow crops of baby-leaf spinach, and the biomass yields and system operational requirements were compared.

Following comparisons of the aeroponics and shallow flow systems, experiments were completed to examine the effects of an initial heavy inoculation of a known virulent strain of pythium zoospores into a continuous production system with a complete range of crop ages present in the same system. This experiment was undertaken to determine how (and whether) a pythium infection would propagate.

In the next experiment, results of the bench top experiment were used to determine operation parameters of
various treatment methods, combined with findings of the inoculation experiment that revealed temperature suppression in the root zone worked as a means of controlling *Pa* in a continuous production system. The four conditions examined were: ultraviolet irradiation, filtration, temperature reduction, and a control. Four benches with these conditions were inoculated with *Pa*, and four were left un-inoculated to determine the effects (if any) these treatments would have on spinach production. The eight benches were populated with spinach of five different ages, spaced at three day intervals. The benches were inoculated 24 hours later to give the *Pa* zoospores a chance to establish themselves in the crop, whereupon the treatment methods were applied. Biomass data were collected for each harvest. When the first part of the experiment was formally terminated, the benches that were still heavily infected had their nutrient solution temperatures reduced. The results showed that temperature reduction to 20 C induced gradual recovery even from a heavily infected state.

Findings indicated temperature reduction was best for continuous production of baby-spinach in a shallow flow system. Additional experiments were completed to examine use of a wild-strain of *Pa* taken from a commercial greenhouse pond solution. In addition, these experiments were conducted in the more traditional deep pond systems wherein previous spinach production development work had been conducted. The purpose of these experiments was to establish whether the deep pond system was as effective as the shallow flow system, and to see how temperature reduction worked against a wild strain of *Pa*. During these experiments several ideas were examined, including shortening the crop duration in a single pond to nine days to recover from a heavy infection, as well as the idea of using two ponds sequentially in a twelve day production cycle. The idea behind the two pond system is that the young crop should spend only six days in the first pond, which is much less time than *Pa* requires to reproduce, whereupon the material is moved to the second pond to grow for another six days before harvest. Even if the initial pond is heavily infected, over time it will clean itself to a point where uninfected material is being moved into the second pond. At this point the second pond would begin to clean itself because only clean material is being supplied for it and, again, six days is not enough time for the *Pa* to reproduce.

The required refrigeration capacity to cool deep ponds is small. The pond nutrient solution recirculation pump was calculated to be the major contributor to the cooling load. The yearly cooling cost of a pond of commercial size was calculated to be no more than $0.20/ft$. Moreover, the opportunity exists to capture heat rejected from the chiller to preheat water used for other purposes in a facility.
CHAPTER 1
BACKGROUND AND INTRODUCTION

Consumer demand for fresh, high-quality spinach has increased dramatically during recent years. Outbreaks of *E. coli* in field-grown spinach (as well as disease outbreaks associated with other fresh produce), coupled with increased transportation and energy costs, make clear the need for commercial-scale, local production of fresh hydroponic vegetables in New York State. The biggest obstacle to hydroponic spinach production is *Pythium aphanidermatum* (*Pa*), a water mold that spreads through the nutrient solution and devastates the roots of a crop. In a previous project funded by NYSERDA, a system was developed using deep-trough (or deep-flow) hydroponics that solved two important hydroponic spinach production problems, germination non-uniformity and premature bolting. The third critical problem, root disease, was the focus of this project.

CHRONOLOGY OF EXPERIMENTS

Objectives of this project were twofold; first to investigate new treatment methods to eliminate *Pa* zoospores from nutrient solution and, second, to compare efficacies of two production systems, aeroponics and a shallow-flow version of deep-flow systems, in terms of suppressing development and spread of *Pa* infections.

The first experiments examined several treatment methods on a bench scale. Bench scale tests allowed various operational parameters to be tested quickly and easily and allowed identification of treatment methods that should be investigated further, on a larger scale. The treatment methods initially tested included the more traditional methods of pasteurization and ultraviolet irradiation, along with sonication and electrochemical disease control. Although considerable research has been published on pasteurization and ultraviolet irradiation, these methods were selected to verify the recommendations and to compare, directly, their efficacies to the newer techniques. To evaluate and quantify the efficacies of all of the methods, a spinach seedling bioassay was developed.

Concurrently with evaluations of the bench-top units, experiments were completed to determine the best growing system to produce baby-leaf spinach. The systems examined included aeroponics, and a modified floating pond (deep-trough) system. Aeroponics uses a spray system to mist nutrient solution onto the crops roots, which dangle into a misting chamber. An aeroponics bench was constructed and several methods of developing the nutrient mist were investigated. The second system examined was a modified pond system. Pond systems were used in the initial investigation of spinach production (previous project). Shallow benches were constructed to reduce the volume of nutrient solution needed. Traditional pond systems use a nutrient solution depth of approximately 30 to 45 cm. The shallow flow benches were constructed with a solution depth of 7 cm. Reduced system nutrient solution volume was chosen as a goal to make the
treatment of this solution easier. In addition, the benches were designed to provide a well-defined direction to the flow, placing new plant material at the inlet end of the bench, immediately following the treatment. Aeroponics and the shallow flow systems were used to grow crops of baby-leaf spinach, and the biomass yields and system operational requirements were compared.

Following comparisons of the aeroponics and shallow flow systems, experiments were completed to examine the effects of an initial heavy inoculation of a known strain of pythium zoospores (Pa 58) into a continuous production system with a complete range of crop ages present in the same system. This experiment was undertaken to determine how (and whether) a pythium infection would propagate.

In the next experiment, results of the bench top experiment were used to determine operation parameters of various treatment methods, combined with findings of the inoculation experiment that revealed temperature suppression in the root zone worked as a means of controlling Pa in a continuous production system. This experiment used eight shallow flow benches. The four conditions examined were: ultraviolet irradiation, filtration, temperature reduction, and a control. Four benches with these conditions were inoculated with Pa, and four were left un-inoculated to determine the effects (if any) these treatments would have on production. The eight benches were populated with spinach of five different ages, spaced at three day intervals. The benches were inoculated and, 24 hours later, the treatment methods were applied. This period was provided to give the Pa zoospores a chance to establish themselves in the crop. Biomass data was collected for each of the harvests. When the first part of the experiment was formally terminated the benches that were still heavily infected had their nutrient solution temperatures reduced. The results showed that temperature reduction to 20 °C induced recovery from a heavily infected state.

**RECOMMENDED SOLUTION**

Findings indicated the temperature reduction method was best for continuous production of baby-spinach in a shallow flow system. To confirm findings of this experiment further, a series of replications was completed that examined only temperature reduction. A variation in this experiment was that following inoculation, the Pa infection was allowed to proceed through several harvests – until the material removed was heavily infected – and had not been present when the initial inoculation was applied. This was to demonstrate how the Pa infection was self-sustaining and did not naturally die out. This also allowed replicates of conditions from previous experiments in which the temperature suppression method was used to recover from a heavily infected state. This experiment was repeated twice; once in winter, and once in summer, to show the effect was not strictly seasonal and that the system functioned properly under the relatively high stress period of summer heat.
During the second replicate of the temperature reduction method, additional experiments were completed in a separate compartment of the greenhouse to examine use of a wild-strain of *Pa* as taken from a commercial greenhouse pond solution. In addition, these experiments were conducted in the more traditional deep pond systems wherein previous spinach production development work had been conducted. The purpose of these experiments was to establish whether the deep pond system was as effective as the shallow flow system, and to see how temperature reduction worked against a wild strain of *Pa*. During these experiments several ideas were examined, including shortening the crop duration in a single pond to nine days to recover from a heavy infection, as well as the idea of using two ponds sequentially in a twelve day production cycle. The idea behind the two pond system is that, because the young crop only spends six days in each pond, which is much less time than *Pa* requires to reproduce, before the material is moved to the second pond to grow for another six days before harvest. Even if the initial pond is heavily infected, over time it will clean itself to a point where uninfected material is being moved into the second pond. At this point the second pond would begin to clean itself for only clean material is being supplied for it and, again, the six days that the crop spends in this pond is not enough time for the *Pa* to reproduce.
CHAPTER 2
DESIGN AND FABRICATION OF TEST FACILITIES

BENCH TOP TREATMENT METHOD DESIGNS
Four treatment methods, Electrochemical Disease Control (EDC), pasteurization, sonication and ultraviolet (UV) irradiation were evaluated, on a bench-top scale, for their effectiveness in reducing root damage due to *Pythium aphanidermatum* (*Pa*). Treatment conditions of pasteurization and UV sterilization that have been demonstrated to be effective against *Pa* were used to verify the effectiveness of literature recommendations and allow comparisons with the new technologies, EDC and sonication. Bench-top systems were used to quickly evaluate the effectiveness of these treatment methods and determine optimum flow rates and other parameters of operation. Rather than attempt to measure the number of zoospores through more traditional means with a hemocytometer or plating, a bioassay using spinach seedlings was used. This simple system, in concert with bench top setups, allowed a greater number of treatment method variations to be quickly and easily tested. (The bioassay technique is detailed in Chapter 3.)

ELECTROCHEMICAL DISEASE CONTROL
Spinu, et al. (1998) describe a method that uses electro-dialysis to adjust the pH of the nutrient solution continuously to a desired level for regular pH control. The EDC treatment system used the same principles, but took the pH to greater extremes.

Figure 2.1. Electrochemical pH adjustment apparatus.
The electrochemical treatment unit (Figure 2.1) consisted of two compartments 10 cm x 10 cm x 15 cm separated from each other by a cation exchange membrane. In the cathode compartment a stainless steel electrode measuring 9.5 cm² was placed against the side of the compartment furthest from the membrane (Figure 2.1). In the anode compartment a titanium plated electrode of identical size was placed on the opposite side. During operation 100 VDC was applied to the electrodes. Voltage across the electrodes was measured with a Greenlee Multimeter (model # DM-200).

PASTEURIZATION
Pasteurization has been used for many years to remove pathogens from many liquids, including nutrient solutions, and considerable research has been conducted as to its efficacy. Several authors recommend a temperature of 95°C for 30 seconds, (Runia et al., 1988, Rey et al., 2000), however others have demonstrated that lower temperatures and longer durations such as; 55°C for 2 minutes, (Tu and Zhang 2000), and even 51°C for 15 seconds (Runia and Amsing 2000) can be effective as well.

In the pasteurization treatment, two liters of nutrient solution was heated on a Thermolyne hotplate (Type 2200), one to a target temperature of 60°C and one to 95°C (measured with a Digi-Sense digital thermometer model # 8528-20). The solution was then thoroughly mixed with a glass stirring rod and at set time intervals 400 ml of solution were removed, placed in an Erlenmeyer flask, and chilled under cold tap water until the solution reached ambient temperature.

SONICATION
Sonication employs the phenomenon of cavitation to lyse cells, rendering them harmless. Cavitation is the formation of pockets of vapor in the solution due to a localized and short-term reduction of pressure in the liquid. A probe vibrating at a high frequency (generally 20 to 40 kHz) causes cavitation in the solution. The rapid formation and collapse of the pockets of vapor create extreme conditions under which cells are destroyed. Tu and Zhang (2000) examined the use of sonication for eliminating Pythium in nutrient solution in a bench top test. They found that after 1.5 minutes of sonication 100% of root disease zoospores and cysts were destroyed. They applied their treatment in a batch system where a probe vibrating with an amplitude of 120 µm and frequency of 20 kHz was inserted into a beaker containing 150 ml of solution.

To test sonication on a flow-through or continuous basis, we used a Misonix flowcell (model # 800B), that attached to our sonicator probe. Sonication was applied with a Misonix 500W ultrasonic generator (Model #2020XL), and converter (model CL4) with a 13 mm (½ inch) probe. The probe was threaded, allowing it to screw into the flow cell. Flow was directed up through the base of the flow cell through a 3.2 mm (1/8”) orifice plate where it contacted the tip of the probe. Solution then flowed around the probe and exited the
flow cell at the highest collection port. The solution was pumped through the flow cell with a Procon pump (model # C01607AFV) at a pressure of 700 kPa (100 psi).

**ULTRAVIOLET IRRADIATION**

Ultraviolet irradiation is a very popular method for disinfecting recirculating nutrient solutions. Stanghellini, et al. (1984) found that a dose of 90 mJ cm$^{-2}$ provided adequate disinfection, and Tu and Zhang (2000) found that 80 mJ cm$^{-2}$ was sufficient to kill one hundred percent of Pythium zoospores and cysts.

Ultraviolet treatment of the nutrient solution was achieved using an 8W, flow-through, ultraviolet reactor (model Aqua Ultraviolet 8W), which used a low-pressure bulb to produce UV-C at a wavelength of 253.7 nm. UV dosage was regulated by varying the flow rate through the reactor. Control of the flow through the UV treatment unit was with a ball valve mounted immediately before the inlet to the reactor. Closing the ball valve caused more of the flow to divert through a bypass and return to the reservoir containing the Pythium infected solution. The action of the bypass also served to keep the solution well mixed and prevented Pythium from settling to the bottom of the tank. UV doses of 120 and 240 mJ cm$^{-2}$ were examined. In normal operation, UV sterilization equipment requires filtration to remove particles that prevent transmission of the UV light. Because our nutrient solution was new, no such sediment was present and filtration was not used. This also allowed measurement of the UV method effectiveness alone, without having to account for filtration effects on the Pa zoospore removal.

**AEROPONIC, SHALLOW-FLOW AND DEEP-POND SYSTEMS**

In addition to comparing the effectiveness of the various treatment methods an additional goal of this project was to examine two different hydroponic production systems: aeroponics and deep flow.

**Aeroponics**

Aeroponics is a method of plant propagation that involves spraying or misting nutrient solution directly onto roots dangling in air. This allows for excellent oxygenation and can lead to greater yields in certain crops. In an aeroponics system, the plant is supported through some (usually inert) structure/media and the roots are allowed to grow down into a misting chamber. Nutrient solution is applied to the roots either periodically, or continuously, by a spray system. Nutrient solution that is not directly absorbed by the roots falls to the bottom and is collected into a reservoir and reapplied.

Aeroponics is currently used primarily for research purposes, to observe root development and conduct precise analyses of plant nutrient uptake. Commercial use of aeroponics has been relatively limited due to higher capital and maintenance costs as compared to other, more traditional, hydroponics systems. Another
A drawback of the aeroponic system is that there is little in the way of buffering if a problem should arise to prevent nutrient solution from being delivered. Without a constant regular application of nutrient solution the crop can quickly wilt and die. Successful operation of an aeroponics production system requires regular monitoring and maintenance.

Aeroponics was considered an excellent candidate for baby leaf spinach production because of how it is fundamentally different from other more traditional production systems. In pond and Nutrient Film Technique (NFT) production, *Pa*can rapidly spread as the mobile zoospores swim in the common pond of nutrient solution, seeking out new roots to infect. Plants don’t have this direct connection through the nutrient solution in aeroponic systems, which allows the plants to be somewhat isolated from one another. Although the nutrient solution for the crop is usually drawn from a common reservoir, into which unabsorbed solution may be returned, aeroponics is well suited for in-line nutrient solution treatment systems. Nutrient solution taken from the reservoir can be treated by any number of technologies before being distributed to the misting nozzles. This type of in-line treatment is not possible in pond and NFT systems because there is still the opportunity for zoospores to swim from one root to another. Of pond, NFT and aeroponic systems, aeroponics is the only system of the three that offers the possibility of isolating infected individual plants and preventing the spread of Pythium to the rest of the crop.

**Aeroponic Bench**

To determine the utility of using aeroponics to produce baby-leaf spinach, an aeroponic unit was constructed. The surface dimensions of the unit mimicked those of the deep flow benches in that they were designed to allow several cohorts of spinach flats in the unit at the same time. However, the aeroponic bench independently supported each of the floats, allowing roots to dangle into the misting chamber beneath. The misting chamber depth was, on average, 50 cm; a slight slope of the bottom facilitated collection of excess nutrient solution (Figure 2.2).

To help guard against temperature swings and light penetration, the misting chamber was insulated with 3/4” polystyrene. A sheet of 5 mil plastic was used as a liner. Collected nutrient solution was allowed to drain into a reservoir beneath the bench, from which nutrient solution could be drawn and replenished. Access panels were placed on the top and sides to allow access to the aeroponic system and to allow for visual inspection of roots.
An important part of an aeroponic system is the equipment to deliver nutrient solution to the roots. In a densely matted root system this can be quite difficult if droplet size is too coarse. Finer droplet sizes allow better penetration of nutrient solution into the root mat. The most common method to generate fine water droplets is through use of nozzles. In the course of this investigation we examined two different styles of nozzles: air atomizing, and low-pressure water.

In the spray industry, a common technique for creating uniform, fine liquid droplets is through using a special nozzle and high-pressure air. In this style of nozzle, high-pressure air is directed against a stream of liquid, which separates droplets from the stream. The water is sprayed out of a center tube and a high-pressure air jet is ejected around the water. This causes the water to separate (atomize) into very fine
The nozzles we investigated were manufactured by Spray, Inc., and used air at 60 psi and nutrient solution at 15 psi which, according to company literature, yielded a mean droplet size of 200 microns. At these settings the nozzles yielded a large volume of spray and 2 nozzles were adequate to fill the misting chamber with nutrient spray. The high-pressure air was supplied through the built-in greenhouse air supply and a pressure regulator. Nutrient solution was provided through a pump (Little Giant 2-MCHD) drawing nutrient solution from the reservoir. Nutrient solution from the reservoir was pre-screened with a 100-mesh filter to remove particles that could clog the nozzles.

The air/water nozzles generated an excellent volume and quality of mist and several crops of baby-leaf spinach were grown in the system. However this style of nozzle required maintenance every 48 hours. Due to the high salt content of nutrient solution (compared to the water regularly used in the nozzles) the nozzles experienced a phenomenon known as "bearding", which was basically a build up of salt around the nozzle that eventually interfered with the generation of mist. The problem was easily rectified by removing the outer part of the nozzle and wiping the salt buildup away. However, this needed to be done every 48 hours or else the water would leave as a steady stream, not as a mist. The original nozzles were a nickel-plated brass material, but stainless steel nozzles versions were found to be just as susceptible. The relative expense of the nozzles, coupled with their required frequent cleaning, precluded them from further investigation. In addition. Moreover, the cost of providing high-pressure air in a commercial setting could make the use of this style of nozzle relatively expensive.

The second type of nozzle we examined was a more traditional style relying on water pressure alone to develop fine droplets. These plastic low flow nozzles, purchased from Ecologic Technologies, use a water pressure of 30 to 250 psi to develop droplets on the order of 100 microns. Nozzles of this style are frequently used in the greenhouse industry for humidity and temperature control purposes. However, these systems typically use very high-pressure water (1500 psi) and are used with pure water. To generate the 90 psi we required for our low-pressure nozzles, a stainless steel Procon rotary vane pump (Model # C01607AFV) was used. Several different plastic nozzle models were available. We selected the wide angle, high flow version. With a supply pressure of 90 psi the mean droplet size was 100 microns. However, flow from each nozzle was considerably less than the air atomizing system, so 10 nozzles were utilized within the system, spaced 20 cm apart (Figure 2.3.) This system proved to be workable, and allowed for the production of several crops with minimal maintenance of the nutrient delivery system.
Floating hydroponics is a proven means of producing high quality fresh leafy vegetables. In this type of system, Styrofoam plug trays hold the growing media and plants float on a pond of nutrient solution. The pond solution is mixed well to ensure uniform nutrient distribution and to provide root oxygenation. This system is well suited to commercial production due to the ease of materials handling (moving floats), the reliability and buffering of having a continuous supply of nutrient solution for the crop, and the ease with which the root zone temperature can be controlled. This type of system is used commercially to produce lettuce, among other crops.

Unfortunately, a pond system as typically designed for lettuce production could not be used to produce baby spinach. Spinach is much more susceptible to Pa, which can reproduce and spread rapidly. The ponds in

\[2.7\]
lettuce production are approximately 30 cm deep to prevent roots from touching the bottom of the pond and being damaged. This corresponds to a large system volume which, while providing an excellent buffer, makes treatment of the nutrient solution prohibitive.

The typical deep flow system for lettuce spinach production was modified and adapted to allow for testing disease treatment methods while attempting to minimize the spread of Pythium. Nutrient solution depth was reduced to 7 cm and flow of the solution was made unidirectional, with treated nutrient solution flowing past the youngest plants towards older material and on to the collection drain. It was hypothesized that such directionality would limit migration of Pa zoospores upstream to new crop material. Flow rates were set to provide an average solution velocity of approximately 1 cm/s below the floating plant trays that, from previous observations under a dissecting microscope, was judged to exceed the maximum self-propelled speed of Pythium zoospores.

For the experiment, 8 identical temperature controlled benches were constructed (Figure 2.4). The interior dimensions of the benches were 235 cm long by 35 cm wide, and 15 cm deep. To set the depth of the nutrient solution (half-strength Hoagland) to a height of 7 cm a standpipe drain was used. The benches were supported at a height of 80 cm above the floor and 150 cm below the lighting array, which provided an average PPFD intensity of 200 µmoles/m² PAR. Nutrient solution from the drain cascaded into a 50 liter
insulated reservoir, and through a coarse filter. The temperature of the nutrient solution in each bench was monitored by RTD (Omega RTD-810), and controlled through a LabView program with a USB DAQ (Measurement Computing USB 1208-LS), switching an immersion heater (Aquatic Ecosystems VT201, 200W), and a cold finger circulating chilled water. Nutrient solution was pumped up to the bench by a positive displacement pump (Little Giant 2-MCHD).

These growing benches were used to test continuous production of baby leaf spinach, both with and without treatment methods. Treatment methods consisted of filtration, UV irradiation, and temperature suppression. The filtration system was installed in-line after the pump and consisted of two 25.4 cm (10") opaque polypropylene filter units (Cole-Parmer C29820-32). The first filter provided filtration to a level of 5 microns (Cole Parmer C-01509-15) and the second to a level of 1 micron (Cole Parmer C-01509-14). Ultraviolet treatment was provided by Aqua ultraviolet aquarium treatment units (Aqua Ultraviolet Classic 15) and provided a dose of UV equivalent to 100 MJ/cm². To achieve the lower nutrient solution temperatures required for Temperature Reduction, cold fingers circulating chilled water were placed in the reservoirs of the systems. A surplus milk refrigeration unit chilled the cooling water, with the cooling water temperature set at 4°C.

**Deep-Pond systems**

Pond systems were originally used in development of baby spinach production. However, extreme levels of hygiene were required to ensure that *Pa* did not establish itself. This required frequent replacement of nutrient solution as well as limiting the number of crops grown in a batch of solution to one or two. Because of its relatively large plant-to-nutrient-solution volume ratio, and the relatively slow movement of the solution, deep pond systems are unsuitable for in-line treatment. However, they have an advantage over shallow-flow pond systems when applying temperature reduction; the large volume of nutrient solution acts as a temperature buffer and is less susceptible to temperature swings in the surrounding environment.

