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101 Ways to Try to Grow Arabidopsis: Materials/Methodology

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Materials/Methodology

Growth of *Arabidopsis* seedlings under differing irrigation and fertilization methods

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(April-May 2004)

Seed of *Arabidopsis thaliana* "Columbia" were stratified 4 days and germinated under a greenhouse mist system for 8 days, then moved to a Purdue greenhouse located in West Lafayette, Indiana. Greenhouse settings were 23C day temperature, 18C night temperature, with a 16-hour photoperiod provided by high-intensity lamps. Once moved to the greenhouse, all plants were sub-irrigated using white 10-cm x 50-cm trays without drainage holes (white" display trays", T.O. Plastics, Minneapolis, MN). Watering with greenhouse fertilizer solution was compared with using clear "tap" water only. Fertilizer solution was our standard greenhouse of (in mg per liter) 200 N, 29 P, 167 K, 67 Ca, 30 Mg, and micronutrients supplied from a commercial fertilizer formulation (Miracle Gro® Excel® 15-5-15 Cal-Mag; The Scotts Co., Marysville, OH)). Adjustment of pH to range 5.7 - 6.0 and alkalinity reduction was achieved via 93% sulfuric acid (Ulrich Chemical , Indianapolis) at 0.08 ml per liter. Draining sub-irrigation trays after saturation was compared with leaving trays filled with solution continuously. Plants that were drained following irrigation were allowed to dry between irrigations but without incurring water stress. Within these treatments, 4 different pot/tray sizes were compared, referred to in this report by their horticultural industry designation: 201 half-flats, 72-cell tray, 3" square pot and 4" square pot.

A second set of plants sown on the same date were grown in growth chambers with 8-hour, 16-hour, and 24-hour photoperiods, natural day greenhouse conditions, and a greenhouse with 16-hour photoperiod provided by metal halide and sodium vapor lamp supplementation. Both the supplemental greenhouse light and the growth chamber illumination were 90-110 $\mu mol/m^2/s$. Temperatues in growth chambers were 23C during the light cycle, 18C during the dark cycle.

101 Ways to (Try to) Grow *Arabidopsis*

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(November-January, 2005-2006)

Arabidopsis thaliana 'Columbia' seeds were sown on 11/16/05, by putting seeds in solution of water and squeezing from laboratory water bottle. Seeded pots were germinated by covering trays with clear plastic domes (Humi-Dome, Hummert Intl., St. Louis, MO) for 5 days. Plants were later thinned to 1-4 plants per pot. Pots ("3 inch square", ITML Horticultural Products, Inc. Brantford, ON, Canada) were filled with one of 6 commercial soilless mixes (Pro-Mixes 'PGX', BX', 'HP', Premier Horticulture Inc, Quakertown, PA; 'Redi-earth', 'Sunshine LA4', SunGro Horticulture, Bellevue, WA; 'Metro Mix 360', The Scotts Company, Marysville, OH) three clay granule components (Profile Greens™ or Turface Atheletics™ MVP, Profile Products LLC, Buffalo Grove, IL; Ecolite™, Western

Organics, Inc, Tempe AZ) or with medium-grade vermiculite. Many treatments consisted of the commercial mixes augmented with the clay granule components, which are reported to improve aeration and nutrient-holding capacity of soils. Sets of all the soils described above were sub-irrigated weekly with complete fertilizer solutions at strengths of 50 ppm N solution or 200 ppm N solution. These fertilizer solutions were drained from the trays after 5 minutes. They were applied alternately with clear water. A third set was kept in a tray of the irrigation solution continuously, alternating clear water with the 200 ppm N complete fertilizer. A small subset of pots were grown on a "self-watering" capillary tray fabricated out of sponges, capillary matting (Hummert International, St. Louis, MO), and modified white display trays (T.O. Plastics, Minneapolis, MN). Several sets of pots were not fertilized with liquid fertilizer, having instead been incorporated with one of four rates of Osmocote® 14-14-14 slow-release fertilizer (The Scotts Company) prior to sowing. The rates were 0.17 g/3" pot (0.5 kg/m3), 0.34 g/3" pot (0.9 kg/m^3), 0.7 g/3" pot (1.8 kg/m^3) and 1.4 g/3" pot (3.6 kg/m^3). The rate of 0.7 g/3" pot (1.8 kg/m^3) is considered the recommended rate, so the treatments were labelled in this report as 1/4X, 1/2X, 1X and 2X for convenience.

