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Technology Transfer, Education, and Applied Research from the Center for Excellence for Controlled Environment Agriculture at Cornell University

Final Report

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Final Report

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Abstract

This project included nine tasks centered on hydroponic production of leafy green vegetables. Each task is represented in a chapter of this report. Seven chapters focus on education and training and Web page improvement, in addition to six summer internships and 20 public workshops.

The eighth chapter involves research to expand the data base concerning interactions and control of the daily light integral and carbon dioxide concentration to make photosynthetic light more efficient in greenhouses, saving money and electrical energy.

The ninth chapter was research to demonstrate a production method readily adoptable by hydroponic greenhouse operators to grow hydroponic spinach while avoiding infection by the root disease, *Pythium aphanidermatum.* Controlling root zone temperature to 20 ºC and adhering to a strict production period of 13 days was successful in a year-long demonstration of deep flow hydroponics, using sequential hydroponic ponds where the plants were moved from a first pond to a second after seven days in the first, and harvested from the second during the fourteenth day from germination. Success was possible because the root zone temperature was low enough to slow the reproduction cycle of the disease organisms sufficiently that they were unable to reproduce and regenerate the disease in either pond. However, the nutrient solution temperature, 20 ºC, was not so low as to impair spinach growth significantly.

Keywords

Greenhouses, controlled environment agriculture, *Pythium aphanidermatum,* spinach, lettuce, carbon dioxide, light integral, hydroponics

Acknowledgements

Much is owed to Research Associate Dr. Timothy Shelford of the Cornell University Department of Biological and Environmental Engineering for his work to design, fabricate, instrument, program, and operate the minichambers used in Task 8. Margery Daughtrey, senior extension associate of the Long Island Horticultural Research & Extension Center, and Mary Ann Karp and Professor Eric Nelson, of Cornell's Plant pathology and Plant-Microbe Biology Section of Cornell's School of Integrative Plant Science are sincerely thanked for their assistance in providing Pythium samples and advice used to complete Task 9 of this project. Finally, many useful suggestions, questions, and different ways of thinking about all aspects of this project were contributed by Professor Emeritus Robert W. Langhans and Associate Professor Neil S. Mattson of the horticulture section of Cornell's School of Integrative Plant Science.

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Acronyms and Abbreviations

CEA	Controlled Environment Agriculture
CO_2	Carbon dioxide
[CO_2]	Carbon dioxide concentration (parts per million)
DLI	Daily Light Integral (mol/m^2)
DO	Dissolved Oxygen
IPM	Integrated Pest Management
EC	Electrical Conductivity (milliSiemens/cm)
HACCP	Hazard Analysis Critical Control Point
L	Liter
NFT	Nutrient Film Technique
P. a.	*Pythium aphanidermatum*
Pa58	*Pythium aphanidermatum* strain 58
RZT	Root Zone Temperature

Executive Summary

Envision that by the year 2020 a vigorous controlled environment agriculture (CEA) industry will emerge in New York State. It would advance plant-based agriculture in ways that greatly decrease dependence on imported horticultural crops and phytochemicals, while enhancing environmental quality; improving energy efficiency for food production, food safety, and security; reducing the carbon footprint of local food production, reducing need for nonrenewable energy resources; and providing year-round and rewarding career opportunities for workers.

The work reported herein addresses these issues through two sets of activities: education and training; and applied research and technology transfer. The work was under the direction of a tenure-line faculty member from Cornell University and included staff as educators as appropriate. Examples of staff included research associates, research support specialists, and graduate assistants. Examples of appropriate expertise include horticulture, engineering, economics, entomology, food science, plant pathology, microbiology, communication, plant sciences, and education.

Nine tasks were completed for the project. Each task corresponds to a chapter in this report. Chapter 1 defines administrative activities. Chapter 2 describes five student summer apprenticeships that were created and financially supported by the project. A sixth apprenticeship was added by request from a self-supported Cornell undergraduate, for which he received three academic credits as a special topics course. Each apprenticeship included introductions to technical subjects in a classroom setting as well as practical “hands-on” experiences. The required minimum length of training was six weeks, although most interns extended their experiences to as much as three months because of their interest in CEA. At least four of the five interns are now working successfully in CEA.

Chapter 3 describes a completely revised and updated CEA website (www.cornellcea.com), which was launched at the end of the first year of the project. New information continues to be added to the website as it becomes available. The website has been and continues to be a long-term commitment of the CEA program and leads to many positive responses and inquiries from users.

Chapters 4 through 7 describe 20 one-day workshops. Subject matter, and depth, depended on the audience. Greenhouse operators and potential operators were presented information in greater depth, with an emphasis on business and marketing aspects of greenhouse vegetable production. Workshops for

secondary school teachers were at the level of the intended student audience (high school, middle school, etc.) and included instructions on fabricating classroom hydroponic units, using instruments such as pH meters. Written materials were provided under the assumption that such teaching aids are unlikely to be readily available to teachers limited by budget restraints in their schools. Public CEA workshops drew substantial audiences, and a sixth workshop was added.

Chapter 8 describes work with optimizing growth of lettuce and spinach. The work grew from previous research and a patented algorithm to control CO_2 concentration optimally to make light (natural and supplemental) more photosynthetically efficient. The original patent considered only butterhead lettuce. Computer simulations in which the algorithm was implemented predicted nearly 50% savings of supplemental light in the cloudy climate of Ithaca. Greenhouse implementation of the algorithm confirmed the computer simulation prediction. The hypothesis of the final task was that vegetative crops such as spinach and arugula respond to CO_2 and light similarly to lettuce. This hypothesis appears to be adequate for leafy greens. More research is needed for confirmation, but preliminary results suggested only one experiment will be needed for each species and each cultivar of a species. This is fortunate because it means other popular vegetative crops are likely to behave similarly, so optimization of their CO_2 and light needs can be readily obtained through experimentation.

Chapter 9 describes work to extend previous CEA research on a difficult pathogen problem with spinach. Commercial hydroponic spinach production has not been successful in the U.S. due to the susceptibility of hydroponic spinach to the root disease *Pythium aphanidermatum* (*P.a.*). Previous research indicated that root zone temperature control could be a method to slow the *P.a.* reproduction cycle to the point where the spinach production cycle is shorter than the *P.a.* reproduction cycle. *P.a.* zoospores attach to plant roots so, if the spinach is harvested before the *P.a.* is able to reproduce, zoospores are removed and disposed of when the plants are harvested. Spinach growth with a root zone temperature of 18 ºC was not slowed perceptibly, while the *P.a.* reproduction cycle was slowed to be several days longer than the spinach seed-to-harvest period. The required production system was deep flow (raft culture) to enable careful root zone temperature control. Spinach is a 14-day crop. Using two ponds sequentially was found to be best. Transferring the rafts from the first to the second pond after seven days reduced the possibility of creating a *P.a.* strain evolved to reproduce in fewer than 14 days. A continuous production system based on this hypothesis (sequential seven-day ponds) was fabricated and operated for one year without a disease outbreak demonstrated this hypothesis.