To simulate commercial pond systems, 400-liter polyethylene tanks (Aquatic Ecosystems, model TP130, mortar style) were used. These tanks are 185 cm by 91 cm by 30 cm deep (73 in. by 36 in. by 12 in. deep), 492-liter nominal capacity. The tanks rested in an insulated support structure consisting of 5 cm (2") thick polystyrene surrounded by 1.3 cm (½ inch) plywood. Mixing in the systems was achieved through a positive displacement pump (Little Giant 2-MCHD) which distributed nutrient solution through input and output manifolds (5 cm (2") PVC pipes with 1 cm holes placed every 30 cm). These manifolds were 150 cm long and were placed on the bottoms of the ponds at each edge, along the pond’s long dimension. Oxygenation of the nutrient solution was achieved through placement of two air-stones (Aquatic Ecosystems, AS3. medium pore diffusers, 2 in. long, 1 in. square cross section) with air supplied by an air pump (Model 5503, Jun adjustable dual air pump, Aquatic Ecosystems). Areas of the surface of the pond not covered by production
flats are covered with 2.5 cm polystyrene insulation to prevent light from penetrating the nutrient solution and to limit evaporation. Temperature control of the nutrient solution was achieved with a similar system to that described above for use in the shallow flow benches. A longer length of cold finger was placed directly in the bottom of each pond.
CHAPTER 3

TASK 3: *Pythium aphanidermatum* CULTURE

THE SOURCE OF THE DISEASE ORGANISM STRAIN

The strain of *Pythium aphanidermatum (Pa)* used in this research was the same as that reported in previous draft final report to NYSERDA (A Commercially-Viable Controlled Environment Agriculture (CEA) Spinach Production System, Contract Number 6257-IABR-IA-00, dated June, 2005). It was the same strain as used by Katzman (2003). The strain was chosen through a recommendation by Dr. Eric Nelson, Department of Plant Pathology, Cornell University (personal communication), because of its high degree of virulence. The strain is identified as Pa58.

METHODS OF MAINTENANCE

Depending on the frequency of use and duration between uses, *Pa* can be stored in a number of forms, and ways. Methods used in this research are described below.

**Long-Term Storage**

For long-term storage, previously sterilized blades of grass are infected with the pure strain of *Pa* desired, and are then stored dry at room temperature (c. 23°C). In this method of storage, *Pa* survives as encysted oospores that remain viable for years. Not only is this a convenient storage means, but it also limits long-term genetic drift in a population. Long-term storage was not required for this project. However, the initial culture was obtained from organisms maintained in this way in the laboratory of Dr. Nelson.

**Storage for Experimental Use**

For our experimental purposes, free-swimming zoospores of Pa58 were used as infective agents and the Pa58 organism was most conveniently maintained in its vegetative form as mycelium, from which zoospores could be produced as needed. To obtain a pure mycelium culture of Pa58, uncontaminated by other microorganisms, a small piece of grass containing the pure strain was placed on agar conducive to mycelium growth, and containing a selective antibiotic to suppress contamination. The mycelium culture produced in this way could be vegetatively propagated on agar for as long as needed. In our case we were provided an initial pure-strain mycelium culture of Pa58 by Dr. Nelson’s lab and, maintained it vegetatively thereafter.

The specific propagation method was to prepare a set of 25 ml petri dishes containing agar gel using 1.5 g CaCO₃, 8.5 g Bacto-agar, 143 ml of V8 juice (1 small can, salted, as a food source), and 357 ml of distilled water, always following sterile procedures. Three petri dishes containing this gel were seeded by 1 cm² blocks of mycelium-covered agar from the lab source, and designated as lines 1, 2 and 3. They were
incubated at 26 C for one week, by which time a thick mat of mycelium covered the petri dish. The procedure was repeated weekly. (The remaining agar filled petri dishes were retained in a sterile environment for future use.) Mycelium thus was kept available for experimental use for the duration of the research. If any line became contaminated it was discontinued and one of the healthy remaining lines was divided into two continuing lines. When mature mycelium was needed on a more frequent basis than weekly, which was the case during Task 5: Preliminary Testing, the schedule of reproduction was adjusted to make mature mycelium available every three days.

**METHOD FOR PRODUCTION OF ZOOSPORE INOCULUM**

To obtain accurate replicable dosages of Pa58 inoculum, free-swimming zoospores are preferable to mycelium because the number of propagules (zoospores) a given amount of mycelium is capable of producing depends on many factors such as age, temperature, growth conditions, and induction conditions, whereas zoospores may be counted. The common practice is to induce mycelium to produce large numbers of zoospores simultaneously when it has reached sufficient age.

The procedure we followed to produce zoospores from mycelium proved to be satisfactory. The first step was to remove two 1-cm diameter discs of mycelium-covered agar from a 25-ml source petri dish, place them in a 10-ml petri dish, cover them with 10 ml of cool, distilled water, and then set them aside at ambient temperature for 18 hours. Immersion in water and the temperature change had the effect of throwing the mycelium into reproductive mode. After 18 hours, the mycelium in the water was inspected under a dissection microscope at 20x magnification to see whether sporangia development was satisfactory, after which the distilled water was changed. The second change of water induced release of zoospores from sporangia. Peak release of zoospores tended to cluster around 4 to 5 hours after the change of water. Progress toward peak release of zoospores was checked under the microscope. When it was judged to have peaked, the liquid with zoospores was removed using a pipette.

**METHOD FOR QUANTIFYING INOCULUM CONCENTRATION**

Depending on how much inoculum was needed, variable numbers of petri dishes of inoculum were prepared. The extracted inoculum was bulked into one container, stirred thoroughly, and 250 microliters was extracted from the middle of the volume, using a pipette, and placed on a hemocytometer for counting. Counting took place in four areas of the grid for each hemocytometer sample. The average of the four counts was calculated and used as the representative concentration for that sample. This procedure was completed three times but if readings were significantly in disagreement, done additional times. The three independent concentrations determined in this way were averaged to obtain a final estimated concentration. Concentrations of zoospores were typically in the range 40 to 60 thousand zoospores per milliliter.
BIOASSAY FOR EVALUATING UNKNOWN LEVELS OF ZOOSPORES IN SOLUTION

To test the efficacies of the various treatment methods it was necessary to develop a method to quantify zoospore concentrations, if any, remaining after disease reduction treatments of the plant nutrient solution were completed. A simple bioassay was developed.

In this assay, arrays of eight spinach seedling radicles, c. 5 cm long, were produced bare-root on moist blotting paper in sealed containers, sloped to have the roots grow in the same direction. Each array of roots was then immersed up to the base of the hypocotyl in a 200 ml aliquot of the solution to be tested, and resealed in the container. After two days, the solution was drained and each radical inspected under a dissecting microscope and rated for the amount and type of disease damage. Typically, two or three replicates were used for each unknown solution. Simultaneously, a dilution series with known concentrations of zoospores was assayed in the same way. The dilution series was used to estimate the concentration of zoospores in the unknown solution, by comparison. A damage index was prepared for each root, in which the types of damage observed were weighted.

The assay is most effective in detecting concentrations of zoospores in the range 2 to 100 zoospore ml\(^{-1}\), but is sensitive to concentrations down to 1 zoospore ml\(^{-1}\). In a variant of the method, after the roots were covered with solution, the container was kept flat. Roots in this case were viewed through the solution at regular intervals to monitor disease progress (if any).
CHAPTER 4
PRELIMINARY TESTING

BENCH TOP TREATMENT METHOD EVALUATION

Bioassay

A preliminary step to evaluate any treatment technology to reduce plant disease is to determine its effectiveness in pathogen destruction. Ultimately, the best test of a system is to construct it to scale and operate it on a growing crop. However, this is not always feasible. Because of the dilute nature of the pathogen in nutrient solutions, it is not feasible to attempt to count zoospores directly in a system by using a hemocytometer, for example. Indirect counting techniques such as serial plating on selective agar, with colony counting, are generally used. However, such methods require a relatively small (~1 ml) sample size that, if the sample is not well mixed, can lead to erroneous results. Plating also requires use of expensive and perishable selective antibiotics to suppress competing organisms. In this research we developed a simpler, inexpensive alternative that uses newly germinated seedlings of the spinach crop itself as a form of a bioassay. We used this bioassay to determine the effectiveness of two new treatment technologies as compared to treatments known to be effective against *Pythium*.

To simulate infected conditions, nutrient solution (one-half strength Hoaglands – see representative analysis in Table 4.1) was inoculated with *Pythium aphanadermatum (Pa)* organisms to a dilution of 10 zoospores ml\(^{-1}\) before treatment methods were applied. Control solutions with counts of 0, 1 and 10 *Pa* zoospores ml\(^{-1}\) were used to provide a comparison to treated solutions collected from the four methods. All conditions were replicated with two bioassays per sample. The four treatment methods evaluated on a bench scale were: Electrochemical Disease Control, Pasteurization, Sonication and Ultraviolet Irradiation.

Table 4.1. Element analysis of typical nutrient solution

<table>
<thead>
<tr>
<th>Element</th>
<th>N</th>
<th>K</th>
<th>Ca</th>
<th>P</th>
<th>S</th>
<th>Mg</th>
<th>Fe</th>
<th>B</th>
<th>Zn</th>
<th>Mn</th>
<th>Mo</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/l</td>
<td>133</td>
<td>215</td>
<td>90</td>
<td>31</td>
<td>18</td>
<td>12</td>
<td>0.12</td>
<td>0.16</td>
<td>0.13</td>
<td>0.14</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

A dilution series was tested to determine the amount of damage associated with each value in the range of *Pa* zoospore control solution concentrations described above. Application of the bioassay procedure is described in Chapter 3.

The graph in Figure 4.1 illustrates the average damage visible on the roots of each plant in the bioassay. In the cases of dilutions of 0 and 1 zoospores ml\(^{-1}\), most damage is fairly minor and constrained to exterior lesions and streaking. In the 10 zoospores ml\(^{-1}\) case, considerably more damage is evidenced by the relatively large amount of dark interior streaking. Presumably, the infection moved into the roots and...
advanced much further than in the other two dilutions. Low level damage in the 0 zoospores ml\(^{-1}\) treatment was unexpected and was most likely not due to \textit{Pa}. In a side experiment to search for the cause of unanticipated infections, damage was present even after surface sterilization of the seeds with chlorine bleach. Because care was taken to minimize the chances of biological contamination, other factors such as mechanical damage or a natural reaction of the root in going from a humid environment of the germination chamber to being submerged in nutrient solution may have contributed to root discolorations, which were then interpreted as lesions and streaking. Root kinking was observed at 10 zoospores ml\(^{-1}\) and to a lesser extent at 1 zoospores ml\(^{-1}\) and seemed to correspond with damage due to lesions. Roots in the uninfected dilution were straight without the abrupt directional changes visible in the more heavily damaged (kinked) roots. A measure of treatment method effectiveness is obtained by comparing damage post-treatment with that measured in the dilution series conducted in parallel.

To facilitate comparing damage present on roots following treatment, an overall damage score was calculated by weighting the four categories appropriately. As lesions are the most minor damage, each lesion contributed a value of 1 (one) to the overall damage score. Exterior streaking was considered to be relatively minor damage and every cm of streaking contributed 2 (two) to the damage score. Light interior streaking was an indication of more serious damage and so each cm was considered to contribute a value of 4 (four). Dark interior streaking was a sign of major root damage and was given a value of 8 (eight) per cm. Summing up the damage on each root from each category provided an overall quantification of damage for the root. Damage present in the uninfected control condition was subsequently treated as due to other factors and the average damage of the control was subtracted from the other conditions so that any remaining damage would be attributable solely to \textit{Pa}. Coefficients used to apply levels of damage to the roots are somewhat arbitrary, however, so adjusting them moderately does not substantially affect the relative overall damage.

Because the primary interest was to reduce damage from \textit{Pa}, the percentage of damage reduction on each root was calculated by subtracting the damage present on the treatment roots from the average damage of the control solution infected at 10 zoospores ml\(^{-1}\) and dividing by the average damage of the control solution infected at 10 zoospores ml\(^{-1}\). Besides giving a percentage reduction in damage, this procedure allowed us to compare treatment systems that were completed at different times, where it would be expected that the initial solution concentration might be slightly different from 10 zoospores ml\(^{-1}\) and/or other factors might influence the amount of damage caused on a particular day.

4.2
Bench Top Treatment 1. Electrochemical Disease Control

One liter of solution with a concentration of 10 zoospores ml\(^{-1}\) was placed in each of the anode and cathode compartments and 100 VDC was applied across the electrodes. During treatment, the solution in each compartment was constantly stirred with glass rods. Treatment times were 2, 5, 15, and 30 minutes. Final pH values in the cathode compartment corresponding to these times were: 4, 3.5, 3, 2.3 and 2. Following the treatment, 400 ml of solution was removed from the cathode chamber and brought to a pH of 5.8 with 1M KOH (potassium hydroxide).

Minute gas bubbles evolved from each electrode (hydrogen gas at the cathode and oxygen at the anode) when voltage was applied across the electrodes. Solution in the anode compartment began to turn cloudy after a few seconds, as the pH rose, and mineral salts began to precipitate out. Precipitate was not formed in the cathode compartment. During operation of the electrochemical unit an average 700 mA of current was drawn by the electrodes and with a supply voltage of 100 VDC resulted in a power use of 70 W. In the 15 minute condition the solution temperature increased to approximately 35C. Figure 4.2 contains a graph that presents a comparison of the percent damage reduction at each electrochemical duration.

Electrochemically reducing pH did not appear to have a large impact on *Pa* zoospores at the durations we examined. This technique might be more successful if durations were greatly increased to the order of several hours. To save power, the current could be turned off when a target pH is achieved and the solution
allowed to remain at that condition for the appropriate duration. However, long treatment durations would correspond to a need for large retention reservoirs. These tests indicated, very strongly, that the electrochemical treatment process had little promise as a Pa zoospore elimination method.

![Graph showing damage reduction](image)

**Figure 4.2. Fraction of damage reduction due to electrochemical disease treatment**

**Bench Top Treatment 2, Pasteurization**

In the pasteurization treatment method, one liter of nutrient solution was heated to 60 C and one to 95 C. Concentrated Pa solution was then added to the heated nutrient solutions to bring the concentrations of zoospores to 10 zoospores ml⁻¹. This was important because Tu and Zhang (2000) demonstrated that Pa can be affected at temperatures above 45 C and the time required to heat to 60 C and 95C with our apparatus was greater than the exposure times we planned to examine. Times of 30, 60, and 120 seconds were used for the 60 C solution, and 15, 30, and 60 seconds were used for the 95 C solution. After adding Pa concentrate and the allotted treatment time had passed, a 400 ml sample was cooled using a 2 l Erlenmeyer flask held under cold tap water. This caused an initially rapid temperature drop, with the solutions reaching ambient temperature within 2 to 3 minutes.

Figure 4.3 presents a comparison of the two heat treatment temperatures and the various treatment durations at these temperatures. Each treatment temperature appears capable of achieving a relatively good reduction in the level of damage. The data demonstrate Pa can survive a short spike in temperature much better than a more sustained increase. Although requiring a longer dwell time and subsequently a larger volume in the treatment system, temperatures of 60 C are easier to attain and work with than of 95 C.
**Bench Top Treatment 3, Sonication**

An important factor in sonication is the contact time of the solution with the vibrating probe in the flow cell. To vary contact time, flow rates of 200, 400, and 600 ml min\(^{-1}\) were used. At each flow rate, amplitude settings of seven and nine, corresponding to amplitudes of 120 and 150 µm, were used. The Misonix XL2020 used to generate the signal that causes the high frequency vibrations in the converter can also display instantaneous power use as a percent of maximum. Larger amplitude corresponds to higher power consumption. At amplitude 9, power consumption was approximately 70% of maximum (~500W), or 350 W. At amplitude 7, power consumption was approximately 50%, or 250 W. The graph in Figure 4.4 illustrates that continuous sonication is capable of successfully eliminating *Pa* zoospores. The best results were achieved with an amplitude of 120 µm and the lowest flow rate tested, 200 ml min\(^{-1}\), although satisfactory results were also achieved with an amplitude of 150 µm. However, to deliver an effective dose of sonic energy, our results indicate that flows greater than 200 ml min\(^{-1}\) are not recommended for this size of generator.

Passage of *Pa*-containing nutrient solution through the pump and flow cell with the sonicator turned off had little effect on damage caused by the *Pa*, indicating that any reduction in *Pythium* activity was due to sonication and not pressurization or flow turbulence.

The stated maximum recommended flow rate for the flow cell is 660 ml/min, and it was developed for use with the XL2020 and other generators of a similar size. Larger generators capable of handling much greater flows exist, so scaling up the flow rate is possible.
Bench Top Treatment 4, Ultraviolet (UV) Sterilization

Ultraviolet doses of 120 and 240 mJ cm\(^2\) were examined. To ensure uniform solution dosing, approximately 3 liters (3 times the volume of the reaction chamber) of nutrient solution were passed through the UV chamber before sampling. The reactor outlet was disassembled between runs and cleaned to prevent cross contamination. Figure 4.5 contains a graph that shows damage reduction due to UV-C. Ultraviolet sterilization worked quite well, especially considering the energy input was only 15 W.
Bioassay and Bench Top Treatments, Conclusions

Although the uninfected control bioassays sometimes displayed low levels of damage symptoms, the concept of using spinach seedlings to estimate Pa levels appears valid. A single bioassay is not able to provide a precise count of how many Pa propagules are present in a solution but, when used in conjunction with a dilution series, a reasonable estimate can be obtained. The spinach seedling bioassay is well suited to comparing the efficacies of different treatment systems and whether they are worth further development. The seedling bioassay has the advantage of being simple, which allows testing of many conditions quickly, without expensive or complicated laboratory procedures, or expertise requirements.

Because the ultimate goal of this research project was to develop a viable commercial hydroponic spinach production system, testing on spinach gives a better indication of the actual damage causing ability of the Pa organism. We performed tests on roots during the initial period when roots are most susceptible to damage. In the production systems, the crop is floated after 48 hours (germination time) and presumably this is when new roots would come into contact with Pythium in the nutrient solution. The bioassays are infected at age 48 hours to correspond to this stage.

The presence of damage on roots even after the solution has undergone treatment does not mean the crop is doomed to failure. In the short crop cycle of spinach, Pythium may not have enough time to produce zoospores to significantly damaging levels.

Of the technologies tested, sonication demonstrated an ability to eliminate Pa. Sonication in a continuous flow mode is effective provided the flow rate does not exceed the capacity of the generator. Unfortunately the electrochemical treatment was found to be largely ineffective at the durations tested. Although the system is very effective at manipulating pH in a nutrient solution and can achieve extreme levels, these levels don’t appear to be sufficiently extreme to eliminate Pa zoospores to a point equivalent to what is achievable using other treatment methods.

COMPARISON OF AEROPONICS AND SHALLOW-FLOW SYSTEMS

Once the plastic low pressure nozzles were selected as a reliable means of generating a nutrient mist, a comparison of biomass yield between aeroponics and the more traditional floating hydroponics was conducted. In this comparison the aeronomic bench and shallow flow bench were placed side by side and flats of spinach seedlings placed in them. All conditions were equivalent with the only difference being the method with which nutrient solution was applied to the crop. Intact spinach seed (cv. “Alright”) was seeded into Redi-Earth growing medium, corrected to a moisture content of 3.1 to 1, in 288 cell Styrofoam plug trays (Beaver Plastic). Once seeded and covered, the trays were placed in darkness in a growth chamber set.
at 25 C, to germinate for 48 hours. Following 24 hours in the growth chamber, one bank of fluorescent lights were turned on to provide a small amount of light to the emerging seedlings to prevent stretching.

After transfer to the greenhouse, target moles were set for 17 per day, and the day/night temperature was set at 23 C. EC and pH were monitored and adjusted to 5.6, and 1400 mS/cm, respectively.

Flats were harvested from the two systems at 14 days of age and the results are shown below in Table 4.2.

In the aeroponic system, areas that showed signs of excessive drying were not included in the harvest statistics. Only position-equivalent rows in the shallow flow system were harvested to more closely match the aeroponic system.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Aeroponic System</th>
<th>Deep-trough System</th>
</tr>
</thead>
<tbody>
<tr>
<td>average, g</td>
<td>3.6</td>
<td>4.95</td>
</tr>
<tr>
<td>minimum, g</td>
<td>0.1</td>
<td>0.30</td>
</tr>
<tr>
<td>maximum, g</td>
<td>7.3</td>
<td>7.90</td>
</tr>
<tr>
<td>number</td>
<td>105</td>
<td>99</td>
</tr>
<tr>
<td>standard deviation, g</td>
<td>1.22</td>
<td>1.61</td>
</tr>
</tbody>
</table>

Although it is possible to produce baby-leaf spinach in an aeroponic system, it was clear that the biomass yield of spinach grown aeroponically was much less compared to the deep flow system. Approximately three days after emergence it became apparent that the aeroponically grown spinach was lagging the growth rate of the shallow flow. At final harvest the average plant weights of the aeroponic spinach were 27% less. We hypothesized that this in biomass decrease was due to drying of the root mass within the cells of the flats. In floating hydroponics, the bottom of the flat is submerged and considerable wicking of nutrient solution up into the Redi-Earth keeps the root mass suitably moist. However in the aeroponics system this wicking either did not occur, or did not occur to the same extent as in the floating system. In figure 4.6 this difference in wicking is clearly visible in the different coloring of the media in different cells of the flats.
Another drawback of the aeroponics system was that patches of dried media developed where the nutrient solution was not sprayed directly onto the bottoms of the flats. Although roots protruding from the bottom of the flat were completely covered by the nutrient spray, and appeared suitably moist upon visual inspection, in Figure 4.7 it is possible to see patches where the media has obviously dried out, resulting in greatly decreased growth.

Considerable effort has gone into the development of a germination protocol to produce seedlings of a consistent size with uniform emergence. Central to this protocol is to use Redi-Earth medium, which allows a precise amount of moisture and air contact with the seeds. However, this medium in the individualized cell trays that we used is apparently incompatible with aeroponic production of baby leaf spinach, for uniform emergence and consistent size are arguably the two most important factors in baby-leaf production.


TESTING OF INOCULATION AND CONTINUOUS PRODUCTION

For fear of *Pythium*, most previous work in developing commercial production protocols for baby leaf spinach was conducted in batch mode where a single crop was grown in the system and then the nutrient solution discarded and the system thoroughly cleaned. Some work was done in which multiple harvests were taken from the same flats, but in this system too, the nutrient solution was not preserved.

To see effects of *Pythium* on a multiple crop system, where spinach seedlings of all ages from seedling to near final harvest share a common volume of nutrient solution, long shallow ponds were constructed. In these ponds young seedlings were placed in one end of the system and then moved along to the other end, until they were old enough to harvest.