All plants described above were grown in a Purdue greenhouse in West Lafayette, Indiana under natural-day conditions during short-day season to keep vegetative until 1/12/06, when a single long day was applied to initiate flowering.

Another set of treatments was conducted in different locations to determine the effect on growth and flowering. Only a single soilless mix, Pro-Mix 'PGX', was used for this set. The locations included: natural day, 16-hour and 24-hour photoperiods provided at Horticulture Greenhouses, the longer days provided by supplemental illumination with high-pressure sodium and metal halide lighting at 100 $\mu mol/m^2/s$; 16-hour light shelf using fluorescent lighting at the same light intensity; 16-hour and 24-hour growth chamber environments using fluorescent lighting at the same light intensity.

All treatments were grown at a day temperature of 21.5C and a night temperature of 18C.

Application of Top-Dressings and Beneficial Nematodes for Fungus Gnat Control in Greenhouse *Arabidopsis* Production

Rob Eddy, Purdue University, West Lafayette, IN
(November-January, 2005-2006)

A second experiment was planted on 11/16/05 and grown as described in the "101 Ways" study to examine effects of top-dressings and beneficial nematodes on fungus gnat infestation. Seeds of A. *thaliana* 'Columbia' were broadcast over pots filled with one of three commercial soilless mixes by tapping out of a creased paper card. Top-dressings were then applied to soil surface consisting silica sand, diatomaceous earth (Voluntary Purchasing Groups, Inc., Bonham, TX), or a Turface/diatomaceous earth combination. One set was left uncovered as a control and two more sets were left uncovered and applied with two rates of *Steinernama feltiae* beneficial nematodes (NemaShield™, BioWorks, Inc., Fairport, NY) on 1/6/06 and 1/16/06. Counts of fungus gnat larvae present in media were made by examining potato wedges (about 2.5cm square) placed on surface of all trays to attract the larvae.

Statistical analysis was performed on the counts using a negative binomial regression with significance determined at the p level of 0.05, using SAS/STAT software.

Determining a Dose Response to Soil-Incorporated Imidacloprid Insecticide in Greenhouse *Arabidopsis* Production

Rob Eddy, Purdue University, West Lafayette, IN
(November-January, 2005-2006)
A third experiment was sown with 'Columbia' on 12/16/05 and grown as described in the "101 Ways" study. Prior to sowing, imidacloprid insecticide (Marathon® 1G, OHP, Mainland PA) was incorporated at recommended rate of 0.25 teaspoon/3" pot (4.2 kg/m^3), and 2X-, 3X-, 5X– and 10X this recommended rate (8.4, 12.6, 21.0 and 42.0 kg/m^3, respectively). Marathon is a commonly used insecticide for systemic control of aphids. One set of pots were left untreated for control comparison. Three additional sets were incorporated with 1X-, 3X-, or 10X-recommended rates but not mixed thoroughly, to simulate careless technique. Plants were examined and observations recorded on damage from over-application and poor mixing.