1 Management, Reporting, and Technology Transfer

A Program Advisory Committee (PAC) was formed to provide advice on tasks primarily related to the internships and workshops. PAC members were:

- Dr. A.J. Both, Rutgers University
- Dr. Mark Bridgen, Long Island Horticultural Research Laboratory (LIHRL)
- Dr. Ray Cross, President, SUNY Morrisville (left after several months for a new job)
- Richard Peterson, NYSEG
- Dr. John Sager, NASA
- Dr. George Crosby, SUNY Cobleskill
- Dr. Preston Gilbert, SUNY ESF
- Viraj Puri, Gotham Greens
- Jennifer Nelkin (now Frymark), Gotham Greens
- Dr. Alan Taylor, Geneva Experiment Station
- Judson Reid, Cornell Cooperative Extension
- William Reinhardt, Project Manager (now retired), NYSERDA

Three yearly Program Advisory Committee meetings were held via teleconference mode. Develop and Conduct an Apprenticeship Program

The original project task list specified that Dr. Ray Cross of SUNY Morrisville would assist in identifying apprenticeship candidates. Dr. Cross subsequently accepted another job out-of-state and did not participate. In his place, Dr. George Crosby of SUNY Cobleskill assisted with identifying three suitable candidates. The other three students were chosen by the Cornell CEA team.

1.1 CEA Apprenticeship Lecture/Discussion Topics

- Types of greenhouses
  - Glass and types of glass
  - Plastic – inflated double poly and twin wall
  - Open roof
  - Fan vented – including roof, wall, distances between inlet and exhaust vents
  - High tunnels
  - Row covers
- Parts of a greenhouse
  - Shade/insulation
  - Insect screening
  - Pumps

- Cooling – pad and fan, mist
- Bench styles

- Hydroponic systems
  - Nutrient Film Technique (NFT)
  - Ebb and Flood
  - Aeroponic
  - Drip
  - Deep trough (pond)

- Plant nutrition
  - Nutrient solution meters (pH, electrical conductivity [EC], and dissolved oxygen [DO])
  - Fundamental chemistry background related to nutrient solutions
  - Solution testing and modification
  - Water quality – reverse osmosis vs. tap water vs. deionized vs. other
  - Charcoal (and possibly other) filters
  - Germination issues
  - Choosing a root medium for seeding

- Light
  - Supplemental lighting
  - Photoperiodic lighting
  - Effects of wavelength on morphology and flowering
  - Day length (night period) requirements of various crops
  - CO_2 supplementation as related to light

- Psychrometrics
  - Plant response to humidity – transpiration
  - Condensation – and disease potential

- Ventilation
  - Fans – types, efficiency, sizing, staging
  - Inlets – sizing
  - Pressure inlet control
  - Recirculation – horizontal air flow, paddle fans, Modine-type heaters

- Heating calculations and heating systems
- Combustion gases and other pollutants
- Energy conservation
- Power
  - Single- vs. three-phase electricity
  - Voltage
  - Current – and wire sizing by current capacity and voltage drop
  - Safety – fuses vs ground fault interrupters, for example
  - Electrical meters and continuity testers

- Pumps – types
- Solar angles – and solar intensity
- Business plans
    - Market analysis
    - Record keeping
- Human nutrition aspects of greenhouse vegetables
- Energy issues: imported vs. local
- Food miles and carbon footprints – positives and negatives
- Harvesting and packaging
- Health and safety, hazard analysis critical control point (HACCP), food safety issues
- Alternative growing systems:
- Aquaponics
- Vertical greenhouses and layered growing systems
- Plant factories and “abandoned warehouses”
- Rooftop greenhouses

1.2 CEA Apprenticeship Hands-On Activities

- Growing system construction
- Set up gutter system for NFT
- Build pond for deep trough
- Set up bag/bucket tomato system
- Nutrient solution management
    - Mix stock solution for nutrient solution – both with easy Hoagland’s recipe and individual salts recipe
    - Calibrate pH, EC, DO meters
    - Dilute acid and mix base
- Add appropriate acid/base for pH correction
    - Take water sample and nutrient solution sample and submit to labs for evaluation
    - Input lab results into spreadsheets and calculate necessary corrections
- Crop cultivation
    - Seed tomato, lettuce, and spinach into rockwool and other artificial media
    - Transplant into grow-out system
    - Daily observations of foliage and roots
    - Integrated pest management (IPM)-style scouting methods and treatments
- Greenhouse Maintenance
    - Furnace testing
    - Testing pump output
    - Fan maintenance (tension belt)

- Seal greenhouse with crack seal and smoker
- Screen cleaning
- Shade spray application/removal
- Evaluating lighting levels, changing bulbs, create a light map
- Measure greenhouse light transmittance

- Miscellaneous
  - Select pump, pressure regulator, header, drippers for drip system
  - Design of wet pads systems for evaporative cooling
  - Create scale model of greenhouse to be used to explore sun angles and shadow movement
  - Test voltage, amperage, and continuity.
  - Graphing pH/EC/temperature/light trends from notes and logger data

2 Expand and Update the Cornell CEA Website

A completely revised and updated website was launched at the end of the first year of the project. New information continues to be added to the website as it becomes available.

Because Dr. Albright retired, the website is now hosted by Cornell's Department of Horticulture under with oversight from Dr. Neil Mattson. Although the website is hosted by a Cornell academic department and can be accessed through that department's webpage, www.cornellcea.com remains active as the preferred entry to the CEA site.

3 Create and Present Public CEA Information Workshops

Location of Workshop	Date
Rochester, NY	Nov 18, 2010
Carmel, NY	February 1, 2012
Chemung County	February 28, 2012
Suffolk County	March 7, 2012
Syracuse, NY	April 10, 2012
Chemung County, NY	April 28, 2012

Limited demand from primary and middle school teachers and heavy demand for public information CEA workshops led to omitting one of the primary and middle school teacher workshops and adding a sixth public workshop. Permission to do was obtained from the NYSERDA Project Manager.

4 Create and Conduct Primary and Middle School Teacher Workshops

Location of Workshop	Date
Ithaca, NY	March 19, 2012
Ithaca, NY	March 22, 2012
Ithaca, NY	April 2, 2012
Ithaca, NY	April 5, 2012

Limited demand from primary and middle school teachers and heavy demand for public information CEA workshops led to omitting one of the primary and middle school teacher workshops and adding a sixth public workshop. Permission to do was obtained from the NYSERDA Project Manager.

5 Complete and Conduct High School Teacher Workshops

Location of Workshop	Date
Ithaca, NY	November 6, 2010
Oswegatchie, NY	June 27, 2011
Hudson, NY	February 2, 2012
Suffolk County	March 6, 2012
Newark, NY	April 4, 2012

Twenty-five kits were created to assist high school teachers to integrate information from the workshops into their science curricula. Kits contained pH and EC meters as well of small hydroponic plant production units that could be replicated by the teachers for their own classes.

6 Complete and Conduct Facility Operators Workshops

Location of Workshop	Date
Albany, NY	December 1, 2010
Syracuse, NY	January 25, 2012
Orange County, NY	February 9, 2012
Ithaca, NY	March 22, 2012
Rochester, NY	April 16, 2012

7 Develop Relationships Between CO_2 Concentration and DLI for Lettuce and Spinach

7.1 Seeding Protocol and Timing

The three crops were grown simultaneously for better use of time. The seeding sequence began with butterhead lettuce (cv. Salvius) and continued 48 hours later with spinach (cv. Space) and another 24 hours later with arugula (cv. Rocket). Every run for each crop was seeded in nine 5×8 (40 cell) Styrofoam flats. Flats were gently filled with Lambert LM-1 germination mix at a 3:1 moisture to dry matter ratio and seeded with two seeds for lettuce and spinach and three seeds for arugula. Flats were lightly covered with medium and sealed in plastic bags to retain moisture as the seeds germinated.