Following our established protocols for seedling production, de-hulled spinach seed (cv. “Alright”) was seeded into Redi-Earth growing medium, in 132 cell Styrofoam plug trays (Beaver Plastic, modified 288 cell tray). Every three days an additional flat was added to the system, until there were a total of five flats present in each system, the youngest being newly emerged seedlings and the oldest ready to harvest baby spinach. At this time, a concentrated solution of *Pa* zoospores (20,000 per ml) was slowly added to two of
the benches, resulting in a final $Pa$ zoospore concentration of 100 zoospores per ml in the system. Four ponds were used, with two ponds inoculated and two ponds not inoculated as controls. In this experiment, nutrient solution temperature was uncontrolled (although monitored regularly as a part of the daily routine.) The temperature closely followed that of the ambient air temperature and averaged approximately 23C.

As was expected, the inoculated benches showed rapid effect of $Pa$ damage. Older material suffered considerably less damage than younger material. Zoospores had less time to work on the older material before it was harvested and the roots they were attacking were already well established. The youngest material that had just been placed in the system suffered the most damage, resulting in dwarfed and wilted plants. It is interesting that the plants did continue to grow slightly following inoculation, but this was most likely due to there being considerable root material within the root plug that was not directly exposed to the nutrient solution and zoospores. Although the bulk of the root material was exposed and heavily damaged, some of the material persisted, allowing a minimum of growth.

The experiment continued with new flats being placed in the system as older flats were harvested. The older flats continued to show damage from $Pa$ but, as the experiment progressed, this damage became less and less severe. The roots showed classic signs of $Pa$ damage, such as extensive browning, but shoot biomass returned to near normal levels. This led us to the conclusion that in this type of system, $Pa$ could not reproduce fast enough before the source of infection (the old roots) were removed. The old highly infected roots did not get the chance to release their zoospores, which would have resulted in the devastation of the younger crop. However the infection did persist, although at a lower level, suggesting that there was some reproduction of $Pa$. Katzman (2003) showed that reduced temperatures slowed, but did not stop, the emergence of $Pythium$ zoospores. In Katzman’s system, adult spinach was the concern, which was generally harvested at a much older age, spending considerably more time in the pond.

Because of the near success in controlling $Pythium$, by reducing the crop duration, it was hypothesized that reducing solution temperature, in addition to a reduced crop duration, might suffice to overcome a crop $Pa$ infection. It is this reason that nutrient solution temperature reduction was added as one of the methods examined in our further experiments, described in the chapters that follow.
CHAPTER 5
TASK 5: PURE-CULTURE CHALLENGE TESTING

INTRODUCTION
In the preliminary testing of aeroponic and deep-flow production systems conducted under Task 4 and described in Chapter 4, crop performance in the aeroponics system was never as good as in the deep-flow system despite best efforts. Aeroponic production of cut greens such as baby spinach differs from typical aeroponic endeavors in that very high plant densities (c. 1500 plants m$^{-2}$) are required. The difficulty we encountered was in keeping the medium wet in all of the many cells needed for high density planting. Wherever the medium in the cells dried out, it had the effect of slowing plant growth in the affected cells, whether the rest of the root system of the same plants was thoroughly wetted, or not. Although we strove to overcome this difficulty by trying a number of different spray systems and planting methods, we were unsuccessful. Faced with these results, we abandoned aeroponics and concentrated our resources on testing in deep flow systems.

Tests to quantify disinfection efficiencies using the pure strain of *Pythium aphanidermatum* (*Pa*), Pa58, were completed in a laboratory setting for all treatment methods of interest, as described in Chapter 4. During this work we encountered an unexpected result: the electro-chemical treatment method was much less effective than anticipated and needed to be replaced as a treatment method. Several good replacement candidates were available so this, in itself, was not a major problem. For example, we had noticed that reducing nutrient solution temperature was effective in suppressing disease. This discovery offered a new and promising treatment method, and was chosen as the replacement. The three root disease treatment methods chosen were: UV Irradiation, Filtration, and Temperature Reduction.

Eight independent deep-trough hydroponic spinach production systems with temperature and flow control of the nutrient solution were fabricated and used in the pure culture challenge experiments. The production system design is detailed in Chapter 2. Data developed through laboratory testing, detailed in Chapter 4, were used to set the levels to achieve the desired production systems efficacy.

The first experiment, Pure-culture Challenge Experiment 1, was conducted during mid-winter for 21 harvests (75 days overall). This experiment was replicated during mid-summer in Pure-culture Challenge Experiment 2, also a 21-harvest experiment (75 days). In this repeat in time, all resources were devoted to testing the most successful method of the first experiment, temperature reduction, which was done with three replications. In both experiments, the production systems were operated for one week with no plants while checking temperatures and nutrient solution flows. Two preliminary harvests were then taken in all eight production systems of healthy crops grown at the same temperature, to check the performance of the
Entering into the pure-culture challenge experiments, it was the intention to explore the effects of different intensities and flow rates for the different treatment methods in a series of tests, as detailed in the Project Tasks. The first experiment revealed that this series of tests would serve no purpose in continuous production mode, because sufficient reproduction of the disease organism was able to take place entirely within the root system, and thus was not accessible to physical treatment methods located outside the root system, in the circulation system. This was not known prior to testing the treatment methods in continuous production; earlier work had shown that, if zoospores were reduced to very low levels in batch crops, productivity was unaffected, meaning that a low level of disease is tolerable. It followed that reducing the numbers of free-swimming zoospores to zero or near zero in the circulating system might be effective in reducing the level of the disease process to the point where productivity was unaffected. Once it was learned that filtration, which removed all circulating zoospores, was ineffective, it became obvious that, in principle, none of the physical treatment methods could work in our production system, regardless of treatment intensity. If we were to pursue filtration or UV irradiation treatment methods, we would need to concentrate effort not on changing intensities and flow rates, but on isolating succeeding cohorts of crops from each other so that upstream infection would not occur. While this is possible in theory, we did not envisage how it could be achieved economically in large ponds under commercial production conditions.

On the other hand, the temperature reduction method, which works on a different principle, proved highly effective. It is essentially a biological control method that depends on manipulation of the length of the reproductive cycle of the disease organism, and the length of the crop cycle. We devoted our remaining time and resources to exploring this method of disease control in Pure-culture Challenge Experiment 2 and in the subsequent 4 wild-culture challenge experiments, described in Chapter 6.

**PURE CULTURE CHALLENGE EXPERIMENT 1**

What follows is a description of an experiment testing UV irradiation, filtration, and temperature reduction of recirculating nutrient solution as possible methods for recovery from root disease in continuous, deep-flow hydroponic production of spinach.

**Introduction**

This experiment was the first of two in which recovery from an established self-sustaining disease process in roots of spinach was affected by reducing the root zone temperature from 27.5 C to 20.0 C, and limiting crop duration in the hydroponic system to 13.75 days. The disease process was induced by inoculating...
continuous-production pond systems with the known strain of *Pa*, Pa58. Nutrient solution temperature was then lowered either shortly after inoculation or after the disease process had proven self-sustaining over many harvests, to see if so doing would eliminate the disease process. This experiment was conducted in mid-winter in contrast to the repeat of this experiment, which was conducted in mid-summer.

Two other treatment methods were also tested to determine whether their use would promote recovery from induced disease. The first was continuous UV irradiation of the nutrient solution. The second was continuous filtration. Each hydroponic production system was set up so that nutrient solution circulated from a reservoir to a pond and back, with a rapid directional flow. UV and filtration units were inserted in the circulation paths upstream of the ponds, and plants in the ponds were organized with the youngest upstream and oldest downstream. If the UV and filtration units eliminated all (or nearly all) zoospores passing through them, as had been shown to be the case in prior work, and the rapid directional flow kept zoospores from migrating upstream, in theory, the disease process should die out.

The temperature reduction treatment method relied on a different principle from the UV and filtration methods. It was based on the empirical finding that *Pa*, when reproducing *in vivo* in the roots of spinach immersed in nutrient solution, requires a significant amount of time (many days) to complete its reproductive cycle from free-swimming zoospore, through mycelium, to free swimming zoospore, the duration depending on temperature (Katzman, 2003). Our supposition was that if diseased roots were removed from the production system before the reproductive cycle was completed, the disease process would die out.

Katzman (2003) suggests estimates of *Pa* reproductive cycles in vivo at 24 C and 31 C. It appears from this and other work that the cycle length is inversely related to temperature (in the temperature range feasible for crop production). In the two experiments described in this section, nutrient solution temperature and crop duration were selected with a view to economic viability for year-round commercial greenhouse production of spinach. Crop cycle duration and solution temperature were not systematically varied, which would be desirable in future research. Rather, the concept feasibility was tested. This work differs from previous efforts in that it relies on coordinated manipulation of the *Pythium* reproductive cycle and the crop production cycle as a means to eliminate, not simply suppress or prevent, an established disease process using a method applicable to continuous long-term production of spinach.

**Methods**

In this experiment, eight continuous-production systems (“ponds”) were brought into steady operation, then four of the systems were heavily inoculated with *Pa* zoospores. Three of the inoculated production systems were used to test different methods by which to create recovery from the disease process, and one was used
as a comparison with no remedial treatment. An additional four production systems were not inoculated and served as various control conditions. Subsequently, additional tests were initiated for the most promising technique, that of temperature reduction. The experiment had four phases.

The experiment started with seeding eight flats of spinach on 12-14-2005 to provide a first set of crops in a continuous succession of crops (to eight independent production systems). The first harvest and data collection was 12-30-2005. From the start of seed imbition to harvest, the crop cycle was 16 days. For each crop, the first 2.25 days were spent in the dark in a growth chamber during germination, after which flats were floated on the production ponds in the greenhouse under light, where they spent 13.75 days. Crops were floated every three days and harvested 13.75 days later. When in full operation, each pond contained five crop cohorts of differing ages, floated 3 days apart. Following each harvest there was a period of slightly more than a day when only four crops were in the system, pending flotation of the next cohort. Natural light was supplemented to provide 17 mol m\(^{-2}\) d\(^{-1}\) of daily integrated PPFD, extending the photosynthetic period as necessary to reach this target. Aerial temperature followed a regime of 23 C in the day and 19 C at night, with a 20-hour thermoperiod. The production system ponds were designed to be long, narrow, and relatively shallow, to enforce fast directional flow. Nutrient solution was withdrawn at one end of the ponds, circulated through a reservoir, and returned at the other end. Younger plants were always upstream of older plants. Nutrient solution recirculation was matched across production systems at c. 7 liters per minute, so solution flowed at 1 cm s\(^{-1}\) in the space below the floating flats. The clearance between the pond bottom and float bottom was 5 cm. Nutrient solution temperature depended on the experimental condition and phase of the experiment.

The first phase of the experiment was to bring the eight independent production systems into full operation in continuous production mode, and collect data from the first two crop harvests to provide baseline data. UV and filtration devices were not activated at this time. Throughout the first phase of the experiment, the nutrient solution was controlled to 27.5 C in all eight production systems.

The second phase commenced immediately after the second harvest with inoculation of half of the production systems (ponds 1, 2, 4, and 5) with sufficient \(Pa\) zoospores to achieve a concentration of 100 zoospores per ml, or approximately 7 million zoospores per production system. To encourage the disease to take hold after inoculation, all pond systems were continued at 27.5 C for 24-hrs without treatment – UV units were not activated and filtration devices were not inserted into the circulation. The newest crops were added to production systems on schedule, 24 hours after inoculation.

The third phase commenced 24 hours after inoculation with application of three types of remedial treatments to three of the inoculated ponds – filtration in pond 1, UV irradiation in pond 2, and temperature
reduction in pond 5. To accomplish filtration, two progressively finer filters were placed in series upstream of the pond, one a 5 micron rated pre-filter, and the second a 1 micron-rated final filter. (Zoospores are usually more than 10 microns in diameter.) To provide UV irradiation, a 15-watt UV system was placed in the recirculation stream upstream of the pond.

Each of three inoculated ponds was matched to a similar un-inoculated pond (ponds 8, 7, and 3), which received the same treatment, to determine the effect of the treatment on its own. The matching pond was symmetrically placed to the treatment pond with respect to the supplemental light array. In winter this was important because the array was in use many hours every day and light mapping had shown the light intensity under the array declined a small amount, systematically, at the edges of the array. The production systems were aligned parallel lengthwise, north to south, under the light array. They were numbered 1 to 8 from east to west. Pond 7 received UV to match pond 2, pond 8 received filtration treatment to match pond 1, and in pond 3 the temperature was reduced to 20.0 C to match that in pond 5, thus achieving the desired symmetry.

In the remaining two ponds, 4 and 6, the temperature was continued at 27.5C. Pond 4, which had been inoculated, served as a control for the temperature reduction method to show what would have happened to the disease process had the ambient root environment continued at the higher temperature. The un-inoculated pond 6 served to show the temperature effect on crop performance alone, without inoculation – essentially, pond 6 was a continuation of the conditions all the ponds had experienced through the first two harvests. Pond 6 was somewhat comparable to ponds 7 and 8, which were also continued at 27.5 C but with the treatment methods applied (and also without inoculation).

Phase four of this experiment was an additional treatment phase, providing two additional replications of the temperature reduction method. It began after the fourteenth harvest from the start of the experiment (02-08-2006), at which time the three initial treatments had produced twelve crops (over 36 days) in which to show their efficacies. By this time, ponds 1, 2 and 4 had developed long-term, self-sustaining disease processes, becoming candidates for the temperature reduction treatment. In pond 4, a self-sustaining disease process had developed as expected (confirming Pa growth is favored at 27.5 C). Filtration and UV were tested in ponds 1 and 2, but with only a small degree of success, thus providing two additional ponds with a self-sustaining disease process. However, the UV pond suffered from iron deficiency as well as disease and so was rejected for testing of temperature reduction to avoid this complicating factor. (UV irradiation is known for its ability to destroy iron chelators and precipitate iron in nutrient solutions.)

In diseased ponds 4 and 1, the temperature was reduced from 27.5 C to 20.0 C as an additional test of the temperature reduction treatment method. Ponds 3 and 5 were continued as un-inoculated comparison
conditions for pond 4, and pond 8 was continued as an un-inoculated comparison condition for pond 1 (with temperature reduced). Data collection was discontinued in ponds 2 and 7, the UV ponds. Seven harvests later, after 21 days, the experiment was discontinued. However, to obtain a characteristic growth curve for the crop, plants stands of all ages were simultaneously harvested from a typical healthy production system in addition to the usual full-term harvest.

**Results**

**Note on Data Collection and Presentation.** In this experiment the sample unit was the individual plant. For each crop stand, a 132-cell flat was seeded (11 rows of 12 cells). After seedling emergence, flats were thinned to 72 plants per crop stand, or 6 to 7 plants per row, following a set protocol to achieve a uniform stand. During harvest, plants from each row were cut through the hypocotyl, disentangled from remaining plants, and weighed immediately. Plant locations within the stand were recorded. For fresh weight (FW) data, all plants in the stand were sampled. Thus, the sample size was c. 72. Plants from four interior rows (rows 4 through 8 inclusive) were oven dried to provide dry weight (DW) values to determine plant water status and growth analysis (sample size c. 25). The number of plants in each row was counted and number and location of noticeably small or wilted plants noted, if any.

Shoots and roots were photographed during each harvest. Roots were photographed hanging down for an indication of length, color and condition, and also close-up in plan view. Additionally, roots were carefully inspected visually for signs of disease and notes taken on quantity and distribution of affected roots. After harvest 2, all roots exterior to the polystyrene floats were removed by cutting, oven dried and weighed.

Data collected from the first two harvests prior to inoculation are presented in full in Table 5.1 along with derivative calculations. Similar data were collected in all harvests of the experiment, with the addition of root data. Row statistics are calculated and included in Table 5.1 for direct comparisons to pure-culture Challenge Experiment 2, in which the sample unit was the row rather than the plant. Total crop mass harvested, yield, and average plant weight are given in Table 5.1. However, for most purposes, it is more helpful to consider crop performance in terms of productivity – plant biomass per unit area and unit time (g m\(^{-2}\) d\(^{-1}\)), or light use efficiency (g mol\(^{-1}\)) – plant biomass per unit of photosynthetically active radiation. Other indices such as dry weight to fresh weight (DW:FW) and Shoot:Root ratios have also been computed. Both were found to be good indicators of root disease problems, sometimes more informative than shoot productivity and will also be used in presenting results.
Table 5.1. Operating and production data from first two harvests prior to disease inoculation.

<table>
<thead>
<tr>
<th></th>
<th>Harvest 1, 30 December</th>
<th>Harvest 2, 2 January</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Production System No.</strong></td>
<td>1  2  3  4  5  6  7  8</td>
<td>1  2  3  4  5  6  7  8</td>
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</tr>
<tr>
<td><strong>Inoculation State</strong></td>
<td>not not not not not not not not</td>
<td>not not not not not not not not</td>
<td></td>
</tr>
<tr>
<td><strong>Pond Temperature</strong></td>
<td>27.5 27.5 27.5 27.5 27.5 27.5 27.5 27.5</td>
<td>27.5 27.5 27.5 27.5 27.5 27.5 27.5 27.5</td>
<td></td>
</tr>
<tr>
<td><strong>Whole Flat FW Harvest (g)</strong></td>
<td>400 453 374 445 415 399 376 378</td>
<td>27.5 27.5 27.5 27.5 27.5 27.5 27.5 27.5</td>
<td></td>
</tr>
<tr>
<td><strong>Whole Flat FW Yield (kg m⁻¹)</strong></td>
<td>3.75 4.24 3.50 4.17 3.89 3.74 3.52 3.54</td>
<td>27.5 27.5 27.5 27.5 27.5 27.5 27.5 27.5</td>
<td></td>
</tr>
<tr>
<td><strong>Number of Plants (n)</strong></td>
<td>80 77 75 76 75 71 76 77</td>
<td>80 77 75 76 75 71 76 77</td>
<td></td>
</tr>
<tr>
<td><strong>Plant Av. FW (g)</strong></td>
<td>5.00 5.88 4.98 5.85 5.53 5.61 4.95 4.90</td>
<td>5.00 5.88 4.98 5.85 5.53 5.61 4.95 4.90</td>
<td></td>
</tr>
<tr>
<td><strong>Plant Av. FW, SD (g)</strong></td>
<td>1.33 1.24 1.22 1.37 1.47 1.72 1.63 1.55</td>
<td>1.33 1.24 1.22 1.37 1.47 1.72 1.63 1.55</td>
<td></td>
</tr>
<tr>
<td><strong>Plant Av. FW, SE (g)</strong></td>
<td>0.15 0.14 0.14 0.16 0.17 0.20 0.19 0.18</td>
<td>0.15 0.14 0.14 0.16 0.17 0.20 0.19 0.18</td>
<td></td>
</tr>
<tr>
<td><strong>Av. No. plants/row</strong></td>
<td>7.27 7.00 6.82 6.91 6.82 6.45 6.91 7.00</td>
<td>7.27 7.00 6.82 6.91 6.82 6.45 6.91 7.00</td>
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<tr>
<td><strong>Plant Density (m⁻²)</strong></td>
<td>750 722 703 712 703 665 712 722</td>
<td>750 722 703 712 703 665 712 722</td>
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</tr>
<tr>
<td><strong>Row Av. FW (g)</strong></td>
<td>36.3 41.1 34.0 40.5 37.7 36.2 34.2 34.3</td>
<td>36.3 41.1 34.0 40.5 37.7 36.2 34.2 34.3</td>
<td></td>
</tr>
<tr>
<td><strong>Row Av. FW SE</strong></td>
<td>1.21 3.10 1.90 2.56 3.20 2.42 2.13 2.17</td>
<td>1.21 3.10 1.90 2.56 3.20 2.42 2.13 2.17</td>
<td></td>
</tr>
<tr>
<td><strong>Flat Area (m²)</strong></td>
<td>0.1067 0.1067 0.1067 0.1067 0.1067 0.1067 0.1067 0.1067</td>
<td>0.1067 0.1067 0.1067 0.1067 0.1067 0.1067 0.1067 0.1067</td>
<td></td>
</tr>
<tr>
<td><strong>FW Productivity, Whole Flat. (g m⁻² d⁻¹)</strong></td>
<td>269 304 255 306 286 274 252 257</td>
<td>269 304 255 306 286 274 252 257</td>
<td></td>
</tr>
<tr>
<td><strong>FW Irradiation Use Efficiency (g mol⁻¹)</strong></td>
<td>15.9 18.0 15.1 18.2 17.0 16.3 14.9 15.2</td>
<td>15.9 18.0 15.1 18.2 17.0 16.3 14.9 15.2</td>
<td></td>
</tr>
<tr>
<td><strong>FW Irradiation Use Efficiency SE</strong></td>
<td>0.47 0.43 0.43 0.49 0.52 0.59 0.56 0.55</td>
<td>0.47 0.43 0.43 0.49 0.52 0.59 0.56 0.55</td>
<td></td>
</tr>
<tr>
<td><strong>Sub Sample FW Prod. (g m⁻² d⁻¹)</strong></td>
<td>268 291 230 280 287 264 225 239</td>
<td>268 291 230 280 287 264 225 239</td>
<td></td>
</tr>
<tr>
<td><strong>Sub Sample FW Prod. SE</strong></td>
<td>14.1 15.1 13.1 13.1 15.1 20.0 17.7 19.3</td>
<td>14.1 15.1 13.1 13.1 15.1 20.0 17.7 19.3</td>
<td></td>
</tr>
<tr>
<td><strong>Ratio. Whole Flat Prod.:Sample Prod</strong></td>
<td>1.01 1.05 1.11 1.10 1.00 1.04 1.12 1.07</td>
<td>1.01 1.05 1.11 1.10 1.00 1.04 1.12 1.07</td>
<td></td>
</tr>
<tr>
<td><strong>Root observations</strong></td>
<td>good good good good good good good good</td>
<td>good good good good good good good good</td>
<td></td>
</tr>
<tr>
<td><strong>Shoot observations</strong></td>
<td>good good good good good good good good</td>
<td>good good good good good good good good</td>
<td></td>
</tr>
<tr>
<td><strong>% wilted</strong></td>
<td>0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0</td>
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</tbody>
</table>
Table 5.1. Operating and production data from first two harvests prior to disease inoculation.