Application of *Steinernama feltiae* Beneficial Nematodes for Fungus Gnat Control in Greenhouse *Arabidopsis* Production

Todd Moulton and Rob Eddy, Purdue University, West Lafayette, IN
(February, 2006)
At termination of the study titled "101 Ways to (Try to) Grow *Arabidopsis"* in January, 2006, 150 pots were moved to an isolated greenhouse and kept continously moist to maintain and increase fungus gnat infestation. 100 pots with green plants at various stages of flowering were randomly selected from the treatments. 50 more were purposely selected from the treatments incorporating calcined clay granules at 50% the final volume to see if that treatment alone had an effect on fungus gnat infestation. On 2/6/06, a single potato wedge approximately 2.5-cm square was placed on each of the 150 pots. On 2/10/06, the wedges were examined and fungus gnat larvae counts recorded for each pot. The 50 pots with 50% incorporated calcined clay granules were then discarded. Later on the same day, the first application of beneficial nematodes (Nemashield™, Bioworks Inc., Fairport, NY) were applied to 50 of the remaining 100 pots at the recommended rate of 50 million nematodes per 1000 sq ft. Clear water was applied to the other 50 pots as an untreated control. The nematodes had been stored for less than a month at suggested temperatures, and the solution prepared and applied acording to manufacturer's instructions in overcast weather. A second application of nematodes was similarily made on 2/17/06. New potato wedges were applied 3 days after each application and trapped fungus gnat larvae counts made at 7 days after first application and 7 days after

second application.

Means were compared using single-samples t-test with significance determined at the p-level of 0.05, using SPSS v12.0 software.

Growth of *Arabidopsis* Seedlings Under Continuous Fluorescent/incandescent Lighting

Rob Eddy, Purdue University, West Lafayette, IN

(April-May 2006)

Arabidopsis thaliana ‘Columbia’ seeds were sown on 4/16/06, by tapping a folded piece of paper containing seeds over the root medium. Seeded pots were germinated by covering trays with clear plastic domes (Humi-Dome, Hummert Intl., St. Louis, MO) and cheesecloth for 5 days. Pots (“3 inch square”, ITML Horticultural Products, Inc. Brantford, ON, Canada) were filled with a 1:1 ratio (by volume) of Pro-Mix ‘PGX’ (Premier Horticulture Inc, Quakertown, PA) commercial soilless mix and Turface Atheletics™ MVP clay granule components (Profile Products LLC, Buffalo Grove, IL). Seeded pots were grown in a Percival model AR-75L plant growth chamber set at a constant 22C, with intensity adjusted to maximum 300 µmol/m^2/s on the upper shelf and a maximum100 µmol/m^2/s on the lower shelf. Lights were on continuously from the start of the experiment. Plants were moved to different positions on their respective shelves every 4-5 days during the experiment to provide more uniform exposure to light. Plants were sub-irrigated continuously with either tap water or fertilizer solution to avoid water stress.

For each light intensity, there were 5 fertilization treatments with 20 replicates each. One set received only the proprietary starter fertilizer that is a component of the Pro-Mix, while the other four treatments were supplemented by either liquid fertilizer or slow-release granules. One set was sub-irrigated continuously with complete fertilizer solution at strength of 200 mg N/liter. Fertilizer solution was our standard greenhouse fertilizer of (in mg per liter) 200 N, 29 P, 167 K, 67 Ca, 30 Mg, and micronutrients supplied from a commercial fertilizer formulation (Miracle Gro® Excel® 15-5-15 Cal-Mag; The Scotts Co., Marysville, OH)). Adjustment of pH to range 5.7 - 6.0 and alkalinity reduction was achieved via 93% sulfuric acid (Ulrich Chemical , Indianapolis) at 0.08 ml per liter. The other three sets were sub-irrigated continuously with tap water, having instead been incorporated with one of three rates of Osmocote® 14-14-14 slow-release fertilizer (The Scotts Company) prior to sowing. The rates were 0.7 g/3” pot (3.5 kg/m3), 1.4 g/3” pot (7 kg/m^3), or 2.1 g/3” pot (10.5 kg/m^3). The rate of 0.7 g/3” pot (1.8 kg/m^3) is considered the recommended rate, so the slow-release rates were quite high.

Plants were observed weekly for signs of stress and progress of growth and flowering.