7.2 Germination

Bagged flats were stored three per tray under the bench in the nursery chamber out of direct light with plastic domes on top of the trays. Temperatures in the trays were held between 20 and 21 °C. Trays were rotated in the chamber at least once daily to account for the 0.5 °C temperature difference from front to back. Lettuce remained under the bench for less than 24 hours before being brought above the bench and floated in direct light in the nursery tub. Spinach spent 48 hours under the bench, followed by 24 hours above the bench in indirect light through its dome and bag before being floated in direct light in its tray. Arugula was given 18-20 hours under the bench before being floated in direct light in the nursery tub.

Unfortunately, repeated poor quality of the arugula seedlings made that data set basically useless. The seed source was inherited from the Finger Lakes Fresh greenhouse operation when they closed. It had had super germination, but a good percentage of the plants did not develop true leaves and did not grow normally. Early on, there was a fungal disease, which was fixed, but it did not solve the problem with the meristem tip. Thus, arugula results are omitted from this report.

7.3 Pre-Float

The nursery tub was a 200-gallon, 2 foot × 4 foot steel tub that floated the 9 lettuce and 9 arugula flats before their entry into the mini-chamber growth system. The tub sat atop a 3-foot × 8-foot plastic bench on stilts 3 feet above the ground. The bench was centered in an 8-foot × 12 -foot walk-in chamber. Spinach flats were floated in their germination trays. Plant growth solution used in the nursery tub was identical in make-up to that in the mini-chamber tubs. It contained a half strength Sonneveld solution formulated for lettuce, made from concentrated stocks with reverse osmosis water to an electrical

conductivity (EC) between 1,300-1,400 microSiemens/centimeter, and with pH adjusted to 5.8. The nutrient solution was made with reverse osmosis water. No temperature control facilities were installed other than Argus^{TM} control of the aerial chamber, controlled to 24 °C. This control kept the nursery tub stable at 22 °C. The Argus' light control program was set to deliver 15 mol/m^2/day in the nursery for each run.

7.4 Floating

On "float" day, plants were transferred from the nursery tub into the experimental system (see Figure 1.) At this point, all crops were reduced to one plant per cell (see Figure 2.) The thinning process favored mid-sized plants, and eliminated plants that were either too big or too small. If necessary, stand adjustments were included to reduce all crops to the same population of plants in the inner and outer rows. At this point, the seven most similar flats out of the nine flats of each crop were selected, and the remaining two flats were designated as nonexperimental, to be excluded from the analysis. (In each run, three mini-chambers were designated as experimental, all receiving the same daily light integral but different levels of CO_2 concentration.) Six of the seven selected crop stands were randomly assigned to a mini-chamber for which the experimental conditions had been predetermined. The seventh selected flat was processed at this time, and individual fresh weights were measured as well as one bulk dry weight for the entire flat.

Two walk-in growth chambers were equipped with two mini-chambers built for an earlier project. The mini-chambers were placed to take advantage of the bilateral symmetry within walk-in chambers in order to assure that equal light integrals were received from the overhead luminaire array. The mini-chambers were 2-feet by 4-feet. Each mini-chamber consists of an 8-inch high wooden base surrounded by a transparent plastic enclosure. The base holds a 15-gallon pond fitted with plumbing for circulation, cooling and aeration and also a variety of electronic gear and control equipment. The pond is covered by a plastic top with cutouts for six floats. Inside the plastic enclosure, an instrument mast holds several sensors aloft for CO_2, humidity, and temperature. It also holds up a horizontal air circulation fan and heater. The Plexiglas enclosure on top of the housing is sealed with a removable sheet of Plexiglas that rests on top of the enclosure and allows access to the crops. The long axes of the mini-chambers are transverse to the long axes of the walk-in chamber they are in, with the sets of three cutouts in the pond covers perpendicular to the long mini-chamber axis: one set close to the instrument mast the other at the far end of the mini-chamber. Crops in the row near the instrument mast were always used for the initial harvest, leaving the row closer to the right wall of the walk in for the final harvest. A flats role as either middle or final harvest was randomly determined by coin flip at float. Crops were arranged within each

mini-chamber in the same layout for each trial: two lettuce flats closest to the door of the walk-in in the mini-chambers closer to the door, two spinach flats in the middle, and two arugula flats near the center. This layout was mirrored in the back mini-chamber so that lettuce was closest to the back wall.

The mini-chamber size imparted limitations on the harvest day sizes of the lettuce and spinach. Each crop was grown to baby-leaf size, corresponding to comparable sizes as commercially marketed. For lettuce, harvest was 16 days from seed, with a fresh weight of 3 grams. For spinach, harvest was 14 days after germination, with a fresh weight of 2 grams. Harvesting spinach after 14 days paralleled the production cycle to prevent spinach root disease as described in Chapter 9.

7.5 Monitoring

During the grow-out, or pre-grow, phases periodic checks on systems and crops were done at least daily. Argus™ control programs managed aerial temperature and light in the three walk-in chambers used during the experiment: the nursery and the two holding the mini-chambers. A LiCor™ Quantum sensor wired into the Argus control panel functioned as the primary light sensor for each walk-in chamber. In the nursery, the LiCor Quantum sensor was placed at crop height with no fear of obstruction by the plants. In the walk-ins containing the mini-chambers, LiCor Quantum sensors were placed in one of the two mini-chambers within the Plexiglas enclosure after the light received in each of the mini-chambers had been carefully matched. These sensors were positioned at crop height and moved up as needed with three-quarter-inch risers to stay at the same height of the growing crop.

Light was delivered by an array of 96, 4-foot long, 40-Watt T5 fluorescent tubes, covering the chamber ceiling 3.5 feet above the crop. The walk-in chamber array allowed for lighting on four possible circuits, each of 24 equidistantly-spaced lamps, and any combination of these sets. Only two circuits could be used concurrently, as initiating a third bank (circuit) raised the temperatures in the mini-chamber environment too high for control purposes. Different combinations of one and/or two circuits were used to achieve the different daily light integral (DLI) conditions.

The Argus light control program featured an accumulation override function to discontinue lights when a target DLI had been reached. Both walk-in chambers were always set to provide the same DLI within each “run,” within roughly the same photoperiod. (There were two independent variables, DLI and concentration of CO_2, each with three levels, to be examined in all combinations. Only 3 of the 9 combinations could be evaluated in each run/trial. Thus three runs were needed per replicate to evaluate all 9 combinations of light and CO_2 concentration (which is denoted as [CO_2]. (There were

two replicates, so 6 runs in all.) In each run, light regimens to achieve the DLI for the run were estimated using instantaneous values for light circuit outputs, then tested and adjusted prior to initiating the experimental trial. The air temperature of the walk-in chambers was kept at 18 ºC to facilitate air temperature control within the mini-chambers by heating.