|                         | Whole Flat FW Harvest (g) | Whole Flat FW Yield (kg m⁻²) | Number of Plants | Plant Av. FW (g) | Plant Av. FW, SD (g) | Plant Av. FW, SE (g) | Av. No. plnts/row | Plant Density (m⁻²) | Row Av. FW (g) | Row Av. FW, SE | Flat Area (m²) | Av. Irradiation Integral (mol m⁻² d⁻¹) | FW Productivity, Whole Flat. (g m⁻² d⁻¹) | FW Productivity, Whole Flat. SE | FW Irradiation Use Efficiency (g mol⁻¹) | FW Irradiation Use Efficiency SE | Sub Sample FW Prod. (g m⁻² d⁻¹) | Sub Sample FW Prod. SE | Sub Sample DW Prod. (g m⁻² d⁻¹) | Sub Sample DW Prod. SE | Correlation. DW:FW | Correlation. DW:FW SE | DW/FW Av. | DW/FW SE | Whole Flat DW Prod. Est.(g m⁻² d⁻¹) | Whole Flat DW Prod. SE | DW Light Use Efficiency (g mol⁻¹) | DW Light Use Efficiency SE | Root observations | Shoot observations | % wilted |
|-------------------------|----------------------------|------------------------------|-------------------|------------------|-------------------|---------------------|-------------------|------------------|----------------|----------------|------------|----------------|--------------------------------------|--------------------------------------|-------------------------------|----------------------------------|-------------------------------|----------------------------------|----------------|----------------|--------------------------------------|-------------------------------|-------------------------------|-------------------------------|------------------------|------------------------|---------|
| Whole Flat FW Harvest (g) | 353 409 344 377 389 406 369 352 | 3.30 3.84 3.22 3.53 3.65 3.81 3.46 3.30 | 71 74 71 72 72 72 71 72 | 665 694 665 675 675 675 665 675 | 6.45 6.73 6.45 6.55 6.55 6.55 6.45 6.55 | 32.05 37.21 31.25 34.28 35.41 36.93 33.55 31.97 | 2.87 3.40 2.12 2.64 1.23 1.74 1.36 0.79 | 0.1067 | 16.83 16.81 16.85 16.88 16.88 16.85 16.84 16.83 | 239 277 237 259 266 277 247 236 | 7.11 8.58 7.40 8.15 9.14 8.72 6.70 7.10 | 4.06 4.14 4.08 4.10 4.12 4.14 4.06 4.10 | 0.046 0.051 0.044 0.048 0.054 0.052 0.040 0.042 | 220 331 243 244 237 257 226 231 | 15.1 18.4 11.4 15.2 15.4 17.7 12.9 16.1 | 1.09 0.84 0.97 1.06 1.12 1.08 1.10 1.02 | 0.87 1.08 0.83 1.07 0.96 1.02 0.88 0.91 | 0.96 0.96 0.92 0.93 0.96 0.97 0.91 0.96 | 0.96 0.96 0.92 0.93 0.96 0.97 0.91 0.96 | 0.051 0.051 0.054 0.051 0.053 0.052 0.054 0.050 | 0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.001 | 12.3 14.0 12.8 13.2 14.1 14.5 13.3 11.9 | 0.94 0.91 0.81 1.14 1.08 1.10 0.96 0.93 | 0.73 0.83 0.76 0.78 0.84 0.86 0.79 0.70 | 0.056 0.054 0.048 0.067 0.064 0.065 0.057 0.055 | 0.055 0.056 0.048 0.067 0.064 0.065 0.057 0.055 | good good good good good good good good | 0 0 0 0 0 0 0 0 | 0 0 0 0 0 0 0 0 | 0 0 0 0 0 0 0 0 |
Phase 1 Results. Please refer to Table 5.1. In harvest 1, whole flat FW productivity averaged 276 g m⁻² d⁻¹, ranging from 252 to 306 g m⁻² d⁻¹, with a standard error of 7.7 and coefficient of variation 7.9%. In harvest 2, whole flat FW productivity averaged 255 g m⁻² d⁻¹, and ranged from 236 to 277 g m⁻² d⁻¹, with a standard error of 6.2, and coefficient of variation 6.8%. In our experience, spinach is prone to a high degree of plant-to-plant variation, so the limited amount of variation between production systems in these harvests may be considered normal and satisfactory. The productivity difference between harvests was likely in part due to a difference in plant density between these harvests (711 to 674 plants m⁻²). After the first harvest plant density was kept very uniform.

In previous experiments using this cultivar and light integral under optimal conditions of temperature and plant density, a productivity of c. 330 g m⁻² d⁻¹ in 13 days growing time was achievable, after edge effects were taken into account. Productivity values of the first two harvests, 250 to 275 g m⁻² d⁻¹, can be explained by a nutrient solution temperature effect (27.5 C). Beyond the first two harvests, the temperature of the nutrient solution in the un-inoculated “control” production system, pond 3, was lowered to 20.0 C, whereupon a productivity of c. 350 g m⁻² d⁻¹ (in 13.75 days) was achieved within 4 harvests and sustained over many crop harvests (see Figure 5.1). At the same time, productivity in another un-inoculated “control” production system, pond 6, in which the temperature was continued at 27.5C, was maintained at the same level as in the first two harvests (c. 270 g m⁻² d⁻¹ – See Figure 5.1), also sustained over many crop harvests.
Phase 2 Results. Zoospores were introduced into the reservoirs of designated production systems on the same day as the second harvest, approximately twelve hours after harvest, when cultured zoospore populations became sufficiently numerous. Proof of successful inoculation came from subsequent effects on crop performance.

Phase 3 Results. Phase 3 commenced with applying the three treatment methods in three pairs of ponds, one of each pair inoculated, one not. UV systems were activated in ponds 2 and 7, filtration cartridges inserted into the circulation systems in ponds 1 and 8, and nutrient solution temperature was lowered in ponds 3 and 5. The first harvest after treatment application occurred 1.5 days later (or 2.5 days after inoculation). The last pair of production systems, one inoculated and one not, went untreated and continued at solution temperature of 27.5 C. These ponds acted as comparison conditions for all three remedial treatment systems, and to assess the separate effects of the treatment methods.

Temperature Reduction Treatment Results. In Figure 5.1, the most important comparison that needs to be made is between the two systems in which temperature was reduced, one inoculated, the other not. In the first harvest after inoculation, productivity was in the 250 to 270 g m\(^{-2}\) d\(^{-1}\) range, as it had been in the harvests prior to inoculation, and there was no difference between the inoculated and un-inoculated
systems. Productivity differences between control and treatment conditions in harvests 2 and 3 were large and highly significant statistically. Productivity went in opposite directions in the two systems in these two harvests, reaching a maximum difference of 150 g m⁻² d⁻¹. Productivity recovered in the fourth and fifth harvests after temperature reduction. By the fifth harvest, productivity was identical in the two systems, at c. 350 g m⁻² d⁻¹. The fifth crop harvested had been floated one day after inoculation and thus exposed to few zoospores.

It took several additional harvests for all visible traces of disease to disappear from the roots, so there may have been a minor secondary wave of infection. Beyond harvest 11 after inoculation, no more traces of disease were seen in the roots and no behavioral evidence for disease remained.

In Figure 5.1 it can be seen that reducing nutrient solution temperature had a large positive effect on productivity by itself in un-inoculated systems (pond 3 vs. pond 6). Four harvests carried beyond all crops exposed for any part of their growth cycle to the higher temperature, after which the effect was consistent over many harvests and the difference was roughly 100 g m⁻² d⁻¹. The production systems in question were matched in position (ponds 3 and 6), so this was unlikely to be a position effect.

Where temperature was left unchanged after inoculation at 27.5 C, pond 4, productivity dropped rapidly from 250 to 75 g m⁻² d⁻¹, after which it stabilized at this level, suggesting equilibrium was established. However, it should be noted that for harvests 5 and 6 to have fared as poorly as they did, they must have been heavily infected and, since neither crop was in the pond at time of inoculation, the most likely source of infection would have been from second-generation zoospores – released from the crops present in the system for the original inoculation.

Crop health can be shown in ways other than fresh biomass, and some indicators appear to be more sensitive to root disease status than fresh weight or shoot productivity. Figures 5.2 through 5.8 show additional measures, the combined use of which better reveal disease status.
Light use efficiency, graphed in Figure 5.2, shows the control and treatment systems closer together after disease recovery than productivity did in Figure 5.1. Dry weight productivity plotted in Figure 5.3, based on a smaller sample than fresh weight productivity, was not on its own very revealing. However, the dry weight to fresh weight ratio (DW:FW), based on the same sample, and graphed in Figures 5.4 and 5.5, appears to be very sensitive to presence of root disease, showing large and highly significant effects in harvests 2 and 3, after which there were no significant differences. (Figure 5.5 is an enlargement of Figure 5.4). In the inoculated untreated production system in which disease became established (pond 4 – see Figure 5.4), DW:FW became almost twice that in a healthy crop. Given that most plants were wilted at harvest, this was not surprising.
Figure 5.3. Effect of temperature reduction on dry weight productivity of inoculated and uninoculated production systems. Error bars +/- 1 SE.

Figure 5.4. Effect of temperature manipulations on DW to FW ratio of shoots in inoculated and uninoculated production systems. Error bars +/- 1 SE.
Figure 5.5. Effect of temperature reduction on DW to FW ratio of shoots in inoculated and uninoculated production systems. Error bars +/- 1 SE.

Figure 5.6. Effect of temperature reduction on dry weight of exposed roots in inoculated and uninoculated production systems.
Figure 5.7. Effect of temperature reduction on shoot to root ratio in inoculated and uninoculated production systems. (Exposed part of root mass outside flat only)

Figure 5.8. Effect of temperature reduction on shoot to root ratio in inoculated and uninoculated production systems. (Exposed part of root mass outside flat only)
DW:FW ratio is also sensitive to solution temperature, so the two effects need to be separated. When the temperature reduction treatment was applied, the DW:FW ratio slowly declined from c. 0.06 to 0.05 in the un-inoculated control system (pond 3), and also in the inoculated system after recovery (pond 5). At lower pond temperature (20.0 C) dry matter was one percent less than at higher temperature (27.5 C), a 20 to 25% difference. (See Figure 5.4).

Figure 5.6 shows that, by the fourth harvests, root mass in the treatment system had caught up with that in the control system. Root mass appears to be another reliable independent status indicator of the disease process. This is not surprising because the disease organism attacks plant roots first. Of considerable interest here is how root mass at 20.0 C was twice root mass at 27.5 C in equally healthy crops.

Finally, Root to Shoot Ratio is shown in Figures 5.7 and 5.8. This indicator is similar in sensitivity to DW:FW ratio. By the same token that root dry mass is highly temperature dependent, so is the Shoot:Root ratio and, to assess disease presence from this ratio, one must take into account the temperature effect at the same time. (Compare un-inoculated systems at different temperatures in Figure 5.7.)

The most sensitive indicator of disease presence in roots, though only a descriptive variable, was visual inspection of the roots for grey discoloration patches. As far as yield and biomass were concerned, productivity recovered in as few as four harvests after application of temperature reduction, but traces of the disease lingered for several more harvests. In the last several harvests of the recovered system there were no grey patches at all, which is a pleasing result despite the fact, as far as yield and biomass were concerned, such lingering traces of disease had no detectable effect whatever in prior harvests.

**Filtration Method Results.** As can be seen in Figure 5.9, productivity in the inoculated system receiving the filtration treatment (Pond 1) fell rapidly to a level approximately 100 g m⁻² d⁻¹ below that of the control condition, in which Filtration was applied in a matched un-inoculated system (pond 8). There was a small recovery in productivity in harvests 4, 5 and 6, but it was not sustained. Productivity in the system treated by filtration (pond 1) ended up little better than if no treatment method(pond 4) had been applied (see lowest plot in Figures 5.9 and 5.1).

Mere application of filtration without inoculation appears to have depressed productivity (pond 8 versus pond 6 in Figure 5.9 – the top two lines). This very consistent effect was probably in part due to differences in light received because of position under the light array. However, it was greater than expected and remains a result without satisfactory explanation. After the experiment was complete, the staining pattern on the filters was carefully inspected. The filter cartridges appeared to be seated well and the filters had functioned properly, so we are confident the filters stopped circulation of zoospores outside the ponds. The
means of infection of follow-on crops was most likely not via the recirculation system but through zoospores (or infected root tissue) that remained within the pond itself. Some infection may have spread through direct contact between diseased and healthy roots. However, we suspect the most likely mechanism for disease dispersal, despite our efforts at achieving fast unidirectional flow, was back eddies carrying newly released second-generation zoospores upstream, or a vertical gradation in flow velocity due to root density differences such that the current was slow enough for second-generation zoospores to swim upstream in part of the flowing stream of nutrient solution. We believe the same considerations apply to the UV irradiation ponds.

UV Irradiation Method Results. As shown in Figure 5.10, UV Irradiation appeared to afford considerable protection from infection to crops in Harvests 4 and 5, or at least slowed the disease process compared to no treatment. Figures 5.11 and 5.12 give alternative measures of root disease status, DW:FW ratio and Shoot:Root ratio, showing the same effect of the UV treatment on harvests 4 and 5 in other ways. Figure 5.11 also shows that in the ninth harvest after inoculation and treatment, the UV control pond itself succumbed to disease, since the DW:FW ratio rises rapidly in this harvest.

After harvests 4 and 5, the UV treatment effectiveness decreased, for reasons unknown. Productivity in inoculated and un-inoculated UV-treated systems did not converge.
Figure 5.10. Effect of inoculation and UV irradiation on fresh weight productivity. Error bars +/- 1 SE.

Figure 5.11. Effect of inoculation and UV irradiation on DW:FW ratio. Error bars +/- 1 SE.
UV treatment had a somewhat different effect on the disease process than filtration, as shown in Figure 5.13, in which effects on productivity are directly compared. Changes from harvest to harvest were abrupt in the UV treated pond, whereas they were gradual and smooth in the filtration pond. The meanings of these differences are difficult to interpret from the limited data of this experiment. Perhaps differences in microbial ecology played a role. Perhaps flow was more nearly laminar in one system than the other. Perhaps the UV system functioned properly only intermittently.

Interpretation of the UV results is made more complicated by the effect of UV on chelators. Iron was gradually eliminated from the nutrient solution in both inoculated and un-inoculated ponds, as reflected in declining productivity in the control pond after the first four harvests (See Figure 5.10). Elemental analysis after harvest 12 showed the solution to be completely stripped of iron in both inoculated and un-inoculated UV conditions. The phenomenon of iron depletion was expected (Acher, et al., 1997) and we had deliberately decided to observe it rather than to counter it in this preliminary experiment.
**Discussion of Phase 3 Results**

**Implications of UV and Filtration Treatment Results for Pa Reproductive Cycle.** In inoculated ponds receiving UV and filtration treatments, and also the inoculated pond receiving no treatment, productivity plunged to a very low level over the first three harvests following inoculation. This was as one would have expected given the infection was already in place in these crops before treatments were applied. Where the systems began to differ was in the fourth and fifth harvest, in which there was substantial recovery in productivity in the case of UV treated system, some recovery in the filtered system, but barely any in the untreated system. If we assume the disease process in the fourth and fifth harvests was in part due to second-generation zoospores, the filtration and UV treatments appeared able to reduce second generation zoospore populations sufficiently to reduce the amount of damage they could cause. This assumption is almost a logical necessity and fits the data reasonably well, but one may ask how likely was it that secondary zoospores were released in time to affect harvests 4 and 5, and what implications are there for the length of the Pa reproductive cycle at 27.5 C?

Considering particularly pond 4 (inoculated but untreated), harvest 1 after inoculation showed that exposure of a crop to a high concentration of zoospores when harvest was 2.5 days away had little effect on biomass. Harvest 2 showed that exposure to a high concentration of zoospores when harvest was 5.5 days away had a

![Figure 5.13. Effects of UV irradiation and filtration on fresh weight productivity in inoculated systems. Error bars +/- 1 SE.](image-url)
large effect on biomass. Harvest 3 showed that exposure to a high concentration of zoospores when harvest was 8.5 days later had an even greater effect. Exactly the same pattern of depression of productivity was found in all four inoculated systems, although muted somewhat in the reduced-temperature system (for the understandable reason that lowered temperature slows pathogen activity). One may draw the conclusion that zoospores can do much damage to a healthy crop if they infect it as little as 5 days before harvest, and more damage yet if given 8 days in which to work, but rather little if only 2.5 days are available before harvest.

Crop size at time of infection appears to have been a limiting factor to the damage that zoospore inoculation was capable of doing in the production system. The smallest crop at time of inoculation, that in harvest 4, fared no worse than the harvest 3 crop, perhaps because a large proportion of its root system was still contained in the seedling media plug and thus protected from infection. With the assistance of any of the treatment methods, the crop in harvest 4 produced moderately to very well. The high level of damage seen in harvest 4 of the untreated inoculated control pond could not have resulted from the original infection alone, for if that was the case we would have seen similar results in UV and filtration ponds, which were at the same temperature, whereas we in fact saw substantial, statistically significant, beneficial effects of the treatment methods in harvest 4. The disease process in the harvest 4 crop of the untreated inoculated pond (pond 4) must have been the result of a combination of original and secondary infections.

The situation for harvest 4 is the limiting case for re-infection. Harvest 2 had only 5.5 days in the pond after inoculation before it was removed, which was 6 days before harvest 4, so it may or may not have had enough in-pond time for the Pa reproductive cycle to be completed. Harvest 3 had 8.5 days in-pond time before removal so any Pa reproductive cycle of less than 8.5 days could have been completed and there would have been 3 or more days for the new zoospores to do damage to the next crop harvested. If the Pa reproductive cycle were as short as 6 days in the harvest 3 crop, for instance, new zoospores would have had 5.5 days in which to infect the harvest 4 crop. Katzman’s (2003) results suggests the length of the reproductive cycle for Pa58, the strain of Pythium in use in our experiment, is c. 6 days at 31 C, with considerable release occurring in as little as 5 days, so it is plausible the harvest 4 crop received additional infection by zoospores released from the harvest 3 crop, and they had sufficient time to do damage.

**Implications of Phase 3 Results for Future Use of Treatment Methods.** The efficacies of filtration and UV systems comparable to those used in this experiment was investigated prior to the experiment in bench-top work. Our expectation was that the systems in use would be effective in eliminating zoospores from recirculating solution or, in the case of UV, reducing concentration to an extremely low level. However, we did not test the efficacy of the systems in place during the experiment itself, so there is uncertainty on this point. Nevertheless, it appears the source of continued infection within these systems was primarily from
second generation zoospores (and further generations) that never left the pond, but made their way upstream to younger un-infected crops, rather than zoospores evading the treatment systems. If that were the case, in order to make either treatment system effective, the task would be to prevent upstream movement. In the case of UV, additionally, chelated iron would need to be continuously replenished as it was being precipitated by the UV irradiation. On the other hand, the temperature reduction method was very successful, leading to further tests of its effectiveness.

**Phase 4 Results**

After the 14th harvest of the experiment, on 2-7-06, temperatures were reduced in ponds 1 and 4, which had long-established self-sustaining disease processes, from 27.7 to 20.0 C. We also reduced the temperature in the un-inoculated filtration control pond, pond 8, to 20.0C, to continue its function as a comparison condition. The comparison condition for pond 4 was pond 3 and which had been at 20.0 C since inoculation. Data from pond 5 are also presented. These are useful as a second comparison, and also of interest because they demonstrate long-term performance after recovery from heavy inoculation.

**Recovery in Pond 1**

Evidence of recovery in pond 1, the inoculated filtration pond, is presented in Figures 5.14, 15, 16, and 17, representing Productivity, Root Mass, DW:FW ratio, and Shoot:Root ratio, respectively. It appears recovery was almost entirely completed by the fourth harvest after temperature reduction, in terms of all four measures. Reductions of the DW:FW and Shoot:Root ratios in the control conditions were normal and expected effects of changing temperature from 27.5 to 20.0 C. The failure of productivity to increase in the healthy control condition (pond 8) as a result of the change to a more favorable temperature was unexpected. It was most probably the result of manganese deficiency beginning to appear. At the end of the experiment, manganese concentration was very low in this production system (5% of target concentration). During final harvests, there was no visible trace of disease in the roots of either the recovered or the control crops, and it appeared the disease process was completely eliminated.

**Recovery in Pond 4**

Evidence of recovery in pond 4, the original untreated inoculated control pond, is presented in Figures 5.18, 19, 20, and 21 representing Productivity, Root Mass, DW:FW ratio, and Shoot:Root ratio, respectively. In this case, convergence of treatment and control ponds in terms of productivity was complete by the fourth harvest after temperature reduction, and also appeared complete according to the DW:FW and Shoot:Root ratios. Productivity, however, and root mass were less than expected.

The steady productivity decrease in the comparison system, pond 5, and leveling off of productivity following recovery from disease in pond 4, was not accompanied by any signs of disease, and is readily
explainable in terms of manganese deficiency, which occurred in most of the systems towards the end of the experiment. Manganese concentration was extremely low in these ponds at the last harvest (5% of target concentration). However, elemental analysis did not show great deficiency in the low-temperature uninoculated control system, pond 3, which leaves unexplained why productivity fell in this system as well. It is quite possible there was a sample or data error in the elemental analysis because, if any pond were to exhaust manganese, it should have been this one because biomass production was maintained at the highest level throughout the whole experiment in this pond.

By the end of the experiment (harvest 7 after the second temperature reduction treatment, harvest 21 from start of experiment) all 3 ponds were producing healthy crops with no visible root disease and had maintained normal expected values for DW:FW ratio and Shoot:Root ratio over several harvests.

![Graph](image)

**Figure 5.14.** Effect of temperature reduction on fresh weight productivity in diseased and disease-free filtration ponds. Error bars +/- 1 SE.
Figure 5.15. Effect of temperature reduction on root mass in diseased and disease-free filtration ponds.
Figure 5.16. Effect of temperature reduction on DW to FW ratio in diseased and disease-free filtration ponds. Error bars +/- 1 SE.

Figure 5.17. Effect of temperature reduction on shoot to root ratio in diseased and disease-free filtration ponds.
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Figure 5.18. Effect of temperature reduction on fresh weight productivity in diseased and disease-free control ponds. Error bars +/- 1 SE.

Figure 5.19. Effect of temperature reduction on root mass in diseased and disease-free control ponds.
Figure 5.20. Effect of temperature reduction on DW to FW ratio in diseased and disease-free control ponds. Error bars +/- 1 SE.

Figure 5.21. Effect of temperature reduction on shoot to root ratio in diseased and disease-free control ponds. Error bars +/- 1 SE.
Discussion and Conclusions

This experiment was a departure in two respects from previous work on spinach disease caused by *Pa* in deep flow hydroponics. The work was conducted in a commercially realistic continuous production mode, and the goal was recovery from a severe self-sustaining disease process without stopping the production system or sequestering and treating of the nutrient solution. As far as we know, all previous work has been conducted in batch production, rather than continuous production, and has aimed at preventing disease rather than recovering from it. Commercially, spinach is currently grown to any large extent only in Japan, where it is grown in trough systems using batch production, the system is thoroughly cleaned between batches, and the nutrient solution is treated between batches or else discarded.