Modifying a Greenhouse Table and a Greenhouse Light-shelf with Portable

Air-conditioners for Improved Cooling

Rob Eddy, Purdue University, West Lafayette, IN

(June-July 2006)

A 5'x12' metal greenhouse table of standard constuction, located near a greenhouse exhaust fan, was modified by adding a layer of 2-inch thick foam-board insulation to the table top and sealing seams with duct tape. A rectangular metal conduit frame was place on top of this that was 24" tall to hold sheets of insulating plastic. This frame was from greenhouse supplies we had on hand to support shade fabric or black-out fabric, and could be constructed out of PVC pipes if necessary. Two 10,000 btu air-conditioners were installed on the table top, using another table and blocks for support, so that the discharge air vents on the back of the unit (the side that would face outdoors in a window unit) were outside the perimeter of the table and close to the greenhouse exhaust fan. The a/c units were plugged into two separate electrical circuits, each that had GFI outlets. Next, two layers of 4ml clear polyethylene plastic were draped over the frame and cut so that they hung to the floor. Care was taken to carefully seal the plastic to the top and sides of the a/c units in a way that the refrigerated air vents would blow into the enclosed table, while the hot air discharge vents were outside the enclosed table. Spring clamps were placed on the bottom edge of the plastic sheeting to weigh down the plastic so that it hung snugly against the perimeter edges of the table, and to facilitate lifting up the plastic during plant loading and care. All the supplies listed above were purchased at a local home improvement store (Menards, Eau Claire, WI).

The light shelf was constructed using 2 plastic shelf units bought at a home improvement store of dimensions 74"x24"x36", assembled end-to-end for a finished dimension of 74" high x 24" deep x 72" wide, its length having a southern exposure to maximize light. The units had four levels of shelves for a total area of about 48 square feet. Two 5,000-btu a/c units were installed on the bottom two shelves in a similar configuration as described for the air-conditioned table. It would have been desirable to place them on the top two shelves but we did not have a suitable support system to hold up the a/c units. Because light was limiting due to the shading of the shelves, wet-location fluorescent fixtures were installed (item # 105447 FarmTek, South Windsor, CT). One four-foot long fixture was installed in the center of each of the four shelves. The lights were plugged into GFI outlets and were on continuously during the experiment. Next, two layers of reflective insultation "bubble wrap" was installed on the north side of the unit. Seams were sealed with duct tape. Three layers of clear, 4-mil polyethylene film were secured to the ends of the unit using duct tape. Finally, two layers of plastic film were placed on south side and then slit top-to-bottom in two places for access, then an additional two-layer "door flap" of the same plastic was secured over the top and south-facing length of the unit. Spring clamps were hung on the bottom of this flap.

Excluding the costs of supplies and components we had on hand such as the greenhouse table, extension cords, timers, thermometers, etc., the cost of the air-conditioned light shelf was $540 and the air conditioned table $470. Construction was completed in a total of 8 hours by one person. Dividing the btu rating of the a/c units by the volume of the air space being cooled in each environment resulted in 166 btu/ft3 for the table and 138 btu/ft3 for the shelf.

Greenhouse and weather data were collected from our environmental control computers (Priva North America, Inc., Vineland Station, Ontario, CA). Temperature sensors were checked for proper calibation prior to experiment. Temperature and humidity data inside the air-conditioned shelf and bench were collected using Hobo data loggers (Onset, Bourne, MA). Light readings were made on June 16, 2006 at 1:00PM with 50% shade curtains drawn in the greenhouse where the a/c bench and shelf were located, and were as follows:

Outdoors:
1811 µmol/m^2/s
Inside greenhouse, 50% shade curtain drawn:
Ambient, 544 µmol/m^2/s
A/C bench, 465 µmol/m^2/s
A/C light shelf with lights on, 60-230 µmol/m^2/s