Environmental parameters within the mini-chambers were controlled and logged by the mini-chamber control computer and custom control programs. Each parameter could be displayed over time for each of the four mini-chambers. The control program logged and accumulated light received from a LiCor Quantum sensor placed in each mini-chamber (additional to the Argus control sensor referred to above), kept at crop height throughout the experiment. Carbon dioxide aerial concentration was monitored using a Vaisala™ Carbon Dioxide Transmitter GMT222 (range 2,000 ppm) and supplemented by a feedback-controlled peristaltic pump gas delivery system. Very tight control over aerial CO_2 concentration was achieved in all conditions. Air temperature was maintained at 25 °C and could be raised by a heater attached to a small circulation fan blowing horizontally above the crop. The ability to reduce relative humidity was achieved by circulation of chilled water from a cold reservoir through exposed stainless steel tubing encircling the perimeter of the pond cover, condensing moisture from the mini-chamber atmosphere. The capability of the humidity control system was limited; the target for relative humidity started at 65% but was raised by about 1% each day as transpiration by the growing plants exceeded the systems control abilities. However, humidity could be and always was matched across all conditions. Pond temperature was monitored using a digital temperature sensor and cooled to 22 °C using a computer-activated pump which circulated water from a cold reservoir through tubing submerged in the pond. Nutrient solution pH was monitored and adjusted either daily or bi-daily using solutions of potassium hydroxide and nitric acid by sampling solution through an access hole cut in the pond cover. EC was not adjusted during the short duration of the experiment. Oxygen was supplied to roots by bubbling air through an air stone submerged in the pond.

7.6 Reflective Barriers

A reflective barrier was applied around the perimeter of each floating 40-cell flat to reduce the disparity in light received between interior and peripheral rows of the stand and limit the amount of light received from the side by the crops. Provisions on the pond cover were made for securing the foil-covered foam reflective barriers in place with metal pins. Plants started with a 1.5-inch barrier with subsequent levels of 1-inch barrier applied to stay approximately even with crop height throughout the experiment.

7.7 Harvest

For the first harvest, one stand of each crop from each mini-chamber was extracted and processed for fresh and dry weights. This process was done in random order by mini-chamber and by crop. Harvest was always executed during the dark period with the perimeter plants in each stand being processed and measured individually as fresh weights, and collectively as a total dry weight for each stand. Plants were placed in paper bags after being weighed and stored in a 70 °C drying oven for 72 hours before dry weights were taken. After completion of the growth cycle the remaining crops were processed for fresh and dry weights in a similar fashion.

7.8 Sanitation

All Styrofoam flats were promptly emptied and thoroughly cleaned with dish soap, then rinsed and dried in a 70 °C drying oven for at least 4 days before being used again for seeding.

Mini-chamber control systems were deactivated and reset for the next experiment. Between runs 1 and 4, ponds were drained, lightly brushed, and rinsed before new solution was added. After run 4, ponds were filled with Green Shield™ detergent and thoroughly cleaned and rinsed before proceeding with runs 5 and 6.

All LiCor™ Quantum sensors were carefully adjusted using a 2-point calibration against a portable calibration sensor between each experimental run. Calibration of water temperature sensors in the ponds was done against a reference alcohol thermometer between each experimental run. Air temperature sensors were calibrated against a handheld digital thermometer with an air temperature sensor attachment. The CO_2 sensors were checked for calibration using a separate handheld CO_2 meter between each experimental run.

7.9 Results

The ultimate goal of the data collection was to use regression to define a two-parameter exponential decay equation to predict the same plant growth as would result from various combinations of [CO_2] and DLI.

Figure 3 and Figure 4 show the results for baby lettuce expressed in two ways: fresh weight and dry weight. Either presentation is valid for plant science research, but the commercial nature of growing spinach to sell on the fresh market led to a preference for presenting results on a fresh weight basis.

The underlying goal of the work was to determine whether the same [CO_2]/DLI parameters apply in general to several or most leafy green crops, or whether each crop, cultivar, harvest date, and growing system will require its own set of parameters based on nine experimental runs per replicate.

The [CO_2]/DLI data show similar shape, indicating the two parameter exponential decay curve shape applies, but the relevant parameters differ from crop to crop, and from growing system to growing system. Only baby-leaf forms of lettuce and spinach were considered due to previously described issues with arugula.

A summary of the regression equation parameters is in Table 1. A summary of [CO_2]/DLI combinations to reach the same fresh weight, based on Table 1 values, is in Table 2. Potential lighting energy savings with CO_2 concentration increased, compared with ambient [CO_2], are in Table 3.

Figure 5 shows the [CO_2]/DLI interactions for two important comparisons. The first comparison is the similarity of the baby lettuce and baby spinach curves. The curves are approximately the same, suggesting an averaged interaction equation can be used for control purposes for the two crops. The exponential decay shape of the interaction curve is defined by Equation 1:

Equation 1 $[CO_2] = a \cdot \exp(b \cdot DLI)$

where b = – 0.21 for baby lettuce and b = – 0.24 for baby spinach.

The third curve in Figure 5 is for mature head lettuce, which was obtained in previous research that inspired this work and gives the relationship shown in Equation 2:

Equation 2 $[CO_2] = 2.66E4 \cdot \exp(-0.26 \cdot DLI)$

One can view “b” in Equation 1 as a “shape” function (or parameter) and “a” as a “scale” function (or parameter). If the shape parameter is unchanged from crop to crop, the curves may be defined as homologous, differing only by scale. More data is needed to confirm the shape parameter value but the similarity of the three numbers suggests that would be a very useful future research objective.

The average of the three values of the b parameter is 0.24, which may be an adequate estimate for leafy greens, if confirmed through future research. Interestingly, the values seem little changed by the harvest date of 14 days for spinach, which is essentially the same as 35 days for mature head lettuce. If this

observation is confirmed by future research, time and effort required to create new interaction graphs and equations will be greatly reduced. For example, in the ultimately simple situation, instead of varying both [CO_2] and DLI over anticipated ranges of values, only one combination of [CO_2] and DLI need be specified and the corresponding value of the scaling parameter “a” can be calculated directly with Equation 3:

Equation 3 $a = [CO_2]/\exp(-0.24 \cdot DLI)$

This equation may be optimistic, but the results of this work suggest it could be a worthwhile path for further exploration. Not only would the research be simplified and shortened, but the number of crops and cultivars could be greatly increased, making adoption of [CO2]/DLI integrated control by the greenhouse industry more likely and saving a significant amount of energy for crop lighting.

8 In vivo Reproduction of *Pythium aphanidermatum (P.a.)* with Spinach

This chapter describes results of an evaluation of the in vivo reproduction time of *Pythium aphanidermatum (P.a.)* with a baby-leaf spinach crop at varying root zone temperatures (RZTs) and demonstrates the efficacy of using RZT as a means to control *P.a.* in commercial greenhouses.

8.1 Experimental Procedure

The first step was to collect nutrient solution from a local hydroponic grower, Finger Lakes Fresh, (FLF) that had recently isolated *Pythium aphanidermatum (P.a.)* in their lettuce crop (determined by the Cornell plant pathology laboratory). Approximately 30 liters (L) of FLF nutrient solution was transferred into a 300-L pond of nutrient solution containing baby leaf spinach of a variety of ages. The intention was to allow the disease process to proliferate into an intense inoculum that could be used to begin in vivo testing of *P.a.* reproductive times of spinach over a range of RZTs.

The *P.a.* did not develop an intense and crippling disease process as intended despite a significant effort to promote favorable conditions and opportunities for disease. Over several months, conditions were changed in the initial 300-L pond, including the following:

- Added a water heater to maintain the RZT at greater than 26 ºC; a high RZT known to favor *P.a*.
- Added a timer switch to provide periods of no bubbling to allow a period of root zone quiescence and reduced air bubbling that may or may not have negatively influenced zoospore chemotaxis.
- Extended growth periods of spinach to allow the *Pythium* process time to develop and multiply.
- Added a second inoculation of FLF nutrient solution that also contained pond sludge.
- Constructed two additional shallow nutrient ponds where roots would touch the bottom to help flagella-less *P.a.* find and penetrate the roots.