Of the three methods tested, two failed and one succeeded. The two that failed, filtration and UV irradiation, relied on infection not being able to spread upstream but evidently our system failed to prevent this from happening. This does not mean it is impossible to avoid upstream infection. However, we now believe this to be a difficult task to accomplish economically in deep flow hydroponics. UV irradiation showed the greater promise of the two that failed but carries along with it the difficulty in that it rapidly degrades the chelator necessary for carrying iron to the plants. UV also upsets microflora equilibria, with unknown consequences. Without a great deal of further work we think UV, filtration, sonication, ozonation and similar methods for eliminating *Pythium* zoospores are best suited to sterilizing reservoirs of nutrient solution between crop batches, or for sterilizing the return stream in NFT or similar systems where the return flow volume is much less than the supply flow volume. In this situation, continuous replenishment of iron to the supply stream overcomes the problem of chelator destruction. This is not the situation for deep flow production systems. In contrast, temperature reduction is highly suited for deep flow production, but not for NFT and similar systems. In a NFT trough, for example, the flowing nutrient solution quickly nears greenhouse air temperature as it flows along the trough and root zone temperatures can not remain properly controlled.

The temperature reduction method was very successful in all three tests. It effected recovery from a heavy inoculation with zoospores, which in other ponds continued on to establish highly destructive, self-sustaining disease processes, and it was used later on to eliminate these same highly destructive self-sustaining disease processes with replicated success. After temperature reduction had eliminated effects of an initial inoculation, the recovered production system was maintained further in continuous production for 16 harvests (7 weeks) without any sign of disease returning.

Temperature reduction was used as a treatment method here but in commercial practice it would be preferable to keep the nutrient solution at a safe temperature continuously, not just when necessary to recover from disease outbreaks. The other critical component of disease management in the production
system suggested by this research is keeping the crop cycle, more precisely, the in-pond crop duration, shorter than the characteristic *Pa* reproductive cycle. For baby spinach, which requires only a short crop cycle, this can be achieved within one pond if supplementary lighting is used in combination with a suitable control program to ensure the crop is large enough to be harvested on a fixed schedule – no later than the allowable in-pond duration. If it is necessary to grow a spinach crop longer than the *Pa* reproductive cycle, a strategy may be adopted of using two (or more) ponds in sequence to grow the same crop, thereby preventing *Pa* reproduction in either pond. This option is evaluated in more detail later in this report.

In this, and other related experiments, only one known virulent strain of *Pa* was used for methodological reasons. It can be expected other strains or species of *Pythium* and *Pythium*-like pathogens will have different requirements for disease prevention and may require shorter in-pond durations and have different temperature optima. The principle applied here was making the in-pond crop duration shorter than the pathogen reproductive cycle. To achieve this it was necessary to determine the characteristic minimum length of the pathogen’s *in vivo* reproductive cycle, as affected by temperature, and then to adjust the in-pond crop duration to be less than that. We feel the principle behind the temperature reduction method in this research will help to combat any pathogens similar to *Pythium aphanidermatum* as long as their *in vivo* reproductive cycle is of significant length.

**PURE CULTURE CHALLENGE EXPERIMENT 2**

**Introduction**

This experiment is a repeat in time of the most useful findings of Pure-culture Challenge Experiment 1, in which recovery from an established self-sustaining disease process in roots of spinach was possible by reducing the root zone temperature from 27.5 C to 20.0 C, and limiting the duration of the crop in the hydroponic system to 13.75 days. The disease process was induced by inoculation with the known strain of *Pythium aphanidermatum*, Pa58. This experiment was conducted in mid-summer in contrast to the previous experiment, which was conducted in mid-winter.

**Methods**

This experiment may be considered in three phases. In the first phase, eight production systems, independent in terms of root environment but sharing the same light array and aerial environment, were brought into full operation, until each contained five crops of different ages, this being the design capacity of the system. The operating schedule was to add crops to the ponds every three days at a fixed time of day and harvesting them when they had been in the ponds 13.75 days. (For 1.25 days the ponds contained only four crops pending addition of the next crop of seedlings. The crops could have remained in the system up to 15 days, but at 13.75 days the spinach leaf size was more than large enough for retail as salad spinach.) The crops consisted of small stands of spinach (c. 77 plants in a square array c. 0.1 m²) planted in
polystyrene trays/floats. Seed imbibition, priming and germination were conducted in a dark growth chamber over a four-day period prior to flotation. Stand establishment was enhanced by selection of sprouted seeds for planting.

Throughout the experiment, natural light was supplemented as needed to achieve a daily light integral of $17 \text{mol m}^{-2} \text{d}^{-1}$. If necessary, the photosynthetic period was extended 24 hours to obtain the required daily light integral. Aerial temperature was 23°C day and night. Greenhouse cooling greenhouse was by venting in conjunction with evaporative pads. (The set point could not always be met during the daytime in midsummer.) During the first phase, the set point for the temperature of the nutrient solution of all eight production systems was 20.0°C.

The first phase concluded with detailed data collection on the first two crops to pass through the system. Thus, the first phase served to obtain baseline data by which to evaluate performance of the production systems and confirm whether they performed similarly, using new, well-balanced nutrient solution.

The second phase commenced with inoculation of half the production systems with a known strain of Pa58, used previously. At the time of inoculation, nutrient solution temperature in the inoculated production systems was raised from 20.0°C to 27.5°C, this being a temperature typical of large ponds in a commercial greenhouse during the summer months – unless cooling methods are deliberately employed. The temperature was raised to foster development of the disease process. A heavy dose of Pa58 zoospores was applied (c. 20 zoospores per milliliter of nutrient solution, or approximately 1.5 million zoospores per pond) to establish the disease process quickly.

In the second phase of the experiment the purpose was to establish a self-sustaining disease process. Accordingly, application of remedial treatment was delayed until all five of the crops in the system at the time of inoculation had been harvested, and only new crops were present in the production system, added three or more days after inoculation. Remedial treatment was applied only after it was determined the follow-on crops were severely infected, as indicated by wilting and stunting, and by very low plant weight and productivity. (Because zoospores are very short-lived unless they invade a host, if the follow-on crops were severely diseased it meant that zoospores in the original inoculation had gone through a complete reproductive cycle and released new zoospores to invade the follow-on crops.) During the second phase of the experiment, the production systems that had not been inoculated were maintained at 20.0°C, this being a relatively safe operating temperature from the point of view of inadvertent disease acquisition.

The remedial treatment – nutrient solution temperature reduction – was applied in the third and final phase of the experiment. Three of the inoculated benches with demonstrated self-sustaining disease processes had
their temperature reduced to 20.0 C. They were matched to adjacent benches which had not been inoculated and in which the temperature had been 20.0 C from the beginning. If the plant size and productivity of the diseased benches could recover to the point of being indistinguishable from the control benches, the treatment would be considered successful.

In the remaining inoculated bench, temperature was continued at 27.5 C to demonstrate the disease process would not die out on its own, given enough time. The remaining un-inoculated bench was devoted to determining the separate effect of warm versus cool nutrient solution temperature, independent of the disease process. In this un-inoculated bench, the temperature, which had been 20.0 C, was raised to 27.5 C for the remainder of the experiment.

Results

Note on Data Collection. In this experiment the sample unit was the row. In each crop stand there were 11 rows of seven plants, staggered row to row. During harvest, plants from each row were cut through the hypocotyl, disentangled from remaining plants, bulked together and weighed immediately. For fresh weight, all rows were sampled and the sample size was 11. Plant materials from five interior rows (rows 4 through 8) were oven dried to provide dry weight data for determination of plant water status and growth analysis. In addition to weighing shoots, several other observations were made at the row level. The number of plants in each row was counted and recorded, and number of noticeably small plants; number of wilted and chlorotic plants and plants with blackened hypocotyls within rows were also noted when appropriate for the treatment and stage of experiment.

All roots exterior to the polystyrene floats were cut off and oven dried. During each harvest shoots and roots were photographed. Roots were photographed hanging down for an indication of length, color and condition, and also close-up in plan view. Additionally, roots were carefully visually inspected for signs of disease and notes were taken on quantity and distribution of affected roots.

Data from the first two harvests prior to inoculation are presented in full in Table 5.2, along with derivative calculations. Similar data were collected in all harvests of the experiment. Row data are included in Table 5.2 because they were the foundation for further calculations. Average plant weights in each row have also been calculated. However, for most purposes it is more helpful to consider data in terms of productivity, such as plant biomass productivity per unit area and unit time (g m⁻² d⁻¹) – or light use efficiency (g mol⁻¹) – plant biomass per unit of photosynthetically active light integral.

5.31
Table 5.2. Typical Spinach Crop Performance at 20°C as demonstrated in two harvests prior to inoculation.

<table>
<thead>
<tr>
<th>Production System No. (Pond)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop Duration in-pond (days)</td>
<td>13.75</td>
<td>13.75</td>
<td>13.75</td>
<td>13.75</td>
<td>13.75</td>
<td>13.75</td>
<td>13.75</td>
<td>13.75</td>
</tr>
<tr>
<td>Pond Temperature</td>
<td>20°C</td>
<td>20°C</td>
<td>20°C</td>
<td>20°C</td>
<td>20°C</td>
<td>20°C</td>
<td>20°C</td>
<td>20°C</td>
</tr>
<tr>
<td>Whole Flat FW Harvest (g)</td>
<td>443.8</td>
<td>465.5</td>
<td>549.7</td>
<td>539.1</td>
<td>516.7</td>
<td>532.7</td>
<td>538.5</td>
<td>512.7</td>
</tr>
<tr>
<td>Whole Flat FW Yield (kg m⁻¹)</td>
<td>4.20</td>
<td>4.40</td>
<td>5.20</td>
<td>5.10</td>
<td>4.88</td>
<td>5.04</td>
<td>5.09</td>
<td>4.85</td>
</tr>
<tr>
<td>Number of Rows (n)</td>
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<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Row Av. FW (g)</td>
<td>40.3</td>
<td>42.3</td>
<td>50.0</td>
<td>49.0</td>
<td>47.0</td>
<td>48.4</td>
<td>49.0</td>
<td>46.6</td>
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<tr>
<td>Row Av. FW, SD (g)</td>
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<td>9.3</td>
<td>14.6</td>
<td>10.5</td>
<td>6.4</td>
<td>16.0</td>
<td>13.0</td>
<td>14.8</td>
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<tr>
<td>Row Av. FW, SE (g)</td>
<td>3.3</td>
<td>2.8</td>
<td>4.4</td>
<td>3.2</td>
<td>1.9</td>
<td>4.8</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Av. No. plnts/row</td>
<td>5.4</td>
<td>6.5</td>
<td>6.5</td>
<td>6.1</td>
<td>6.2</td>
<td>6.1</td>
<td>6.3</td>
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<tr>
<td>Plant Density (plnt m⁻²)</td>
<td>558</td>
<td>671</td>
<td>671</td>
<td>633</td>
<td>643</td>
<td>633</td>
<td>652</td>
<td>652</td>
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<tr>
<td>Plant Shoot Av. FW (g)</td>
<td>7.52</td>
<td>6.56</td>
<td>7.74</td>
<td>8.05</td>
<td>7.60</td>
<td>7.95</td>
<td>7.80</td>
<td>7.43</td>
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<tr>
<td>Plant Shoot Av. FW SE</td>
<td>0.62</td>
<td>0.44</td>
<td>0.68</td>
<td>0.52</td>
<td>0.31</td>
<td>0.79</td>
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<td>0.010</td>
<td>0.010</td>
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<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
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</tr>
<tr>
<td>Av. Irradiation Integral (mol m⁻² d⁻¹)</td>
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<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>FW Productivity, Whole Flat (g m⁻² d⁻¹)</td>
<td>305</td>
<td>320</td>
<td>378</td>
<td>371</td>
<td>355</td>
<td>366</td>
<td>370</td>
<td>352</td>
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<tr>
<td>FW Productivity, Whole Flat SE</td>
<td>25</td>
<td>21</td>
<td>33</td>
<td>24</td>
<td>15</td>
<td>37</td>
<td>30</td>
<td>34</td>
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<td>FW Irradiation Use Efficiency (g mol⁻¹)</td>
<td>17.9</td>
<td>18.8</td>
<td>22.2</td>
<td>21.8</td>
<td>20.9</td>
<td>21.5</td>
<td>21.8</td>
<td>20.7</td>
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<tr>
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<td>1.5</td>
<td>1.3</td>
<td>2.0</td>
<td>1.4</td>
<td>0.9</td>
<td>2.1</td>
<td>1.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Sub Sample FW Prod. (g m⁻² d⁻¹)[n=5]</td>
<td>275</td>
<td>322</td>
<td>351</td>
<td>334</td>
<td>326</td>
<td>307</td>
<td>324</td>
<td>260</td>
</tr>
<tr>
<td>Sub Sample FW Prod. SE</td>
<td>18</td>
<td>26</td>
<td>46</td>
<td>29</td>
<td>19</td>
<td>35</td>
<td>44</td>
<td>29</td>
</tr>
<tr>
<td>Ratio. Whole Flat Prod :Sample Prod</td>
<td>1.11</td>
<td>0.99</td>
<td>1.08</td>
<td>1.11</td>
<td>1.09</td>
<td>1.19</td>
<td>1.14</td>
<td>1.35</td>
</tr>
<tr>
<td>Sub Sample DW Prod. (g m⁻² d⁻¹)[n=5]</td>
<td>12.8</td>
<td>15.0</td>
<td>15.3</td>
<td>14.8</td>
<td>14.5</td>
<td>13.5</td>
<td>14.0</td>
<td>11.5</td>
</tr>
</tbody>
</table>
Sub Sample DW Prod. SE | 0.92 | 1.19 | 2.17 | 1.30 | 0.85 | 1.57 | 1.92 | 1.28  
Correlation. DW:FW | 0.98 | 0.98 | 1.00 | 1.00 | 0.97 | 1.00 | 1.00 | 0.99  
DW/FW Av.[n=5] | 0.0465 | 0.0465 | 0.0434 | 0.0443 | 0.0445 | 0.0439 | 0.0431 | 0.0441  
DW/FW SE | 0.0007 | 0.0006 | 0.0008 | 0.0003 | 0.0007 | 0.0005 | 0.0006 | 0.0009  
Whole Flat DW Prod.Est.(g m-2 d-1)[n=5] | 14.2 | 14.9 | 16.5 | 16.4 | 15.8 | 16.1 | 16.0 | 15.5  
Whole Flat DW Prod. SE | 1.0 | 1.2 | 2.3 | 1.4 | 0.9 | 1.9 | 2.2 | 1.7  
IW Irradiation Use Efficiency (g mol-1) | 0.84 | 0.88 | 0.97 | 0.97 | 0.93 | 0.95 | 0.94 | 0.91  
IW Irradiation Use Efficiency SE | 0.06 | 0.07 | 0.14 | 0.08 | 0.05 | 0.11 | 0.13 | 0.10  
Exposed Root DW, whole Flat (g) | 3.32 | 3.04 | 3.50 | 3.73 | 4.15 | 4.43 | 3.54 | 3.09  
All Root DW Est. (g) | 4.53 | 4.15 | 4.78 | 5.09 | 5.67 | 6.06 | 4.84 | 4.22  
Shoot:Root ratio (DW basis) | 4.6 | 5.2 | 5.0 | 4.7 | 4.1 | 3.9 | 4.8 | 5.4  
Root observations | VG | VG | VG | VG | VG | VG | VG | VG  
Shoot observations | VG | VG | VG | VG | VG | VG | VG | VG  
% wilted | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  

Harvest 2, 4 July

Production System No. (Pond) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8  
Harvest No. - from start of Expt. | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2  
Harvest No. - from inoculation | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1  
Harvest No. - from remedial treatment | -6 | -6 | -6 | -6 | -6 | -6 | -6 | -6  
Crop Duration in-pond (days) | 13.75 | 13.75 | 13.75 | 13.75 | 13.75 | 13.75 | 13.75 | 13.75  
Pond Temperature | 20C | 20C | 20C | 20C | 20C | 20C | 20C | 20C  
Whole Flat FW Harvest (g) | 499.7 | 513.3 | 467.2 | 488.7 | 463.6 | 469.5 | 426.8 | 459.2  
Whole Flat FW Yield (kg m-1) | 4.72 | 4.85 | 4.42 | 4.62 | 4.38 | 4.44 | 4.03 | 4.34  
Number of Rows (n) | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11  
Row Av. FW (g) | 45.4 | 46.7 | 42.5 | 44.4 | 42.1 | 42.7 | 38.8 | 41.7  

5.33
<table>
<thead>
<tr>
<th></th>
<th>8.9</th>
<th>10.6</th>
<th>9.1</th>
<th>10.5</th>
<th>11.7</th>
<th>10.8</th>
<th>13.0</th>
<th>13.6</th>
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<tr>
<td>Row Av. FW, SD (g)</td>
<td>2.7</td>
<td>3.2</td>
<td>2.7</td>
<td>3.2</td>
<td>3.5</td>
<td>3.3</td>
<td>3.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Av. No. plnts/row</td>
<td>7.9</td>
<td>7.3</td>
<td>6.7</td>
<td>6.4</td>
<td>6.2</td>
<td>6.1</td>
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<td>6.3</td>
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<td>Plant Density (plnt m-2)</td>
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<td>756</td>
<td>700</td>
<td>662</td>
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<td>Plant Shoot Av. FW (g)</td>
<td>5.74</td>
<td>6.42</td>
<td>6.31</td>
<td>6.98</td>
<td>6.82</td>
<td>7.01</td>
<td>6.47</td>
<td>6.66</td>
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<tr>
<td>Plant Shoot Av. FW SE</td>
<td>0.34</td>
<td>0.44</td>
<td>0.41</td>
<td>0.50</td>
<td>0.57</td>
<td>0.53</td>
<td>0.66</td>
<td>0.65</td>
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<tr>
<td>Row Area (m2)</td>
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<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
</tr>
<tr>
<td>Av. Irradiation Integral (mol m-2 d-1)</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>FW Productivity, Whole Flat. (g m-2 d-1)</td>
<td>344</td>
<td>353</td>
<td>321</td>
<td>336</td>
<td>319</td>
<td>323</td>
<td>293</td>
<td>316</td>
</tr>
<tr>
<td>FW Productivity, Whole Flat. SE</td>
<td>20</td>
<td>24</td>
<td>21</td>
<td>24</td>
<td>27</td>
<td>25</td>
<td>30</td>
<td>31</td>
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<tr>
<td>FW Irradiation Use Efficiency (g mol-1)</td>
<td>20.2</td>
<td>20.8</td>
<td>18.9</td>
<td>19.8</td>
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<tr>
<td>FW Irradiation Use Efficiency SE</td>
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<td>1.4</td>
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<td>1.6</td>
<td>1.4</td>
<td>1.7</td>
<td>1.8</td>
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<tr>
<td>Sub Sample FW Prod. (g m-2 d-1)[n=5]</td>
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<td>350</td>
<td>333</td>
<td>356</td>
<td>298</td>
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<td>337</td>
<td>311</td>
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<td>Sub Sample FW Prod. SE</td>
<td>23</td>
<td>32</td>
<td>29</td>
<td>34</td>
<td>41</td>
<td>16</td>
<td>44</td>
<td>39</td>
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<td>Ratio. Whole Flat Prod :Sample Prod</td>
<td>1.16</td>
<td>1.01</td>
<td>0.96</td>
<td>0.94</td>
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<td>15.4</td>
<td>16.0</td>
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<td>15.6</td>
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<td>1.12</td>
<td>1.57</td>
<td>1.40</td>
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<td>0.88</td>
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<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>1.00</td>
<td>0.96</td>
<td>1.00</td>
<td>0.99</td>
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<tr>
<td>DW/FW Av.[n=5]</td>
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<td>0.0446</td>
<td>0.0461</td>
<td>0.0451</td>
<td>0.0452</td>
<td>0.0442</td>
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<td>0.0431</td>
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<tr>
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<td>0.0006</td>
<td>0.0006</td>
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<td>15.1</td>
<td>14.5</td>
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<tr>
<td>DW Irradiation Use Efficiency (g mol-1)</td>
<td>0.92</td>
<td>0.93</td>
<td>0.87</td>
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<td>0.08</td>
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<td>Exposed Root DW, whole Flat (g)</td>
<td>3.06</td>
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<td>3.19</td>
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<td>3.56</td>
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<td>2.96</td>
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<td>All Root DW Est. (g)</td>
<td>4.18</td>
<td>4.72</td>
<td>4.36</td>
<td>4.66</td>
<td>4.52</td>
<td>4.86</td>
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<td>Shoot:Root ratio (DW basis)</td>
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<td>VG</td>
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<td>VG</td>
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<td>VG</td>
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<tr>
<td>% wilted</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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</table>
**Phase 1 Results.** With reference to Table 5.2, comparing productivity across benches in the first two harvests, we see in Harvest 1 whole flat FW productivity averaged 352 g m⁻² d⁻¹, and ranged from 305 to 378 g m⁻² d⁻¹, with a standard error of 9.2, and coefficient of variation 7.5%. In Harvest 2, whole flat FW productivity averaged 326 g m⁻² d⁻¹, and ranged from 293 to 353 g m⁻² d⁻¹, with a standard error of 6.5, and coefficient of variation 5.6%. If the five rows in the sub-sample for which dry weights were obtained are considered, the productivity values were reduced, as also was the variation in productivity across benches (312 and 329 g m⁻² d⁻¹). The sub-sample consisted of interior rows, which minimized edge effects, particularly the effect of plants in the leading and trailing edges of the pond system. In our experience spinach is prone to a high degree of plant-to-plant variation, so we consider the limited amount of variation between production systems in these harvests normal and satisfactory.

In absolute terms, the FW productivity values were also satisfactory. Previously, using this cultivar and light integral under optimal conditions of temperature and plant density, a productivity of c. 330 g m⁻² d⁻¹ in 13 days growing time was achieved, after edge effects were taken into account. In this experiment plant density (c 700 plants m⁻²) was roughly half what would be optimal in commercial production (1500 plants m⁻²), and yet productivity still reached this level. Productivity in fact was so high it requires explanation. Part of the explanation is that edge effects were not eliminated, either in the growing phase or in the harvest. This was a deliberate choice. The high productivity is also partly due to the plants being allowed to grow slightly longer and bigger than is ideal for baby spinach. At time of harvest the crop was growing very fast and productivity (g m⁻² d⁻¹) was still increasing on a daily basis.

We conclude that productivity was satisfactory and in the range expected. (For comparison purposes, lettuce productivity for 5-ounce heads of butterhead lettuce under similar light conditions but in a 35-day crop cycle with plant re-spacing to increase efficiency, is roughly 315 g m⁻² d⁻¹.)

**Phase 2 Results.** Inoculation and temperature manipulation took place directly after the second harvest. Ponds 1, 3, 5, and 8 were inoculated and the temperature raised to foster establishment of disease. As Figure 5.22 shows, biomass was only slightly reduced in the first crop harvested after inoculation, presumably because plants were already large at the time of inoculation and already had a large, established root system. Only three days were available to the *Pa* to degrade roots. Subsequently, productivity decline was rapid, ending almost at zero. Follow-on crops (harvests 6 and 7 in Figure 5.22) to those originally inoculated (harvests 1 to 5) also were severely afflicted, indicating successful reproduction of *Pa*.