For a test conducted June 23-July 23, 2006, *Arabidopsis thaliana* 'Columbia' seeds were sown by tapping a folded piece of paper containing seeds over the root medium. Seeded pots were germinated by covering trays with clear plastic domes (Humi-Dome, Hummert Intl., St. Louis, MO) and cheesecloth for 5 days. Pots ("3 inch square", ITML Horticultural Products, Inc. Brantford, ON, Canada) were filled with a 1:1 ratio (by volume) of Pro-Mix 'PGX' (Premier Horticulture Inc, Quakertown, PA) commercial soilless mix and Turface Atheletics™ MVP clay granule components (Profile Products LLC, Buffalo Grove, IL). One set of plants was placed in each of the air-conditioned environments, and a third set on a table without air-conditioning in the same greenhouse. Each set of plants consisted of 60 pots, divided into three fertilizer treatments. One fertilizer treatment was our standard practice of irrigating with 200 ppm N fertilizer solution, alternated with irrigation using clear water. The fertilizer solution is described in other experiments on this webpage. A second group was irrigated at each irrigation with the 200 ppm N solution; no clear water. A third group was irrigated each time with the fertilizer solution diluted to 100 ppm N; no clear water. Observations of plant growth and flowering were made each week.

Phytotoxicity of Three Commercial Greenhouse-labelled Fungicides Applied to *Arabidopsis thaliana* 'Columbia'

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(Summer 2006)

Rectangular, 601-style cell packs of dimensions 12-cm x 15-cm x 6-cm deep (ITML Horticultural Products, Inc. Brantford, ON, Canada) were filled with Pro-

Mix PGX soilless mix (Premier Horticulture Inc, Quakertown, PA). *Arabidopsis thaliana* 'Columbia' seeds were sown on 8/4/06, by by tapping a folded piece of paper containing seeds over the root medium. Seeded pots were irrigated with clear water as needed to avoid excessive drying of root medium until germinated. Following germination, plants were irrigated once per week using a fertilizer solution at strength of 200 mg N/liter. Fertilizer solution was our standard greenhouse fertilizer of (in mg per liter) 200 N, 29 P, 167 K, 67 Ca, 30 Mg, and micronutrients supplied from a commercial fertilizer formulation (Miracle Gro® Excel® 15-5-15 Cal-Mag; The Scotts Co., Marysville, OH). Adjustment of pH to range 5.7 - 6.0 and alkalinity reduction was achieved via 93% sulfuric acid (Ulrich Chemical , Indianapolis) at 0.08 ml per liter. Plants were grown in a glass greenhouse with a temperature setpoint of 22.5C day/ 18C night until 9/8/06, when they were transferred into a controlled-environment room with similar temperature setpoints but artificial lighting using metal halide and sodium vapor lights combining for an intensity of 100 $\mu mol/m^2/s$ for 16 hours per day.

On 9/8/06, spray applications were made, each treatment consisiting of six replicate pots. Controls were pots sprayed with reverse-osmosis water only, while treatments consisted of three products applied at three rates, all mixed using reverse-osmosis water. The products and rates were as follows: kresoxim-methyl (Cygnus, BASF, Research Triangle Park, NC) at 0.23, 0.69 and 1.38 g/l; piperalin (Pipron, SePro, Carmel, IN) at 0.32, 0.96 and 1.92 g/l; and 1-(4-Chlorophenoxyl)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone (Strike 25 WDG, OHP, Mainland, PA) at 0.64, 1.9 and 3.84 ml/l. It should be noted that the first rate of all three products was the label-recommended rate for powdery mildew control.

Observations and photographs were made 7 days later.

Local Supply Sources:
Turface, Profile, Ecolite: Turf Specialties, Zionsville, IN, 317-875-9225

Pro-Mixes, Metro Mix, Redi-earth, Sunshine LA4, Excel fertilizer, Marathon 1%G insecticide, silica sand, pots and web-bottom trays, white display trays: Carl Brehob & Son, Brehob Rd., Indianapolis IN

Nemashield beneficial nematodes: BFG Supply Co.,
Portage, MI, 800-883-7234

Diatomaceous Earth, slow release fertilizer, capillary matting: Hummert International, St. Louis, MO 800-365-3055