One of the two shallow ponds was given greater than 50% by volume FLF nutrient solution, including pond sludge, and the other shallow pond was mixed with various samples of outdoor soil. All of these, as well as the previous attempts, failed to produce significant and consistent damage to the roots, either failing to inoculate, failing to effectively proliferate, or not existing in the inoculum samples.

Running out of options to develop a natural *P a*. population as intended, and by permission of the NYSERDA Project Manager, plates of cultured *P.a.* were obtained. These inoculations were all successful initially. However, they failed to proliferate in the systems after the initial inoculation. Next, a specific strain, *P.a.58,* which is known to be virulent to spinach and which had been used extensively for previous spinach disease research, was obtained. This strain reduced the generality of the work, but assured disease would proliferate. It is possible that the strains of disease to this point were not adapted to spinach crops or were less virulent in nature. The known virulent strain of *P.a.58,* maintained in the laboratory of Professor Eric Nelson at Cornell, was used successfully for this experiment.

A channel system was used to obtain important supplementary information during the time that researchers were attempting to overcome difficulties starting the disease process. See Figure 6 for an overview of the channel system. In addition to confirming consistency of spinach biomass yields in all plant positions within each stand and identifying and correcting minor issues with the system, several important experiments regarding the effects of root zone temperature on spinach biomass yields were run.

The first biomass tests evaluated RZTs of 15 ºC and 20 ºC and found that fresh shoots weighed 24% less in 15 ºC RZTs than in 20 ºC RZTs but only 18% less in dry weights. This difference signifies that lower water content was maintained in the 15 ºC RZT plants than those at 20 ºC. During this experiment, there were issues with maintaining pH at the targeted 5.8 ± 0.2, frequently finding pH dropped approximately 0.75-1.2 overnight or during the course of a day. Nitrifying populations of bacteria were identified in the ponds that break down the relatively large starting concentrations of ammonium in the nutrient solution (8.75 mg/L NH_4-N).

Due to the significant time investment made in the initial data, and interest in the effects nitrifying populations had on the pH, a 2 × 2 experiment was designed to determine whether there was an interaction between pH and temperature that would invalidate our previous work. The four conditions were pH 4.8 at 15 ºC, pH 4.8 at 20 ºC, pH 5.8 at 15 ºC, and pH 5.8 at 20 ºC. The experiment was repeated with channel positions switched. The nitrification process was allowed to continue to completion in new nutrient solution before the experiments began, to ensure pH could be controlled consistently within the target ranges. The results showed no effect of pH on the biomass yields at either RZT evaluated, and the 24% biomass reduction in shoot fresh weight from 20 ºC to 15 ºC was confirmed.

The *P.a.58* disease was initially developed in two of the six hydroponic channels (frequently referred to in this report as channels due to their shape), using a continuous production cycle at 24 ºC RZT. Germinated spinach flats were floated in the channels every three days, harvested after 13 days in channel, and moved from end to end in three-day steps from entry to harvest. An intense disease process was developed for several weeks and then used to inoculate the other four clean ponds. Reproduction times for *Pa58* on spinach were evaluated at 16, 18, 20, and 24 ºC RZTs. Results are summarized in Table 4.

The presence of the *Pythium* disease process was evaluated visually and based on brown, dark, and/or decaying roots. (See Figure 7 and Figure 8 for a contrast between plant growth with healthy versus diseased roots.) The reproduction life cycle on spinach at 24 ºC was not evaluated for crop cycles shorter than seven days due to the lack of commercial utility even in a two pond system and, likewise, the reproductive cycle was not evaluated for durations longer than 13 days at 16 and 18 ºC RZT because 13 days produces baby leaf spinach of desirable size for marketing. A RZT treatment of 20 ˚C was able to reduce the disease process to a small and fluctuating population in a 13-day crop cycle, but unable to fully clear it. The time spent by flats in the pond was next reduced to 10 days and the disease was very rapidly cleared from the system. The data collected on spinach reproduction times suggested a (sequential) two pond system at a moderately reduced RZT would be a viable system for disease prevention. A two-pond system works by placing the plants in the first pond for seven days, and then transferring them to a second pond for the rest of the production time. The hypothesis is that, in this way, infected spinach roots are removed to a clean pond before the disease zoospores can reproduce for more than a small number of cycles. Plants are harvested from the second pond, carrying immature zoospores with them on their roots, which are ultimately disposed of as compost.

As the season moved into winter, a marked decrease in *Pythium* virulence was noted. Several pathologists confirmed a seasonal effect on *Pythium* populations, but no published paper or mechanism could be found in the extant greenhouse research literature to identify how or why *Pythium* populations consistently decrease in virulence in winter and increase during spring and summer. This *P.a.58* strain, for example, was maintained in a climate controlled room, at a cool room temperature, in a drawer, for many years without light. Nevertheless, seasonal decrease in virulence was observed in all experiments in the greenhouse, on Petri plates, and in several different growth chambers.

During the winter of 2014-2015, when both the disease aspects could not be evaluated any further and at risk of accidental re-inoculation of the ponds with *Pythium* was low, the time was used to evaluate the effect of root zone temperature on growth/yield in healthy plants across the range of RZT of interest, namely 16, 18, 20, and 22 ºC. (Previous work had shown that 20 and 25 ºC were not statistically different in biomass yields and, as 25 ºC would not be viable in spinach production, it was not included.) Lee and Takakura (1994) found that at high ambient air temperatures, best yields occur at a RZT of 22 ºC.

This experiment continued over the course of five months, with four replicates, running four cohorts of spinach through each channel in each repeat. In case of channel effect, plants were grown in each channel at each temperature over the course of the experiment. A consistency experiment (all channels at the same temperature) was run at the end of the experiment to identify any position effects. As for previous occasions, no statistically significant effect of channel position was found.

Average fresh mass decreased in a close to linear fashion in response to decreased temperature, with the biggest decrease in growth between 18 ºC and 16 ºC RZT (Figure 9). Table 5 and Table 6 data show the average yields and percentage comparisons to 22 ºC. The difference in plant weights between 18 ºC and 20 ºC RZT is a mere 2% during winter growth conditions. Given the significantly longer reproduction times of *P.a.* at lower temperatures, 18 ºC RZT may be the desirable compromise between growth and disease risk.

8.2 Two-Pond System (Continuous Production)

Earlier work on spinach disease funded by NYSERDA demonstrated the possibility of using a two-pond system for spinach production. It involved splitting the growing time for the crop between two ponds, one for the early part of the crop cycle and another for the later part. It has the advantage of radically reducing the time available in which the reproductive cycle of *Pythium* species can occur; in theory, halving the time. In a 13-day crop cycle (in-pond time of 13 days from initial flotation), any species of *Pythium* would need to be able to reproduce in less than 6.5 days in one or the other of the ponds, or it would die out from failure to sustain the disease process because diseased roots would be removed before releasing propagules. If clean seedling flats enter the first pond, they will be quickly infected by any existing disease process but be physically removed, roots and all, before they have time to release zoospores as

long as the temperature is low enough that the zoospore to zoospore cycle cannot be completed in time. Therefore, even if a disease exists, the first pond will clean itself in due course and material entering the second pond will be clean. The process repeats in the second pond until both ponds are disease-free. In contrast to an in-pond time of 13 days in one pond, the much shorter residence time in each pond presents a strong barrier to mutations that could shorten the *P.a.* reproduction cycle.