In Figure 5.22 an indication is also present of the effect of increasing temperature from 20.0 C to 27.5 C without inoculation. (These data actually derive from the third phase when the temperature was changed,
but are put here to answer the natural question of what effect raising temperature would have had alone.)

Roots of the crop grown at 27.5 °C solution temperature were sparser than at 20.0 °C, crinkled and slightly tan in color. From the level of fresh weight productivity in comparison with crops at 20.0 °C, it appears nutrient solution at 27.5 °C is less satisfactory for spinach growth than at 20.0 °C, a finding duplicated in our first experiment, and in the literature. The difference is considerably reduced on a dry weight basis, however, because much of the apparent reduction in fresh weight productivity was due to a significant change in dry matter content of the plants (compare Figures 5.24 and 5.25 following, and see Figure 5.26).

![Figure 5.22. Effect of Inoculation with *Pythium aphanidermatum* on FW productivity of spinach](image)

Regardless of these considerations, the depressing effect of raising solution temperature on productivity was much less than the depressing effect of inoculation in addition to raised temperature. In the latter case the crop was virtually destroyed, while in the former it was still useable, but reduced 10 to 20 percent on a fresh weight basis.

In Phase 2, only half the ponds were inoculated. Averaged data are plotted in Figure 5.22 for three ponds in which production was continued at the original temperature of 20.0°C without inoculation. Although there was some variation from harvest to harvest, productivity remained extremely high in these ponds. The variability seen was most probably due to heat-wave conditions affecting the greenhouse aerial environment. In Figure 5.22 the fall off in productivity as a result of inoculation is dramatically portrayed, all four inoculated ponds acting in synchrony.
**Phase 3 Results.** Phase 3 commenced immediately after the seventh harvest of the experiment, with reduction of solution temperature from 27.5 to 20.0 C in ponds 3, 5, and 8. The temperature was kept at 20.0 C in ponds 4, 6, and 7. Pond 1 was continued at 27.5 C for comparison purposes, and pond 2 was raised to 27.5 C.

**Productivity.** Figure 5.23 shows the changes in fresh weight productivity that occurred in each of the diseased ponds in successive crop harvests following temperature reduction. Restored of productivity was in synchrony in two of the systems (ponds 3 and 5), but lagged slightly in the third system, pond 8. In Figure 5.24 fresh weight productivity is again presented, but with the three treated production systems (diseased ponds 3, 5, and 8, averaged, and three comparison/control systems – uninoculated ponds 4, and 7 – see below concerning pond 6). Similar dry weight productivity values are presented in Figure 5.25.

![Figure 5.23. Effect of temperature reduction treatment on fresh weight productivity of 3 diseased production systems. Error bars +/- 1 SE.](image-url)
Figure 5.24. Effect of temperature reduction treatment on fresh weight productivity of diseased production systems. Error bars +/- 1 SE.

Figure 5.25. Effect of temperature reduction treatment on dry weight productivity of diseased production systems. Error bars +/- 1 SE.
As can be seen in Figures 5.23, 24, and 25, diseased production systems in which temperature was reduced recovered to match or exceed the productivity of the uninoculated control systems within 10 or 11 harvests following temperature reduction, and continued at this level over the final 3 harvests. The diseased production system remaining at 27.5 C showed no recovery and continued with close to zero productivity. Productivity in the uninoculated comparison pond in which temperature was raised fell to approximately 75% of the cooler temperature ponds on a fresh weight basis (Figure 5.24) but, as Figure 5.25 shows, was much closer on a dry weight basis. Figure 5.26 shows the DW:FW ratio of shoots was consistently and significantly higher at 27.5 C than at 20.0 C. However, most importantly, it shows the DW: FW ratio was identical between the control system at 20 C and the systems that had recovered from disease at 20 C.

Figure 5.26. Effect of temperature reduction treatment on dry weight to Fresh weight ratio of diseased production systems. Error bars +/- 1 SE.
In Figures 5.23, 24, 25, and 26, for the sake of clarity data for harvests 13, 14, 15, and 16 from the start of the experiment have been omitted for the following reason: In harvest 11 from the start of the experiment, four harvests after temperature reduction began, leaf chlorosis was noticed in one of the ponds recovering from disease. It became more visible and pervasive in all three ponds recovering from root disease (ponds 3, 5, and 8) in the next three harvests, leading to depression of plant shoot growth. By harvest 14 we had diagnosed the chlorosis as due to manganese deficiency and made a correction to the nutrient solution to bring Mn concentration up to target. Subsequent recovery in productivity was rapid. The depression in productivity due to manganese deficiency is shown graphically in Figure 5.27.

![Figure 5.27. Effect of nutrient solution temperature reduction on Pythium Infected spinach crops: Infection at harvest 2, temperature reduction at harvest 7](image)

Data from one uninoculated control production system, pond 6, have also been omitted in these figures. Pond 6, that had been nominally at 20.0 C throughout the experiment, spontaneously developed a persistent disease process in the roots that looked typical of Pa, but may in fact have been another species or different strain than that used for inoculation. As a result productivity in this pond dropped noticeably, which negated any usefulness of the pond as a control. In other concurrent experiments in the same greenhouse range as this experiment, we deliberately introduced wild unspecified pathogen mixes and fostered their development into full-blown disease processes for the purposes of challenging this disease remediation technique. These foreign introductions could have been the source of disease in pond 6. Evidence of this
disease process first appeared in the seventh harvest after temperature reduction (fourteenth of the experiment) and persisted to the end of the experiment, in the form of small patches of discolored roots. The disease process did not appear to reach high into the roots, and depressed productivity only a small amount, similar to the effect of raising nutrient solution temperature to 27.5°C, but invalidated this production system for purposes of comparison.

**Traces of disease in the roots.** Although over the final four harvests, all three inoculated production systems had recovered from root disease sufficiently to either match or exceed the control systems in terms of fresh and dry weight productivity, only one system showed itself consistently clean of any visual trace of disease in the roots. Pond 5 crops showed no sign of disease in the roots for the final six harvests. Ponds 3 and 8 showed only small or tiny isolated patches of disease at the root ends during the final six harvests, and each gave one harvest with no visual signs of root disease at all. Pond 3 productivity ended identical to that of pond 5, so evidently whatever disease process was left was insufficient to affect biomass significantly.

Productivity for the final two harvests in pond 8 was slightly depressed, which may have been due to lingering disease, but its productivity still matched that in uninoculated benches. After the disease process took hold following inoculation, by harvest time only blackened traces of roots remained, with negligible mass. Figure 5.28 shows the recovery of root mass following temperature reduction. It is of interest that the dip in biomass productivity found during the period of manganese deficiency did not occur in the roots.
Control Pond productivity. Although at first it might seem a good thing to have the recovered ponds exceed the control ponds in productivity in the final two harvests, the difference was in fact significant and begs explanation.

Elemental analysis of nutrient solution made towards the end of the experiment showed the three control ponds to be very low in potassium – one tenth of the target of 5.50 mM. Other ponds were in the 2 to 3 mM range – which our experience has shown to be typical and satisfactory, for luxury consumption of potassium is the norm. Nutrient solution imbalance would seem a likely explanation of the small depression in productivity evidenced in the control ponds as compared to the recovered diseased ponds. An immediate correction was made, but it was too late to affect plant performance.

Discussion and conclusions
This experiment was the first time we had attempted long-term continuous production of spinach in the greenhouse during mid-summer using cooled nutrient solution, and it was a very hot summer. It was not always possible to maintain the 20.0 C set point for the nutrient solution with the equipment available.
Cooling was required even for the higher set point of 27.5°C. Cooling needs were at times prioritized in favor of the recovering ponds versus the control ponds. The situation with aerial temperature was similar. For many days in a row, aerial temperature set point was exceeded during the middle part of the day.

None of these temperature departures were likely to seriously affect productivity, or plant quality, but they did add stress to the plants and possibly favored survival of the pathogen by at times speeding up its reproductive cycle.

Our inexperience with long-term solution management in continuous production of this crop also showed itself. Although we conducted two elemental analyses of the nutrient solution during the experiment, which lasted close to 10 weeks, we failed to anticipate the shortages of manganese and potassium that occurred differentially, and rather abruptly, within the production systems.

Despite these shortcomings, the experiment was largely successful. Functionally, we eliminated effects of a devastating self-sustaining disease process in all three replicates, restoring productivity to control values (or higher) and producing a high-quality saleable product. In one replicate we completely eliminated any sign of the disease in the roots over a final six harvest periods. In the other replicates, only traces of the disease remained at the end of the experiment, and only sporadically. We expect these would have disappeared given time.

The fact in this experiment we were unable to eliminate the final traces of the disease in every one of the replicates in the time available, whereas in the previous experiment we succeeded in doing so, suggests several design and operating considerations for year-round production of spinach in a single pond system. First, frequent nutrient solution analyses should be part of the production protocol to avoid shortages of nutrients such as manganese and potassium. Second, the nutrient solution chilling system should have sufficient capacity for at least the 97.5% summer design temperature conditions (and the system of solution delivery should be well insulated). Perhaps the temperature needs to be a slightly lower or the crop cycle needs to be slightly shorter than the values we used here, at least in the summer. This could be achievable with the same productivity using a slightly higher daily light integral, which would be of little cost due to greater solar availability in summer.

We know that in an ongoing Pa colony, new zoospores are released over time, most probably with something like a normal distribution. The length of the reproductive cycle has a mean and standard deviation. It is possible our combination of crop duration and solution temperature may have permitted some part of the Pa colony, that responsible for one tail of the distribution, to reproduce successfully. It
could also be that our principle of removal of the diseased plant material before it had time to reproduce was circumvented; shreds of broken roots could enable a small-scale reproductive cycle to be conducted outside the plants. These remain speculative at this time.

The mysterious disease process that spontaneously developed in pond 6 may have simply been the result of inadvertent contamination from adjacent diseased ponds, and the fact the disease gained a foothold may have been simply because of our inability to hold set-points for temperature perfectly under summer conditions. However it serves to raise the question of how to deal with disease organisms such as *Pythium dissoticum* that actually have a temperature optimum in the vicinity of that we are using to control *Pythium aphanidermatum*.

We have identified *Pythium dissoticum* in our area so we do not know if it will be a future problem. However, the same approach could be used for *P. dissoticum* as for *P. aphanidermatum*, that of removing plant material before the reproductive cycle is complete, by drastically shortening the time the crop is in any one pond system. We tested this approach (a two-pond system) in one of the wild-culture challenge experiments to be described in the next chapter, and it appears to be feasible. However, a pressing research need remains to explore the precise nature of the *in vivo* reproductive cycle of this important spinach root disease organisms with a view to circumventing its propagation.
INTRODUCTION

The wild culture challenge experiments were the culmination of the project. By this time we had eliminated a number of possibilities for treating *Pythium* disease and discovered one that worked well against our pure strain of the organism. This we called the Temperature Reduction Method.

The disease process caused by *Pythium* organisms in spinach requires some explanation and overview. It consists of a mass of mycelium growing in and on the spinach roots, which acts as its food source. The growing infection gradually destroys the roots and, thus, eventually the shoot. But the disease process is more than this; it includes an on-going reproductive cycle between mycelium, fixed in place, and free-swimming zoospores by which the organism spreads itself. When one refers to *Pythium* disease, it connotes this entire phenomenon - a living and changing process cycling through time, not just the short-lived free-swimming propagules called zoospores. Neither is it only mycelium, the vegetative stage.

The key to our control method was the revelation by Katzman (2003) that a characteristic time interval exists, which varies with temperature, between invasion/infection of roots with zoospores and release of new zoospores into the solution to spread the disease. One may call the interval between infection/inoculation by zoospores and release of new zoospores the length of the *Pythium* reproductive cycle *in vivo*, understanding this characterizes the relationship of a particular organism to a particular production system and protocol and which must be determined empirically. The cycle depends strongly on temperature. Katzman (2003) showed the *in vivo* reproductive cycle for the Pa58 strain at 24 C in a tub system is between twelve and nineteen days. The principle behind our treatment method is to keep the in-pond crop duration shorter than the pathogen reproductive cycle, while at the same time growing the crop to a useful size. In this protocol, the mycelium is removed from the production system before a new generation of zoospores is released to spread the infection.

It was deemed essential that, whatever successful treatment method/methods we succeeded in developing, we needed to test it on a “wild” mixture of microorganisms derived from an established, commercial production pond, using a commercially-realistic spinach production protocol. To increase the commercial applicability and validity of the wild-culture testing of the disease prevention method, deep ponds with large, gently-stirred nutrient solution volumes were used instead of the shallower fast-flowing production systems used for the pure-culture challenge experiments. (Fast directional flow is not necessary for the
temperature reduction method.) In the deeper, slower-flowing ponds used for the wild culture challenge experiments, one might expect any free-swimming zoospores to reach roots of all ages unimpeded by currents, for the speed of water flow within the ponds was relatively slow. This mimics the slowly recirculating water flow movement present in commercial production ponds. The specific design of the deep flow systems used in the wild-culture challenge experiments was described in Chapter 2.

The wild-culture challenge experiments were conducted in long-term, continuous, production mode – the nutrient solution was replenished and adjusted for pH and nutrient concentrations, but not changed out. To establish a full complement of crops in continuous production takes time. After establishment there were five cohorts of crops of different ages in the ponds at all times except briefly between harvest and transplant of new plants. In continuous production, crops necessarily must be removed routinely to make room for new crops to be added – in this case we adopted a three-day schedule for crop removal and addition.

After the systems were filled with crops, additional time was needed to establish a self-sustaining disease process and prove it to be self-sustaining. We considered the latter accomplished when biomass production (spinach growth) was near zero in a completely new set of flats that had been entered into the ponds subsequent to the inoculation event. Only at this point – when the disease process was full-blown and robust – was the temperature reduction treatment applied.

In the first two experiments, data collection began with the harvest following application of the temperature reduction treatment. Subsequently, detailed data were collected in eight to twelve additional harvests to examine the long-term effects of the treatment. In the final two wild culture challenge experiments, detailed data were collected only when the treatment began to take effect – namely, four to five crops after application of the treatment. Each crop/harvest consisted of a polystyrene flat of approximately 75 plants. These flats were floated when seedlings emerged (a two to four day process depending on the type of seed used, temperature and exact germination method). The length of time flats were kept in the ponds depended on the experiment’s objectives, and was reduced in the final two experiments. This length of time was a critical variable in the treatment method, for it is the time available to the disease entities in which to complete their reproductive cycle if they are not to die out. (It is referred to as “in-pond duration” to distinguish it from crop age or length of crop cycle, which normally includes germination time.) In the final two experiments, the treatment included shortening the in-pond crop duration in addition to temperature reduction.

For purposes of data collection and analysis, a sample unit was a row of plants within a polystyrene flat. Fresh weights (FW) were obtained for the whole crop (also termed flat/float/harvest) bulked by row, thus
the sample size was generally eleven, corresponding to the number of rows used in the flat. The number of successful plants in each row was recorded so that plant density and average individual plant size could be determined, as well as the number of wilted and small-sized plants in each row. Dry weights (DW) were obtained for a sub-sample of five rows, again bulking by row. Thus, n, the sample size, for DW and DW:FW ratios was equal to 5. Root dry weights were obtained as bulk figures for the parts of the roots growing outside the flat. These procedures were followed in all four wild-culture challenge experiments with the exception that shoot DW values were omitted in the final experiment.

Results are presented in terms of in-pond productivity (g m$^{-2}$ d$^{-1}$). This statistic is neutral as to the sample unit used and is the most useful metric for comparing crop performance across species and different plant densities and production protocols if a consistent daily light integral is maintained (as it was in these experiments). In-pond productivity is used rather than complete-crop-cycle productivity because all light-assisted growth of the crop took place in the ponds and the cropping in-pond duration was consistent. Care was taken to float flats immediately after seedlings emerged. There was some variability in length of the germination phase of the crop cycle, on the other hand, because we switched between de-hulled and intact seed and tried a number of methods to obtain the most reliable germination performance using intact seed.

Within a given experiment, in-pond duration was the same, daily light integral was the same and plant density varied little and, thus, whether the results are presented in terms of average plant size, yield, productivity, or irradiation light use efficiency, makes little difference in patterns presented, for all these measures were highly correlated, if not simply factors of one another.

**SOURCE OF WILD CULTURE TEST ORGANISMS**

Wild-culture challenge testing, Task 6, was a necessary “proofing” part of the project. All prior experimental work of the project was done using a pure strain of *Pythium aphanidermatum, Pa58*, with known properties. But many strains of *Pa* exist, some more virulent than others and, in reality, in commercial production one must contend with a complex mixture of mostly unknown microorganisms working in concert. Additionally, the scientific literature recognizes another species of *Pythium* as being troublesome for spinach, but it has a different, lower temperature optimum than *Pa*. This species, *Pythium dissoticum*, is less common and less damaging than *Pa*, but it could potentially cause a problem for a treatment method developed solely to handle *Pa*.

The goal of the wild-culture challenge experiments was to determine whether the temperature-reduction method of disease control for *Pa*, previously demonstrated successfully on a pure, known pathogen (Pa58),
would prove effective if the pond system in which the plants were grown was inoculated with a mixture of organisms existing in typical commercial lettuce-production ponds. The inoculum source ponds were located in the commercial lettuce production greenhouse operated by Challenge Industries in Ithaca, NY (and previously by Cornell University CEA Program, see www.fingerlakes.fresh.com). Temperature in these ponds is normally held between 24 and 28°C. Previous microscopic examination of the micro flora of these commercial ponds showed several strains of *Pythium aphanidermatum* to be present, strains capable of invading and completely destroying spinach crops once stimulated to increased populations by the presence of spinach roots (Katzman, 2003).

Virulent occurrences of what was most likely *Pa* appeared spontaneously in two of the un-inoculated control conditions in earlier experiments of the current project. Solution samples from these sources were incorporated in the pond nutrient solution of the wild-culture challenge experiments, in addition to the inoculum from the commercial production ponds. (The spontaneously appearing disease entities may or may not have been identical to the pure strain used throughout the research; in any case they had shown themselves to be highly effective in rapidly destroying spinach crops at warm temperatures.)

Following the practice developed in the Pure-culture Challenge Testing Experiments using the pure strain of *Pa*, (See Chapter 5), the wild culture was allowed to reproduce for weeks in the presence of growing spinach in which to increase its presence to a highly destructive level in the microflora mix.

**OVERVIEW OF WILD CULTURE CHALLENGE TEST EXPERIMENTAL RESULTS**

Full descriptions of the wild-culture challenge test experiments are presented below. Here the results are briefly summarized and discussed. The first two wild culture experiments closely followed the pure-culture challenge experiments in design.

After the wild inoculum had established a self-sustaining disease process in two ponds simulating commercial production systems in continuous production mode, the temperature was reduced from 27.5 C to 20.0 C in one system but continued at 27.5 C in the comparison system. With development of the self-sustaining disease process, productivity had effectively ceased. In the comparison system held at 27.5 C, productivity continued to be close to zero. In the pond treated with temperature reduction, initially it appeared there would be a recovery to normal levels of productivity for a healthy crop (in the 4th, 5th and 6th harvests following start of the treatment), but after the initial promising response (change from 1 to 250 g m⁻² d⁻¹), productivity fell to a level approximately one-half of what would have been the case had the crop fully recovered (150 g m⁻² d⁻¹ versus 330 g m⁻² d⁻¹). Although the temperature reduction treatment had a
considerable effect in restoring biomass production, plants were wilted and unacceptable for commercial purposes so, in effect, the treatment failed. In view of the stressful mid-summer conditions concurrent with the experiments, the experiments were extended after application of the treatment to allow an opportunity for recovery, but all indications were that recovery would not take place.

A logical and possible explanation of the failure of the first two wild-challenge experiments was that a new pathogen had been introduced in the wild culture that was capable of reproducing faster than the in-pond duration of the spinach crops (which in the first two experiments had been 15 days and 13.75 days respectively). Following up this hypothesis we ran two additional experiments, one in which a two-pond production system was used with an overall crop cycle of twelve in-pond days, but just six in each of the two ponds (Wild-culture Challenge Experiment 3), and one in which the in-pond crop duration was reduced to nine days (Wild-culture Challenge Experiment 4). The starting point for each of these experiments was a self-sustaining disease process. The reduction of in-pond duration was successful in restoring a high level of productivity and, in each case, rapidly eliminated all signs of disease in the roots.

COMPARING RESULTS OF PURE STRAIN Pa TESTING VS. WILD-CULTURE TESTING

In the first two wild-culture challenge experiments, which formally repeated the methods of the pure-strain challenge experiments, productivity following the temperature reduction treatment ended up no better than half of that found in the pure-culture challenge experiments. What plant production was harvested was of no commercial value; many plants were wilted and not marketable. (As reported previously, in the pure-culture experiments, recovery of biomass production was fully up to healthy crop standards.)

In the final seven harvests of wild-culture experiment 1, covering a period of three weeks, average productivity was 176 g m⁻² d⁻¹, with a standard error of 10.6 g m⁻² d⁻¹ (see Figure 6.1 and Table 6.1). In the final four harvests of wild-culture experiment 2, covering a week and a half, average productivity was 159 g m⁻² d⁻¹, with a standard error of 13.5 g m⁻² d⁻¹. (See Figure 6.7 and Table 6.2). By comparison, in the first pure-culture challenge experiment, for six harvests after recovery from inoculation, productivity was 340 g m⁻² d⁻¹, with a standard error of 8.6. In the second pure-culture challenge experiment, for the final six harvests after recovery from inoculation, productivity was 336 g m⁻² d⁻¹, with a standard error of 31. (See Tables 5.1 and 5.2.)

Many factors influence precise productivity levels in healthy spinach crops, but the figures for productivity after recovery from disease cause by the pure culture, Pa58, were in accordance with a large body of previous research in healthy crops and certainly were satisfactory, whereas results for the wild culture
challenges experiments were clearly deficient (see the report: A Commercially-Viable Controlled Environment Agriculture (CEA) Spinach Production System, Contract Number 6257-IABR-IA-00, dated June, 2005, draft final project report submitted to NYSERDA). Based on this work, in a healthy crop, we expected productivity should be in the vicinity of $330 \text{ g m}^{-2} \text{ d}^{-1}$.

The final two wild-culture challenge experiments were successful in eliminating all visible signs of disease in the roots, and other indicators of crop and root health such as DW:FW ratio and Shoot:Root ratio, also indicated healthy plants.