A long-term demonstration of a two-pond system was accordingly launched in late summer of 2014 and continued for a year so as to be subjected to all four seasons of weather conditions. The concept was that if the system could be operated, disease-free, for a year, the demonstration would provide confidence in the method and possibly be adopted by commercial growers. The overall in-pond duration for each cohort was 13 days, six days in the first pond, and seven in the second.

New flats of germinated seedlings were added to the first pond twice weekly, three or four days apart. At that time, flats that had been afloat for six days were transferred to the second pond and 13-day old flats were harvested from the second pond. Root zone temperature was maintained at 20 ºC as this temperature was discovered to be suitable for plant growth during the previously discussed biomass work, although it could have been maintained at 18 ºC with very little difference in yield. Earlier work in this project had shown that the disease process caused by the strains used in this system (probably predominantly *P.a.58*) disappeared convincingly if the crop cycle was reduced from 13 days to 10 days at 20 ºC. However, it could linger at a low level in a 13-day crop cycle, so it was not unreasonable to adopt an RZT of 20 ºC for a six to seven day in-pond dwell time. Fortuitously, 20 ºC was a stricter test of the system.

In the two-pond system demonstration, roots and shoots were examined and photographed at each harvest. Throughout the one-year period, no disease in the roots was observed, and shoots also appeared to be productive and healthy (see Figure 10.) For the latter part of the trial, a second cultivar was introduced *(*cultivar *Space*-with seeds that have not been treated*)* alongside the first *(*cultivar *Eagle – with treated seeds);* it expressed no disease process either. No inoculum was introduced, and there is no conclusive proof of inadvertent infection by stray propagules of the strain that had been used to test the effect of temperature on the duration of the Pythium reproductive cycle.

To test the efficacy of the two-pond system at resisting an introduced commercial mixture of *Pythium* infection, it was challenged it during the final two months of the year-long experiment in high summer when *Pythium* is most virulent.

Samples were collected from Bright Farms, a company with multiple hydroponic greenhouse operations in the United States producing salad materials. The Yardley, PA, location specializes in production of mixed leafy greens, including spinach, using a raft system. They had known problems with *Pythium* outbreaks in their spinach crops, unlike the local Finger Lakes Fresh who did not grow spinach and apparently had no virulent *Pythium* species present in their ponds.

The samples were used to inoculate this two-pond system demonstration trial in two ways. First, approximately 10 L of their nutrient solution and a small handful of just-harvested baby leaf spinach roots were added to each of two tubs that each held a 132-cell flat of seven-day-old spinach seedlings. This procedure was done on site at Bright Farms so that inoculation of the roots could begin prior to long-distance transportation. These two flats would be used to host the disease process and allow it to develop before being floated in the two-pond demonstration project. Secondly, an additional 20 L of nutrient solution was transported back to Cornell and poured into the first pond of the two-pond system for an immediate inoculation.

The inoculation of the spinach flats allowed the existing disease organisms to start the disease process on the roots before the return drive. Because *P.a.* has flagella that are easily detached by jostling and bumping, an immediate inoculation and a shallow reservoir was used to give zoospores the best chance to successfully inoculate the roots. Minimally filling the reservoir enabled even encysted zoospores (flagella-less) the opportunity to inoculate the roots that rested on the bottom of the tub. The Bright Farms diseased roots were added for good measure. Should the initial nutrient solution inoculation fail, they might release additional zoospores.

The spinach inoculation that was started in plastic tubs at Bright Farms was allowed to continue and develop on a bench in the greenhouse for an additional four days (absent RZT control). Nutrient solution was supplemented as necessary to replace evapotranspiration but the tubs were kept at their shallow starting level to ensure that any encysted zoospores had a chance to successfully inoculate roots touching the bottom. (10 L in a tub was less than five centimeters deep).

The inoculation protocol was extremely successful. Roots showed signs of disease within two days of inoculation, an incredibly rapid progression given that the strain of *P.a.* in the ponds at Cornell had been taking over seven days to show definite disease process. Very little to no shoot growth occurred after the inoculation of the seedling flats. On the fourth day, the heavily diseased flats of spinach were transferred to the first pond of the two-pond system (Figure 10 and Figure 11.) Once environmental and secondary factors possibly contributing to the continued disease presence were tightly within control, a disease presence was observed well beyond when significant reductions in quantity and intensity of *P.a.* infection would have been expected. The first harvest following implementation of the improved cooling capacity showed significant reductions in disease presence. However, subsequent harvests had similar to pre-improvement levels of a consistent and regenerating disease presence. Figure 7 and Figure 8 show the capability of a 20 ºC RZT to significantly reduce a *Pythium* disease process' intensity and virulence, however long term growth with a maintained presence introduces risk of selection for more virulent *Pythium* within the user systems.

A commercial mixture of diseases may have many strains and species of pathogenic organisms present and each may have different reproduction times at different temperatures. One hypothesis is that a form of *Pythium,* either a different strain of *P.a.* or an entirely different species such as *Pythium dissotocum,* a species known to also infect spinach, could have maintained itself within the ponds.

The RZT was reduced to 18 ºC in both ponds in an attempt to overcome the disease presence via further slowing the disease reproduction process. At 18 ºC RZT, the biomass significantly improved to expected yields with significantly higher percentage of roots showing no signs of disease (Figure 12). The less than 10% of roots showing disease were also less necrotic, suggesting the *Pythium* process may have been maintaining itself but was not sufficiently vigorous to significantly damage the host spinach plant.

9 References

Lee, Y.D. and Takakura, T. 1995. Root Cooling for Spinach in Deep Hydroponic Culture Under High Air Temperature Conditions. *Acta Hort.* 399, 121-126, DOI: 10.17660/ActaHort.1995.399.12, http://dx.doi.org/10.17660/ActaHortic.1995.399.12

10 Figures

Figure 1. Three environmentally controlled mini-chambers located within a walk-in plant growth chamber

Lettuce, spinach and arugula crops are growing simultaneously.

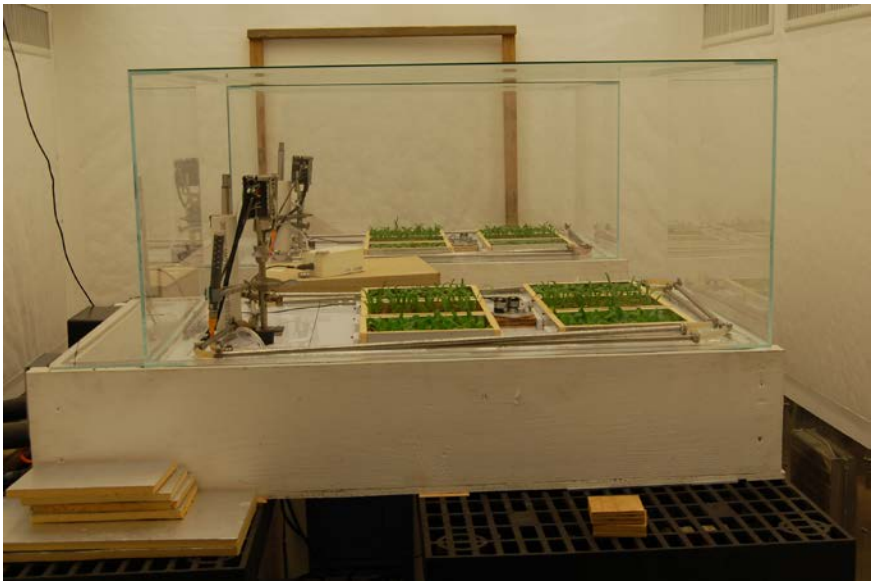

Figure 2. Three crops after thinning, floating in mini-ponds within the mini-chambers, within the walk-in plant growth chamber

Plants are lettuce, spinach, and arugula (left to right).

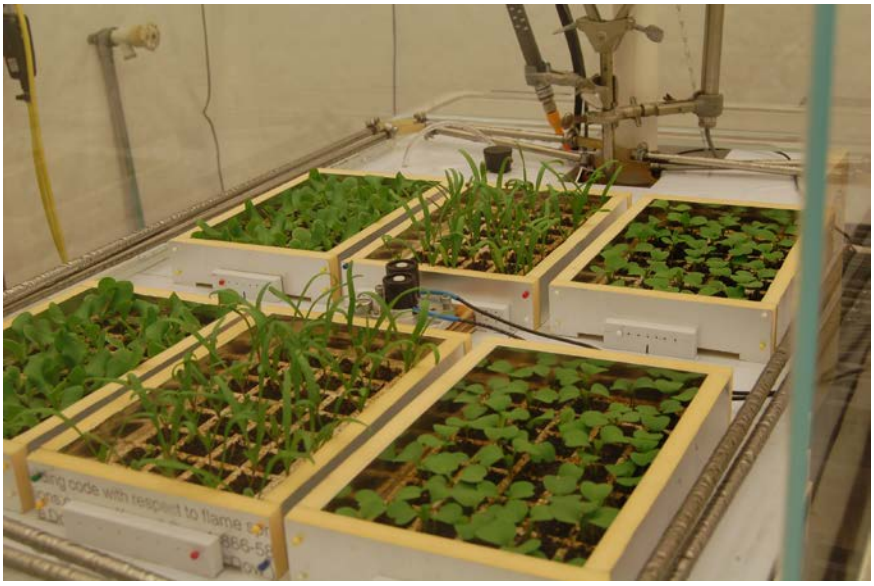

Figure 3. DLI and CO_2 combinations required to reach a 3.0-gram fresh weight baby lettuce crop in 16 days under light

Curve represents a two-parameter exponential fit: a = 1.98E4, b = -0.21.

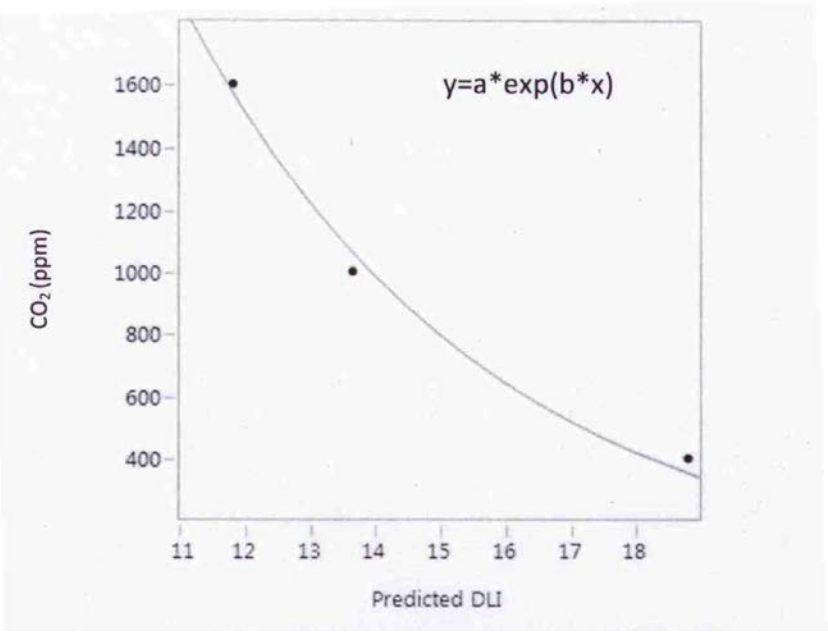

Figure 4. DLI and CO_2 combinations required to reach a 0.12-gram dry weight baby lettuce crop in 16 days under light

Curve represents a two-parameter exponential fit: a = 2.39E5, b= -0.372.

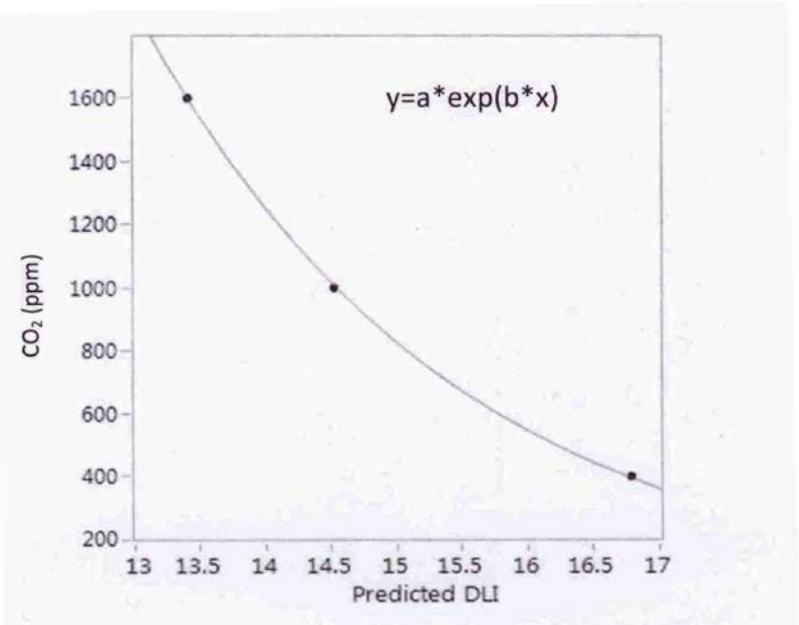

Figure 5. Comparison of baby lettuce (3 g/16 days) to baby spinach (2 g/14 days) and mature head lettuce (150 g/35 days)

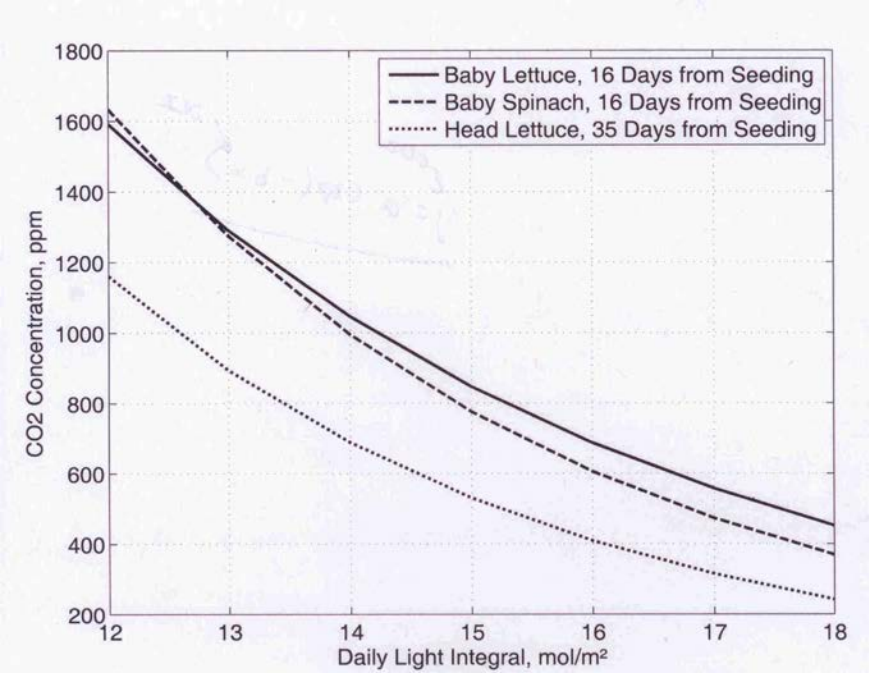

Figure 6. Experimental setup with six hydroponic channels, with each channel allowing up to five flats of 132 spinach seedlings each