In the 2-pond system of wild culture experiment 3, with in-pond duration of six days in each pond, for the final two harvests of the experiment (after recovery from disease), productivity in a 12-day in-pond crop cycle was $222 \text{ g m}^{-2} \text{ d}^{-1}$, with standard error $9 \text{ g m}^{-2} \text{ d}^{-1}$ (see Figure 6.12 and Table 6.3). Additionally, at the end of this experiment, two crops were allowed to continue to grow to an in-pond duration of 13.75 days. Productivity for these crops was 290 and 335 $\text{ g m}^{-2} \text{ d}^{-1}$. In previous work using the same deep pond system, 330 $\text{ g m}^{-2} \text{ d}^{-1}$ was considered a reliable and achievable productivity for this size of spinach plant, as noted above. Also, as noted above, productivity after recovery from disease in the two pure-culture challenge experiments was approximately $340 \text{ g m}^{-2} \text{ d}^{-1}$. Considering that the transfer step between ponds likely

![](Figure 6.1. Wild-culture challenge experiment 1. Fresh weight productivity. Response of diseased spinach crops to reduced pond temperature. Cultivar Whale, in-pond duration 15 days, +/- 1 SE)
caused some shock to the roots and productivity reduction, the value for productivity achieved after recovery from disease in the two pond cultural system of wild culture experiment 3 was satisfactory.

Time and resources did not permit running control conditions in the fourth wild culture experiment, the final experiment of the project, in which a single pond production system was used but with an in-pond duration of nine days. Data collected at the end of the wild-culture challenge experiment 3 were used to develop a growth curve for the recovered crops, as shown in Figure 6.17 below. Reading from this curve, it can be seen for a nine days in-pond crop duration, productivity of a healthy crop might be expected to be 160 g m⁻² d⁻¹. In Wild-culture Challenge Experiment 4, in which the in-pond crop duration was 9 days, the productivity over the final three harvests of the experiment were 152, 135 and 147 g m⁻² d⁻¹, following recovery first from disease and then from deficiency of manganese (see Figure 6.18 and 6.19).

Discrepancies between measured and expected productivity values were small and within experimental error in two of the final three harvests. Nevertheless, productivity was lower than expected for a one-pond system, which should be higher than for a two-pond system because no transfer and root disturbance is required. While the productivity was lower than expected, it achieved a level in nine days similar to that achieved in 13.75 days in the same pond before the crop duration was lowered to nine days (see Figure 6.18) and, additionally, plants looked healthy in every way. Whatever its cause, it apparently was not due to root disease because two crops left in the production system a further two weeks grew to be very large and their root systems remained completely healthy after in-pond durations of more than 20 days.

CHALLENGE EXPERIMENT 1

Introduction

The goal of this experiment was to see whether the temperature-reduction method of disease control for Pa demonstrated previously would prove effective if the pond system in which the plants were grown was inoculated with the mixture of organisms existing in typical commercial lettuce-production ponds; the inoculum source ponds used were located in the commercial lettuce production greenhouse operated by Challenge Industries in Ithaca, NY. Temperature in these ponds is normally held between 24 and 28C. Previous microscopic examination of the microflora of these commercial ponds showed several strains of Pa to be present, and that they were capable of invading and completely destroying spinach crops once stimulated to increased populations by the presence of spinach roots (Katzman, 2003). Virulent occurrences of what was most likely Pa appeared spontaneously in two of the un-inoculated control conditions in earlier experiments of the current project and solution samples from these sources were incorporated in the pond nutrient solution of the challenge experiment, in addition to inoculum from the commercial production.
pond. (The spontaneously appearing disease entities may or may not have been identical to the pure strain used throughout the research; in any case they had shown themselves to be highly effective in rapidly destroying spinach crops at warm temperatures.)

**Methods**

The first challenge experiment was conducted between mid April and mid June of 2006 using the spinach cultivar Whale, and a 15-day in-pond duration. Two ponds were used, each inoculated with pathogen-laden solutions as described above. After inoculation the ponds were maintained at a warm temperature (27.5°C) and spinach crops were rotated through them to foster the disease process. After disease was proven self-sustaining in both ponds, one of the two ponds was randomly selected to receive the experimental treatment and the temperature of the nutrient solution was reduced to 20°C in this pond. This occurred 5-12-06. Temperature was continued at 27.5°C in the infected comparison pond. An uninfected cool-temperature control pond was not needed, in view of having an abundance of performance data for disease-free crops grown under similar conditions in other experiments.

Detailed data collection began in the harvest following application of the treatment, namely 5-15-06, one month or 10 harvests after inoculation. It continued for 13 more crop harvests through 6-23-06. When it became apparent the recovery process was stalled, with a persistent self-sustaining disease process remaining in the roots, the experiment was discontinued.

**Results**

Figure 6.1 shows a fast recovery in biomass productivity when the temperature was changed, then a decline to moderate productivity with some up and down performance. No recovery occurred in the control condition in which the temperature was continued warm. Not shown in the numerical data is that widespread disease of the roots was present throughout the month-long test period in the treatment condition, even if the disease process allowed considerable root and shoot growth and did not devastate the plants to the extent it did in the control condition. (Roots and shoots were photographed in every harvest in which data were collected.) Also charted (Figure 6.2) is the percentage of plants observed to be wilted. Plants tended to wilt late in the crop cycle, in the final few days before harvest.

Early in the production system recovery period, the number of wilted plants in the harvest fell from a starting point of 100% of all plants before treatment (not graphed) to 5 percent in Harvest 7 after treatment, but crept back above 70% by the end of the experiment (Harvest 14 after treatment). DW productivity, DW:FW ratios, and root biomass data support these findings (Figures 6.3, 6.4, 6.5 and 6.6). In healthy crops at 20.0°C, one would expect DW:FW ratios below 0.05. In this experiment, after treatment, DW:FW ratios
made a dramatic descent from 0.40 to 0.054, but the ratio then trended upward in the latter part of the experiment, indicating increasingly water-stressed or wilted plants (see Figures 6.4 and 6.5). Following the same pattern, root DW drifted down in the latter part of the experiment after recovering from near-zero mass at the start of treatment (Figure 6.6). Shoot DW productivity (Figure 6.3) remained relatively steady, although at no point did it reach the value expected of a healthy crop (approximately 16 g m⁻¹ d⁻¹).

Figure 6.2. Wild-culture challenge experiment 1. Wilting response of diseased spinach crops to reduction of pond temperature. Cultivar Whale, in-pond duration 15 days
Figure 6.3. Wild-culture Challenge Experiment 1. Dry weight productivity. Response of diseased spinach crops to reduced pond temperature. Cultivar Whale, in-pond duration 15 days, +/- 1 SE.

Figure 6.4. Wild-culture challenge experiment 1. Dry weight: fresh weight ratio. Response of diseased spinach crops to reduced pond temperature. Cultivar Whale, in-pond duration 15 days. +/- 1 SE.
Figure 6.5. Wild-culture challenge experiment 1. Dry weight:fresh weight ratio. Response of diseased spinach crops to reduced pond temperature. Cultivar Whale, in-pond duration 15 days.

Figure 6.6. Wild-culture challenge experiment 1. Root dry weight per harvest response of diseased spinach crops to reduced pond temperature. Cultivar Whale, in-pond duration 15 days. Only exposed roots included.
**Discussion**

The steady dry weight performance and occurrence of wilting late in the crop cycle suggested the disease process had a fairly long reproductive cycle, thus enabling good early plant growth but diminishing growth later in their cycle. Probably only small amounts of inoculum were released by each crop, late in the crop cycle (just before removal from the system), to infect succeeding crops. However, the crops did wilt substantially and were poor in appearance despite considerable growth. Because the *Pa* organism is biological, any parameter that characterizes its life cycle is unlikely to be a constant. Instead, all parameters will show a probability distribution (perhaps normal) with a mean value but a spread to each side of the mean. This includes the cycle time from zoospore to mycelium to zoospore. The two-pond system described later in this report is a method to avoid appearance of the occasional outlier that occurs with a small, but finite, probability.

The failure of the treatment method to eliminate disease led to three lines of speculation as to why it did not work. The first was that the change of spinach cultivar, the inadvertent lengthening of the in-pond duration, and the change in type of pond used with a different solution flow rate might be sufficient cause. Secondly, it was possible that we encountered a new organism such a *P. dissoticum*, that could reproduce successfully in less time than the in-pond duration of the crop, or an outlier (as mentioned above) was the cause. Thirdly, it was possible that stresses imposed by mid-summer heat and periodical loss of aerial temperature control might have weakened the plants, making them more susceptible to *Pa* invasion.

In summary, despite biomass having recovered well, disease caused by the unknown mix of organisms introduced into the solution was not eliminated and it was unclear why. Even though biomass production was reasonably good, the crop was useless commercially for many plants were wilted and unattractive.

**CHALLENGE EXPERIMENT 2**

**Introduction**

In view of the failure of the treatment method to eliminate the wild strain disease process in the first wild culture challenge experiment, a second sequence of trials was initiated with stricter adherence to conditions under which disease recovery took place in the original discovery research (i.e., the pure culture challenge testing experiments reported in Chapter 5). The cultivar Eagle was used, the in-pond duration was kept to exactly 13.75 days, and nutrient solution temperature was closely controlled during fluctuating greenhouse heat loads throughout the experiment. We stayed with the deep, slow-flow, production system, however.
Methods
The experiment was run between mid May and mid July, 2006. In a pond in which a self-sustaining disease process had been established at 27.5°C, (the infected, warm-temperature control pond of the previous experiment) temperature was reduced to 20°C on 6-17-06. Data collection commenced on a limited basis immediately, then detailed data were collected in every harvest from 6-29-06 to 7-19-06, when the experiment was terminated. Because it had been previously well established that, in a warm temperature background, pathogens in this mix reduced crop productivity to virtually nothing, a warm temperature diseased control condition was omitted.

Results
Results were very similar to the first Challenge experiment, although this time recovery was not as large and the decline after recovery was faster. As can be seen in the figures following, peak recovery occurred in the sixth harvest after the treatment was applied. Fresh and dry weight productivity values were a maximum and wilting was a minimum (Figures 6.7, 6.8 and 6.9). The DW:FW ratio momentarily reached 0.05, an almost normal value for a healthy crop (Figures 6.10 and 6.11). (Root data were incomplete and were not graphed, but also trended downward after harvest 7; see Table 6.2.) Interestingly, dry weight productivity did not decline rapidly although fresh weight did. Photosynthesis was apparently not affected greatly although more wilting made the crop unmarketable and explains the noticeable fresh weight reduction. (A DW:FW ratio of 0.07, Figure 6.11, indicates noticeable wilting. The ideal is 0.04 to 0.05 and an increase from 0.05 to 0.07 with the same DW means the FW has decreased by nearly thirty percent.)
Figure 6.7. Fresh weight productivity. Response of diseased spinach crops to reduced pond temperature. Cultivar Eagle, in-pond duration 13.75 days, +/- 1 SE.

Figure 6.8. Wilting response of diseased spinach crops to reduction of pond temperature. Cultivar Eagle, in-pond duration 13.75 days.
Figure 6.9. Dry weight productivity. Response of diseased spinach crops to pond temperature reduction. Cultivar Eagle, in-pond duration 13.75 days, +/- 1 SE.

Figure 6.10. Dry weight to fresh weight ratio. Response of diseased spinach crops to reduction of pond temperature. Cultivar Eagle, in-pond duration 13.75 days. +/- 1 SE.
Discussion

Three hypotheses are listed above as possible explanations of the limited success of the first two wild culture experiments. One was explored further. To explain these data it is plausible, and in accordance with literature on spinach disease, to speculate that a different set of microorganisms came to dominate the microbial ecology when the temperature changed from 27.5 °C to 20 °C, and perhaps including some Pa58 pathogens that could reproduce successfully at 20 °C within the time allotted and, thus, sustain a disease process and persist in doing significant damage. For example, Stanghellini et al. (1984) and Bates and Stanghellini (1984) have shown that \( P. \text{dissotichum} \) completely takes over from \( Pa \) as the dominant pathogen harming spinach in solutions containing both pathogens when the temperature is changed from warm to cool.

That the pattern of recovery, followed by relapse, was so similar in the two experiments strengthens the hypothesis that a different set of pathogenic microorganisms came to dominate the microbial ecology when the temperature was changed abruptly from higher to lower, and that this strain of microorganisms was present in the "wild" inoculum sources added to the pond solution. Initial recovery could be attributed to preventing the high-temperature-adjusted microbial mix initially present from reproducing, due to the temperature change. The later relapse can then be attributed to a lower-temperature-adjusted microbial mix becoming more numerous, eventually dominant, and eventually capable of causing significant damage. In
each experiment sufficient time was available for the microflora population to adjust to the lower temperature following the change.

However, we can only speculate about the microorganism mix in the solution at either temperature, so this explanation is not certain. An alternative explanation is that *Pa* still predominated (or lower temperature outliers were selectively encouraged to reproduce) after temperature reduction, and it was not eliminated due to imposition of unusual environmental stresses. Both experiments were conducted in the heat of midsummer and aerial temperature control in the greenhouse was lost for several hours in the middle of the day on several occasions (hot, sunny, humid afternoons). Temperature control of the nutrient solution was good and improved as the experiments proceeded, but we may hypothesize that when aerial temperature was higher than intended, heat penetrated into the medium bridging the shoot and root zones, providing a suitable breeding ground for heat loving disease organisms such as *Pa*. Additionally, after these experiments were concluded we became aware that manganese concentration probably had been steadily falling throughout the experiments. We did not take note of chlorosis at the time, but nor were we looking for it. A developing deficiency could have contributed to the biomass trends found in this experiment.

On the other hand, although manganese deficiency could have exacerbated damage from the disease process, it requires additional assumptions to explain how it might have changed the length of the reproductive cycle of the predominant disease organisms, which evidently became shorter than the in-pond duration of the plants, for otherwise they would have disappeared.

In summary, despite the 1.25 day reduction of in-pond duration and the change of cultivars in this second experiment, the temperature reduction treatment method again failed to eliminate the disease process (although there was a rapid initial recovery). From a commercial point of view the crop was useless despite the initial recovery due to wilting and poor appearance.

**CHALLENGE EXPERIMENTS 3 AND 4 OVERVIEW**

In view of the failure of the initial two wild-culture challenge experiments to eliminate root disease using 15 or 13.75 days in-pond durations, and in view of the possibility that a pathogen or set of pathogens that we had not previously encountered was present in the solution (which was capable of reproducing within these durations) we decided to intensify measures to eliminate the disease. The two most obvious options were to lower the root zone temperature further or to shorten again the in-pond crop duration. However, if the problem derived from organisms with lower temperature optima than *Pa*, lowering the temperature further would be unlikely to lead to success and, in addition, might hurt productivity and increase production costs. We decided first to explore the second option, shortening again the in-pond crop duration.
The literature suggests that pathogens such as *Pythium dissoticum* that flourish and become dominant in cool temperatures are not as destructive as those operating at higher temperatures (such as *Pa*). This may be a temperature effect on rate of biochemical processes of all kinds, including reproduction. Even at 31°C, Katzman (2003) demonstrated *Pa* needs approximately six days to reproduce *in vivo* (from free swimming zoospore to free swimming zoospore). We reasoned that even if nutrient solution temperature was near optimum for some pathogens at 20°C, they would still need considerable time to complete their reproductive cycle if they were at all like *Pa*. By reducing the in-pond duration sufficiently, it should be possible to prevent even low-temperature-adapted disease organisms from reproducing in time to become established. Accordingly, the production process was split between two ponds, with each crop passing sequentially through the ponds. This halved the time plants (and associated mycelium) were in any given pond. For convenience we decided on a 12-day crop cycle, which meant harvest of the old crops and flotation of the new would occur on the same days. Thus, all root material would be removed from each of the ponds every six days and harmful pathogens would need to reproduce faster than six days to become self-sustaining in any given pond. (It is certainly possible to produce sufficient leaf size in twelve days by application of additional light, if need be; the crop is close to sufficient size in twelve days under 17 moles m⁻² d⁻¹ already.) We designated this experiment Wild-culture Challenge Experiment 3.

If the damaging species and strains of pathogens included in the wild culture micro-organism mix behaved in a way similar to *Pa*, they would require a minimum length of time to reproduce in the ponds, which we could determine experimentally. The first two experiments suggested this time was less than 14 days, and it seemed likely the time would be longer than six days. In our final challenge experiment (number 4) we began the process of pinpointing the minimum time required. That is, in Wild-Culture Challenge Experiment 4 we tested whether diseased crops could recover health using a nine day in-pond duration.

**WILD CULTURE CHALLENGE EXPERIMENT 3**

**Methods**

A self-sustaining disease process in one pond (hereafter pond 1) was created in the same manner as in the first two challenge experiments, while at the same time a second pond (pond 2) was prepared with a healthy crop. Both ponds were operated on a 14-day in-pond crop duration, with crops entering and leaving on a three day schedule. When ready to launch the experiment, the oldest flats containing the most highly diseased plants were transferred from pond 1 into pond 2. We considered this inoculation process a valid and stringent test of what might happen in commercial production. After this initial redistribution of crops, flats were transferred from pond 1 into pond 2 every three days, following a normal production schedule. New flats were fed into pond 1 to occupy the places opened up by the transferal of floats to pond2. The first two cohorts of flats to leave pond 1 after the initial rearrangement were diseased, as evidenced by observed root condition. Their addition to pond 2 can be considered additional inoculation.
Temperature set point was raised to 27.5 C in pond 1 on 6-23-06 to bring the disease process to a maximum state. By 7-15-06 crops were reduced to virtually zero productivity (as recorded photographically). Pond 2 was established during the same time period, and a healthy crop-production cycle established in it at 20 C by mid-July using a 14-day cycle. The cultivar Eagle was used. On 7-15-06, temperature was dropped in the highly diseased pond 1, and its contents rearranged for a two-pond production system. Older flats in the diseased pond were transferred to pond 2 so that the oldest flat in pond 1 from then on would never be older than 6 days, and the oldest in pond 2 never older than 12 days nor younger than 6 days. To keep roughly the same ratio of plants to nutrient solution volume, the crop size was subsequently doubled in both ponds i.e., two flats of the same age were floated and harvested every 3 days after a transition phase.

Harvests for the twelve days in-pond crop schedule commenced on 7-17-06. Detailed data collection was begun 8-1-06, after the temperature reduction treatment and shortening of the in-pond duration began to show effect, and was continued on a three day-schedule for nine more harvests to 8-27-06. Root condition was also closely examined.

No control conditions were used. At the end of the experiment, two crops were left growing in the ponds an extra 1.75 days, for 13.75 days in-pond duration. Performance of these crops was used as an indicator of the level of productivity and health of the precondition system. At the time of the final harvest, crops of all ages were simultaneously harvested and used to develop a growth curve.

**Results**

In evaluating the temperature reduction effect on the disease process it helps to remember that there were four infected crop cohorts in place at the time of temperature reduction, cohorts 1 and 2 in pond 2 and cohorts 3 and 4 in pond 2, which were harvested in harvests 1, 2, 3, and 4, respectively, after application of the treatment. Crop cohorts 5, 6, and subsequent cohorts, entered pond 1 clean.

In the sixth harvest following temperature reduction (8-1-06, cohort 6), the roots appeared completely disease-free in one of the pair of flats harvested but showed some minor infection in the other flat. Cohort 6 was the second new crop cohort to enter the production system after the temperature reduction treatment began and it was expected to show some root infection if cohort 4, the last original infected cohort, released zoospores before it was removed from pond 2 in harvest 4. Immediately after harvest 4, cohort 6 was transferred from pond 1 to pond 2, so this eventuality was plausible. Zoospores could well have been alive and motile in pond 2. Another route by which the cohort 6 could have become infected would be if cohort 5 became infected when originally put into pond 1 (possible if the older infected flats were releasing zoospores) AND itself released zoospores during the 3-day interval it overlapped with cohort 6 in pond 2, when it would have had 9 to 12 days in which to breed more zoospores. Roots in the seventh harvest
(cohort 7, the third new cohort to enter the system, harvested 8-4-06) appeared to be completely clean, and this was true for all subsequent harvests/cohorts.

Biomass production decreased for four harvests following the six cohort, harvested on 8-1-06, despite the roots remaining completely healthy (in appearance) and without there being any wilting (i.e., harvests 7 through 10). (See Figures 6.12 and 6.13.) It appears this was due to manganese (Mn) deficiency, rather than root disease, for the following reasons. At the outset of detailed data collection in this experiment (8-1-06, harvest 6 after the application of temperature reduction), leaf chlorosis was observed and noted despite the roots of affected plants appearing completely disease free. Pond 1 had been in continuous production for 15 weeks by this time, pond 2 for 6 weeks. Through elemental analysis of solution samples taken from the ponds, results showed manganese was severely depleted in pond 1 and at half strength in pond 2, suggesting the most likely cause of the chlorosis was manganese deficiency. Corrective steps were initiated on 8-10-06 after harvest 9 was completed. All signs of chlorosis disappeared rapidly and biomass production dramatically increased, suggesting the diagnosis was correct. Because Mn deficiency affected productivity level in earlier harvests, particular emphasis should be placed on the final two harvests. (From this chain of events, we concluded the nutrient solution composition and manner of solution maintenance we had been using in continuous production in this particular pond system led slowly to Mn deficiency, and the effect of Mn deficiency in spinach was manifested in reduced growth and leaf chlorosis. This is a problem relatively easy to fix, and it will be investigated further and fixed.)

Figure 6.12. Wild-culture challenge experiment 3. Fresh weight productivity (12 day production cycle) in a 2-pond system. Cultivar Eagle, in-pond duration 6 days each pond, temperature 20C, +/- 1 SE. Crops 7-10 were Mn deficient. Correction made at harvest 9.
Because of the problem with Mn deficiency, biomass data in this experiment were not a reliable indicator of root health at all times during the experiment. Mn deficiency also appears to have affected the DW:FW ratio (Figures 6.14 and 6.15) and Shoot:Root ratio (Figure 6.16). However, when both the disease problem and the Mn deficiency had been addressed, the ratios became typical of healthy crops.

At the end of the experiment, two crop stands from different cohorts were continued on for 13.75 days in-pond growing time and comprehensive data were collected for all ages and stages of the crop, by which to compare biomass performance to fully healthy crops in other production systems. Productivity of the full-term crops was 290 and 335 g m\(^{-2}\) d\(^{-1}\) (see Figure 6.17). As recorded in Chapter 5, productivity following recovery from disease in the two pure-culture challenge experiments was approximately 340 g m\(^{-2}\) d\(^{-1}\). In view of the expectation that transfer between ponds likely causes some root shock and reduced productivity, the productivity achieved after recovery from disease in the two-pond cultural system was respectable. In previous work using the same pond system, 330 g m\(^{-2}\) d\(^{-1}\) was considered a reliable achievable productivity for this size of spinach plant. There are some differences in the manner of calculation of productivity in the two instances, but the values are close and, thus, these results are typical of healthy spinach crops.
Figure 6.14. Wild-culture challenge experiment 3. Dry weight to fresh weight ratio, 2-pond system. Response to splitting crop cycle between 2 ponds, with temperature reduction. Cultivar Eagle, in-pond duration 6 days, +/- 1 SE.