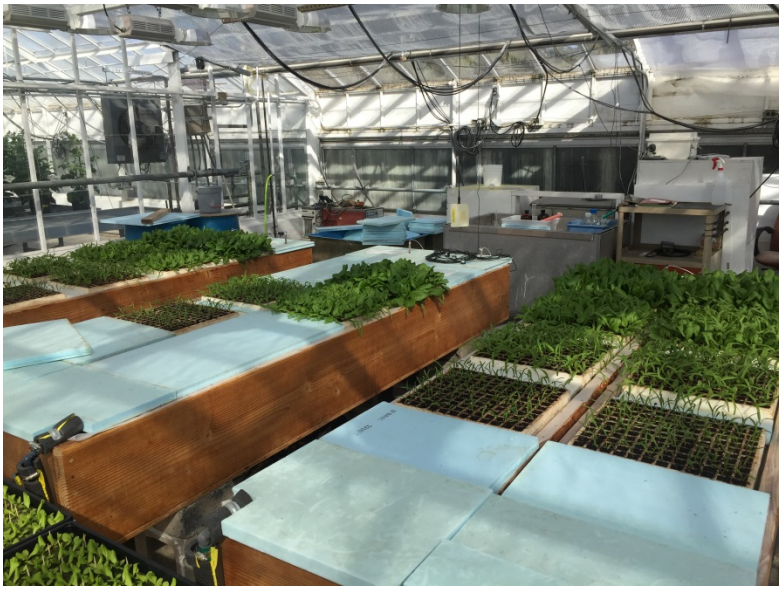

Figure 7. Two examples of heavy *P.a.* infection

Significantly decreasing yields (top row), and two examples of healthy spinach crop (bottom row).

Figure 8. Healthy and diseased roots visual comparison

The top row shows healthy spinach roots with pearly white roots. The top left plants were grown at 16 ºC RZT for an extended period to confirm absence of disease presence. The bottom row shows two intensities of the *Pythium* disease process.

Figure 9. Average spinach plant fresh mass as a function of RZT

Different letters signify significance at the 5% level.

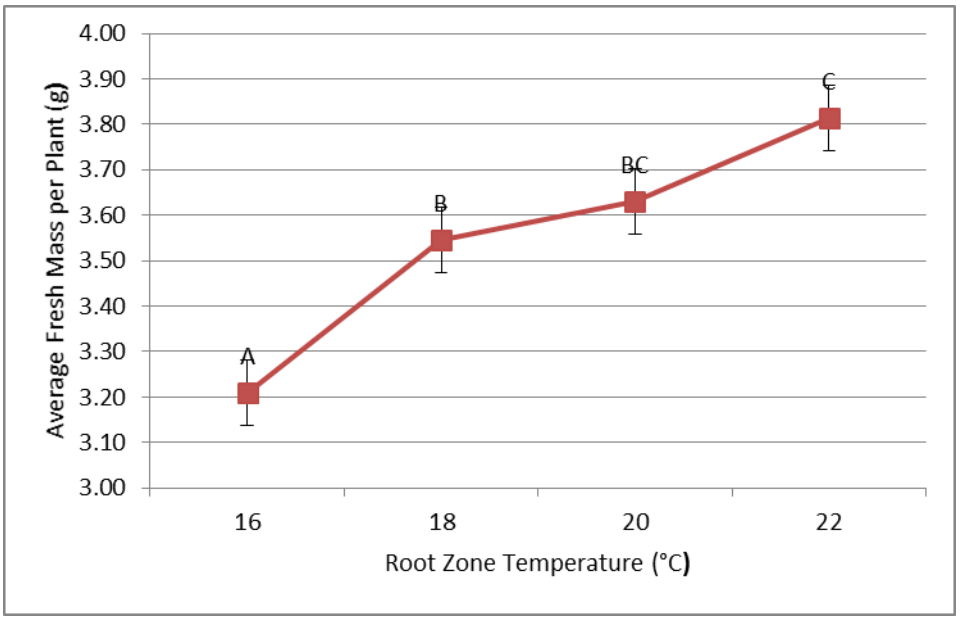

Figure 10. Flat #1 of inoculated spinach with Bright Farms nutrient solution and diseased roots

Shown at day four before floating into the two-pond system.

Figure 11. Flat #2 used to inoculate the two-pond system

Same preparation and treatment as Flat #1 in Figure 10.

Figure 12. A representative harvest at 18 ºC in the two-pond system

The biomass yields are excellent. The roots are generally long and mostly healthy. Although most plants have a small portion of their root containing *Pythium* (darker grey portions of the roots), root tips are white, indicating health.

11 Tables

Table 1. Summary of CO_2/DLI exponential decay parameters

Crop	Applicable conditions	Scaling parameter	Shape parameter
Baby-leaf lettuce	3 g fresh weight in 16 days	1.98E4	-0.21
Baby-leaf spinach	2 g fresh weight in 12 days	3.20E5	-0.24
Mature lettuce heads	150 g fresh weight in 35 days	2.66E4	-0.26

Table 2. Combinations of [CO_2] and DLI to achieve same growth in same number of days

Baby-leaf lettuce, 16 days		Baby-leaf spinach, 12 days		Mature lettuce heads	
[CO_2]	DLI	[CO_2]	DLI	[CO_2]	DLI
400	18.8	400	16.1	400	17.3
1,000	14.7	1,000	15.6	1,000	16.0
1,600	13.5	1,600	12.4	1,600	15.7

Table 3. Anticipated lighting energy saved by supplementing CO_2 to make lighting more efficient

Baby-leaf lettuce, 16 days		Baby-leaf spinach, 12 days	
[CO_2]	% Energy Saved	[CO_2]	% Energy Saved
1,000	21.8	1,000	3.1
1,600	28.2	1,600	23.0

Table 4. *Pythium aphanidermatum* (P.a.) in vivo reproduction time of mature deep flow spinach hydroponic ponds

Root Zone Temperature (ºC)	Time to Reproduce
16	>13 days
18	>13 days
20	10<x<13 days
24	<7 days

Table 5. Pond culture spinach yields of treated *Eagle* cultivar with a consistent daily light integral of 17 mol/m^2, comparing 16, 18, 20 and 22 ºC RZT

Root Zone Temperature (ºC)	Average Fresh Weight Yield (g)	Growth Comparison (% of 22 ºC)
16	3.21	84%
18	3.55	93%
20	3.63	95%
22	3.81	100%

Table 6. Pond culture spinach yields of treated *Eagle* cultivar with a consistent daily light integral of 17 mol/m^2, comparing 20, 22, and 24 ºC RZT

Root Zone Temperature (ºC)	Average Fresh Weight Yield (g)	Growth Comparison (% of 22 ºC)
20	4.00	*99%*
22	4.06	100%
24	3.96	*98%*

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