Figure 6.15. Wild-culture challenge experiment 3. Dry weight to fresh weight ratio, 2-pond system. Response to splitting crop cycle between 2 ponds, with temperature reduction. Cultivar Eagle, in-pond duration 6 days, +/- 1 SE.
Figure 6.16. Wild-culture challenge experiment 3. Shoot to root ratio, DW basis. Response to splitting crop cycle between 2 ponds, with temperature reduction. Cultivar Eagle, in-pond duration 6 days.

Figure 6.17. Wild culture spinach fresh weight productivity as a function of growing time in a two pond production system.
THEORETICAL COURSE OF DISEASE ELIMINATION IN A TWO-POND SYSTEM

Consider the first, second, and third new crops entering the two-pond system, (cohorts 5, 6 and 7, harvested as 5th, 6th and 7th crops following establishment of the two-pond system and temperature reduction). Assume the pathogens doing damage require more than six days in which to carry out their reproductive cycle.

In the two-pond production system of this experiment, each pond only ever contained crops of two different ages. The initial four crops in the two-pond system originated from pond 1 when it was in self-sustaining disease mode and all were infected. The first new crop placed into pond 1 (cohort 5) could not be infected by zoospores released by the crop/flat already in the first pond (cohort 4), since in this flat the disease process would have had only 3 to 6 days in which to become infected and, thus, would not be ready to release zoospores before being transferred to pond 2. The same is true for the just-departed 6-day-old flat. It was transferred to pond 2 before it could release zoospores.

If the first new flat placed into pond 1 were to be infected, it would have to be by previously-released freely swimming zoospores. Crop rearrangement to launch the experiment occurred just two days before the first new flat was put into pond 1, so it is possible zoospores that had been released from older flats before rearrangement survived for long enough to infect the first new flat. However, this was likely to be a minor infection because zoospores are short-lived, and most would have already attached to roots or become encysted.

When the second new flat (cohort 6) entered the first pond, it was in the presence only of the first new flat and long after any zoospores had been released, so it had no chance of being infected in pond 1 in the normal way, and would enter the second pond completely clean – as would all subsequent flats. Thus we see that the first pond “cleaned up” for all subsequent crops entering it after just one clean new crop went through it. The statement generalizes to the second pond. It, too, should have “cleaned up” entirely for all subsequent crops after only one clean crop had passed through it.

To recapitulate: the first clean flat put into pond 1 (cohort 5) might get infected in pond 1 and become further infected in pond 2. The second new flat put into pond 1 (cohort 6) would enter pond 2 clean, but might be infected in pond 2. The third new flat (cohort 7) would not get infected in either pond and we would expect to be completely clean at harvest. The results fit with this explanation. Cohort 7 and all subsequent crops showed no signs of root disease.
WILD CULTURE CHALLENGE EXPERIMENT 4

Introduction
In testing the two-pond system, we had little doubt that the disease process would be interrupted because we thought it unlikely pathogens causing the damage would reproduce faster than in 6 days. Even at warm temperatures, *Pa* is unlikely to reproduce that quickly *in vivo*. In an effort to bracket more closely the in-pond duration required for the reproductive cycle of the unknown pathogen/s in the wild culture nutrient solutions, an experiment was undertaken in which the in-pond duration was nine days. If the plants recovered, it would mean the reproductive cycle was between nine and fourteen days.

Methods
The fourth wild culture challenge experiment was an extension of the second wild culture experiment. When the wild culture challenge experiment number 2 was deemed to have failed to show recovery from disease, the crop cycle was reduced from 13.75 days to 9 days by removing the older flats and adopting an earlier harvest time (enacted 7-21-06). Remaining flats were 1, 4, and 7 days old). The experiment was initiated on 7-21-06 by removing flats older than 9 days (in the pond), leaving flats of age 1, 4, and 7 days in the ponds. The oldest cohort the remaining flats had been exposed to was approximately 13 days old, so some infection could have happened. Two days later (7-23-06) the oldest flat, now 9 days old, was removed and the first new clean flat added under the new harvest schedule. Harvests and flotation continued but data collection was omitted during the recovery phase. Data collection commenced 8-1-06 when the first new flat in the system came due for harvest, and continued until 8-25-06. Dry weight data were not obtained in this experiment, except in the final harvest.

Results
Roots were perfectly clean and white in the fourth harvest after switching to a 9-day in-pond crop duration. This harvest represented the first clean crop to enter the system after the switch. Roots remained clean in all further harvests, except for one dark root in one plant two harvests later. We judged this to be a different phenomenon than typical root disease caused by *Pythium* species.

The production system used in this experiment had been in use as long as the production system of pond 1 in wild-culture experiment 3, and manifested Mn deficiency as leaf chlorosis at the same time as it was found in the two-pond system of experiment 3 (if anything, worse, because in the two-pond system plants found some relief when they moved to the newly constituted second pond at age 6 days). After adjustment of Mn on 8-10-06, shoot appearance and biomass recovered, as can be seen in Figures 6.18 and 6.19.
Figure 6.18. Challenge Experiment 4. Response of diseased spinach crops to shortening of crop cycle: Fresh weight productivity for a 9-day in-pond duration in-pond duration shortened after harvest 11. Manganese concentration adjusted after harvest 16.

Figure 6.19. Challenge Experiment 4. Response of diseased spinach crops to shortening of crop cycle: Fresh weight productivity in a 9-day in-pond duration. Manganese concentration adjusted after harvest 5.
**Discussion**

As can be seen in Figure 6.18, productivity in the final three harvests of the experiment averaged approximately 150 g m\(^{-2}\) d\(^{-1}\), achieving in nine days what had taken fourteen days when lingering disease was present before shortening the in-pond duration. (See Figures 6.7 and 6.18.) With reference to the growth curve presented in Figure 6.17, derived from data obtained in the third wild-culture challenge experiment, one might have expected a slightly higher productivity – of approximately 159 g m\(^{-2}\) d\(^{-1}\). Without further experimentation we cannot explain the reason for the slightly lower than expected productivity. It could have been a position effect, it could have been a lingering asymptomatic disease process, or merely within the margin of error of the experiment. After experimental data collection was terminated, the younger crops remaining were continued on until the production system was emptied of crops, and some crops were retained in the pond for durations of 18 and 19 days. Root conditions of these crops were closely observed and bulk shoot production measured. No signs of disease were present in either root or shoot. This result is of considerable interest because the immediate question that occurs is: “Does the disease entity go away or is it poised ready to come back at the first opportunity?” We can at least say it appears to require some considerable time to re-manifest.

In conclusion, it appeared that a full recovery to healthy roots took place and was sustained by reducing the crop cycle to 9 days. Biomass production recovered completely or nearly completely. We conclude the critical time required for the Challenge microorganism mix to reproduce and sustain itself in the pond system is probably longer than 9 days and shorter than 14 days.

**DISCUSSION AND CONCLUSIONS: ALL WILD-CULTURE CHALLENGE EXPERIMENTS**

This set of experiments showed how critical in-pond duration was for disease control in crops exposed to the wild-culture challenge microorganism mix for longer than 9 days but shorter than 14 days. There was not sufficient time within the project to pinpoint, more exactly, the critical in-pond duration for disease control. As it is, there appear to be no rules to decide, a priori, whether a single-pond or two-pond system will be more satisfactory for general production of baby spinach. If, for instance, one could reliably control disease using a 13-day in-pond duration, then a single-pond system might be more desirable than a two-pond system to avoid the work of transferring plants between ponds. (Sufficient leaf size can be obtained in this length of time without excessive demand for supplementary lighting.)

On the other hand, a two-pond system proved to be very effective in eliminating disease quickly and the mid-term transfer between ponds did not appear to set back crop productivity greatly. It is easy to conceive of a two-pond production system in which crops dwelled in each pond 7 days, for an in-pond crop duration of 14 days. In certain geographic locations it might be profitable to grow spinach in a two-pond system.
using a longer crop cycle, such as 9+9 days, to reduce investment and operating costs required for an extensive installation of supplementary lighting equipment.

Alternately, an asymmetric production schedule wherein a first pond holds the crop for perhaps nine days, followed by a three-day, second pond with limited nitrate in the nutrient solution to reduce plant tissue nitrate and oxalic acid concentrations for greater human health reasons. Previous work (Johnson, 2000) at Cornell has shown this protocol can be implemented to produce spinach with greatly reduced nitrate concentrations.

Another intriguing possibility of multiple-pond production systems is that higher pond solution temperatures, perhaps 23 to 24 C, might be tolerable, eliminating the need for any major cooling of the pond solution. It is possible that no species of *Pythium* can complete its reproductive cycle in a pond system in seven days at 24 C. A two-pond system with seven days in each pond and solution held at 24 C would provide sufficient time to grow baby spinach crops with minimal requirements for cooling and, in certain cultivars, biomass production would be enhanced.

Regardless of the ultimate answers to these questions, coordinated management of disease reproductive cycles and crop in-pond duration appears to be a robust and versatile method to control root disease in baby spinach.
CHAPTER 7
TASK 7: ANALYSES, DISCUSSION AND CONCLUSIONS

EFFICACIES OF THE PROPOSED TREATMENT PROCESSES

**Electrochemical Disease Control**

This method proved to have no promise as a *Pythium* control method. Even though the oxidative-reduction potential could be driven to a very low value, as well as pH, zoospores survived in sufficient numbers to cause unacceptable damage. Additionally, the electrical energy required to achieve these (inadequate) conditions was excessive and heated the solution excessively.

**Ultraviolet Radiation**

Ultraviolet radiation has been used for nearly a century to treat drinking water in the U.S. The U.S. Department of Health, Education and Welfare established guidelines (1966) of a minimum acceptable treatment dose of 16 mJ cm\(^{-2}\) for UV light disinfection of drinking water. Other standards, such as the National Sanitation Foundation International set the minimum at 38 mJ cm\(^{-2}\). Each of these standards, in reality, apply to visually clear water, for particulate matter or light absorbing pollutants in the water can block the UV radiation. Pre-filtration to at least 5 micron size is part of the standard UV system design. In the bench top study, UV treatment following filtration down to 1 micron particle size provided a dose equivalent to 100 mJ cm\(^{-2}\), well above drinking water standards. In spite of this intensity of treatment, root damage was reduced by no more than 93 percent. Complete sterilization by UV is never guaranteed, which creates a problem in trying to prevent any *Pa* outbreak with spinach in continuous, deep-trough hydroponic production because lingering zoospores can reproduce in the production system and create an endemic infection, or worse. Therefore, a reasonable conclusion, based on these results, is that UV treatment for *Pa* control in deep-trough spinach production is not to be recommended.

**Pasteurization**

Heat treatment provided nearly, but not totally, complete reduction of *Pa* damage to spinach roots. Residence time of at least one minute, and heating to at least 60 C, provided the best results. However, the energy required to provide heating to this temperature (even with heat exchangers to recapture most of the applied energy), coupled with incomplete control, makes pasteurization an inadequate treatment method for deep-trough hydroponics.

**Sonication**

Sonication showed some promise as a method to eliminate *Pa* damage to roots. The best results were achieved with an amplitude of 120 µm and the lowest flow rate tested, 200 ml min\(^{-1}\), although satisfactory results were also achieved with an amplitude of 150 µm. However, to deliver an effective dose of sonic
energy, our results indicate that flows greater than 200 ml min$^{-1}$ are not recommended for this size of generator. The energy input was large to accomplish $Pa$ control even at this very low flow rate. With the small sonication unit tested, 250 W input of electric energy was needed. Sized to commercial production and treatment, the conclusion was that energy concerns made sonication inappropriate regardless of its $Pa$ treatment efficacy.

**Aeroponics**

Although it is possible to produce baby-leaf spinach in an aeroponic system, it was clear that the biomass yield of spinach grown aeroponically was much less than that in the deep flow system. Approximately three days after emergence it became apparent that the aeroponically grown spinach was lagging the plants in the shallow flow system. At final harvest the average plant weights of the aeroponic spinach were 27% less. This biomass decrease was thought to be due to root mass drying within the cells of the flats. In floating hydroponics, the bottom of the flat is submerged and considerable wicking of nutrient solution up into the Redi-Earth keeps the root mass suitably moist. However in the aeroponics system this wicking either did not occur, or did not occur to the same extent as in the floating system.

Additionally, the original hypothesis that $Pa$ infections could not spread readily from root mass to root mass in an aeroponic system was not confirmed by experiment.

**THE TEMPERATURE REDUCTION METHOD**

Several factors combined to make the root zone temperature reduction method successful. They are based on the biology of $Pa$. *Pythium aphanidermatum* reproduces by a multi-step process that is temperature dependent. Zoospores infect root tissue, creating a vegetative state, mycelium, that feeds on and eventually destroys the root tissue. After some period of time, the mycelium begin to shed new zoospores that are motile and spread to new plant roots, where the cycle is repeated. The time period from zoospore to zoospore depends on temperature. When this process was quantified, the possibility was opened of using root zone temperature as a treatment method.

Baby spinach is typically harvested no later than 15 days from seeding in the production method used for these experiments. Harvest can be sooner, but an optimization process intrudes. The relative growth rate of spinach increases rapidly between 12 and 15 days, and continues to increase for some days after (although leaf size become unacceptable). Thus, to harvest early is to lose growth and profit. $Pa$, on the other hand, shows an average reproduction cycle time of approximately 15 days at 20 C. Once new zoospores are released, infection spreads unchecked. Biological variability suggests plants should, in fact, not remain in the same nutrient solution for more than several days less than 15, the mean reproduction period. Extended experiments showed two possibilities. One was to remove the plants several days before 15 days of age,
which removed any infected roots and prevented disease spread. Another was to consider a two-pond production system where residence time in any one pond would be nine days or less, preventing disease cycling. The second method requires careful system design to coordinate pond sizes to assure they are filled at all times, but not difficult to do. Additionally, the concept lends itself to possible operation wherein the second pond is devoid of nitrate in the nutrient solution, permitting production of a crop with no nitrates in the leaf tissue, and lowered oxalate content.

ENERGY IMPLICATIONS

Electrochemical Disease Control

This method of *Pythium* control proved to be so ineffective that thorough analysis of the energy implications is worth little. However, at the highest efficacy tested, approximately 35% damage reduction in the bench tests (Chapter 4), 70 watts of power for 15 minutes were needed to treat one liter of water. Extrapolating this to a CEA facility such as the lettuce greenhouse operated for several years by Cornell (www.cornellcea.com), with a surface area of 6400 ft² and a spinach shallow flow pond depth of 3 inches (7 cm), a total of 1600 ft³, or 12,000 gallons, would require treatment, perhaps on a daily basis. If the bench top test data is applied, where 17.5 watt-hours were needed to treat 0.264 gallons (or 66 watt-hours per gallon), the energy need is calculated as $(0.066)(12,000) = 790$ kWh daily, or 290,000 kWh/year. This would be a significant production cost, with little benefit and, in reality, a greater cooling load because of the temperature of the nutrient solution after treatment.

Temperature Reduction Method

The primary energy concern related to the temperature reduction method is chilling the nutrient solution. The analysis shows this to be a very limited energy burden.

The energy cost is not to chill the water itself – fresh water added to a deep trough system will almost always be colder than the target of 20°C nutrient solution temperature. The amount of water added daily will be small, which will provide minor cooling of water already in the ponds. As a perspective, fresh water needed for butterhead lettuce production in the demonstration greenhouse operated for several years by the Cornell CEA program was 4 mm (1/6 inch) per day, reflecting transpiration from a relatively dense canopy. Spinach produced in baby form will transpire less, for it has noticeably less canopy and smaller leaf area index. For every mm of water added, entering perhaps at 20°F (at the most) below pond temperature (68°F), the daily energy required to heat the water, $Q$, will be approximately

$$Q = m c_p A t = (0.2 \text{ lbft}^2)(1.0 \text{ Btu lb}^{-1} \text{F}^{-1})(20 \text{ F}) = 4 \text{ Btu ft}^2 \text{ of pond area}$$
Two energy calculations are needed for system design. The first is maximum capacity of a chilling system, a value needed for design and which impacts demand charges. The second is the kilowatt-hours required to operate the system, reflecting average operating conditions. Each calculation requires a heat transfer analysis. For that, some assumptions are needed, as follow in Table 7.1.

Table 7.1. Assumptions for the temperature reduction method (inch-pound units)

1. Floats have insulation value equivalent to 1" of expanded polystyrene, thermal resistance of 5.26 per inch of thickness. Actual floats are more than 2" thick, but their thermal resistance is reduced by conduction through the root medium. Assume no added thermal resistance due to the crop canopy.
2. Average pond temperature is 68 F (20 C).
3. Average greenhouse air temperature is 75 F in summer and 72 F in winter. Set points are 75 F day and 69 F night.
4. Production area is 6400 ft², equivalent to the lettuce greenhouse operated at Cornell.
5. Pond edges are 1 foot high and made of 4" of concrete, having an R-value of 0.08 per inch thickness. The outside surface resistance (concrete to air) is assumed to equal half that of a vertical, 8' wall, or 0.34.
6. Heat exchange with the ground, even at the perimeter, is minimal due to reaching thermal equilibrium with the ground under the ponds soon after operation starts.
7. Electricity costs $0.10/kWh.
8. The chiller seasonal energy efficiency ratio (EER) is 10.

Heat gain from greenhouse air to the water, through the top surface and sides of the pond, is

\[ Q_{\text{surface}} = UA \Delta t = (A/R) \Delta t = (6400/5.26)(72 - 68) = 4900 \text{ Btu/hr} \]

\[ Q_{\text{side}} = UA \Delta t = ((400/(0.32 + 0.34))(72 - 68) = 2400 \text{ Btu/hr} \]

for a total of 7300 Btuh (Btu/hr), or approximately 2.1 kW.

Added to the heat gain by conduction will be heat added by the recirculating pump. In the lettuce greenhouse that is the model for this example, four, 3 HP pumps operate continuously to recirculate nutrient solution, adding oxygen and sometimes pH control and nutrient concentrate, as well as assuring uniform conditions in the root zone. A conservative estimate is that all of the energy into the pumps is added to the water, either through direct contact with the pump or turbulence decay in the recirculating water. Pump
efficiency has not been directly measured but, to be conservative, 3 HP of pumping capability could require 3 kW input, leading to a maximum possible cooling load of

\[
Q_{\text{pump}} = (4 \text{ pumps})(3 \text{ kW/pump})(3412 \text{ Btu/h/kW}) = 41,000 \text{ Btu}
\]

The total heat input is, thus, 4900 + 2400 + 41000 = 48,000 Btu, or 14 kW. With an EER of 10, this translates to an input (continuous) power of 1.4 kW to the chilling unit. Operating continuously, this is 34 kWh/day, at a cost of $3.40/day at the assumed conditions. The pump is the major contributor to the cooling load, meaning that calculations for a warm summer day (increased heat conduction) will make little difference. Moreover, a chiller drawing less than 1.5 kW will have little effect on demand charges. Finally, viewed as a yearly cost per square foot of production pond area, the cost would be approximately $0.20/year. This value is based on a number of conservative assumptions and, even at that, is a small expense, which suggests nutrient solution chilling cost in a deep trough production system will be small compared to other production costs. Finally, the opportunity exists to capture heat rejected from the chiller to preheat water used for other purposes in a facility.

SUMMARY AND CONCLUSIONS

The objective of this work was to develop an energy-efficient and operationally-effective disinfection method to treat recirculating nutrient solutions for hydroponic spinach production. The principal organism to be eliminated was identified as Pythium aphanidermatum (Pa). A hydroponic spinach production system based on deep-trough hydroponics had been developed previously at Cornell and tested thoroughly on a research scale. Plant growth appeared to be suitable for commercial production at a profit except for one aspect, the need for extremely thorough and time-consuming cleaning between (batched) crops because of a continuing danger of devastating Pa outbreaks. The cleaning process between crops added significantly to production costs, obviated continuous production, added an unacceptable degree of risk and, even at that, was not guaranteed to prevent all disease outbreaks.

Three water disinfection methods were proposed for possible application to continuous production in deep-trough systems, electrochemical disease control, ultraviolet radiation, and pasteurization heat treatment. At the time of the original proposal, complete sterilization was thought not to be desirable because it eliminates potentially useful microbes. Additionally, a comparison of deep-trough hydroponics and aeroponics for spinach production was included for evaluation, as was evaluation of a sonication unit thought perhaps to destroy the fragile Pa zoospores.

Heat treatment using a heat exchanger for better energy efficiency was to be the standard; research literature suggested a residence time of two minutes at 55 C provides 100% lethality for Pythium.
Ultraviolet (UV) irradiation is a standard water treatment method but can be a problem in hydroponic solutions. Lethality may be incomplete due to shading of organisms by floating debris (thus, filtration normally included in the system). Moreover, UV radiation interacts with recirculating hydroponic solutions to precipitate certain plant nutrients, particularly iron. However, it was included due to its popularity and wide acceptance. Electrochemical water treatment was an experimental treatment technology and not widely applied to hydroponic production systems. Primary application has been for pH control and removing certain unwanted ions, but the method can reduce the oxidative/reduction potential to the point where microbes may be destroyed.

Research often leads into unexpected territory and this project was no exception. None of the proposed treatment methods proved suitable as a means to eliminate of Pa damage completely. Instead, as understanding of the Pa life cycle increased, a new method was developed based on the simple concept of controlling the nutrient solution/plant root zone temperature. This proved able to defeat Pa infections and even to cleanse already-infected systems. Root zone control to 20 C, with very short crop cycles, was the key. This method is suitable only for deep-trough, not NFT, production. Additionally, the method must be accompanied by supplemental lighting to assure rapid production, in addition to accurate daily light integral control such as provided by the algorithm previously patented by Cornell (Albright, et al., 2000). The method appears ready for evaluation in spinach production systems on a scale that reflects potential commercial applications.
REFERENCES


## SYMBOLS AND ACRONYMS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Area, ft²</td>
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<tr>
<td>CEA</td>
<td>Controlled-Environment Agriculture</td>
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<tr>
<td>(c_p)</td>
<td>Specific heat, Btu lb⁻¹ F⁻¹</td>
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<td>EC</td>
<td>Electrical Conductivity, mSiemens/cm</td>
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<td>EDC</td>
<td>Electro-chemical Disease Control</td>
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<td>DO</td>
<td>Dissolved Oxygen, PPM</td>
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<td>DW</td>
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<td>FW</td>
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<td>NFT</td>
<td>Nutrient-Film Technique</td>
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<td>NYSERDA</td>
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<td>(Pa)</td>
<td><em>Pythium aphanadermatum</em></td>
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<tr>
<td>PAR</td>
<td>Photosynthetically-Active Radiation, mol m⁻² d⁻¹</td>
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<tr>
<td>Q</td>
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<td>SD</td>
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<td>(\Delta t)</td>
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<td>U</td>
<td>Thermal Conductance, Btu hr⁻¹ ft² F⁻¹</td>
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<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>VDC</td>
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