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NCSU PHYTOTRON PROCEDURAL MANUAL*

For Controlled-Environment Research at the
Southeastern Plant Environment Laboratory



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TABLE OF CONTENTS

INTRODUCTION.....	3
USE OF FACILITIES	3
REVIEW PROCESS.....	3
FACILITIES	4
Greenhouses.....	6
A and B Chambers	8
C-Chambers	9
H-Chambers	10
Miscellaneous Chambers.....	10
Pathology (BSL-3)	10
Utilities	10
CULTURAL PRACTICES.....	11
Plant Density.....	11
Nutrition and Substrate.....	11
OPERATIONAL PROCEDURES	13
Pest Control.....	13
Responsibility of the Investigator.....	13
Reserving Phytotron Equipment	14
Role of the Phytotron Staff.....	14
Phytotron Operating Schedule	14
Program Changes	14
Project Termination.....	14
Visitors	14
Standard Methods of Reporting Environmental Conditions.....	14
MEASUREMENT OF ENVIRONMENTAL CONDITIONS	16
Temperature	16
Radiant Energy.....	17
Air Velocity	19
Relative Humidity	19
LITERATURE CITED.....	19
PHYTOTRON STAFF DUTIES	19
CONVERSION FACTORS	20
NATURALLY-OCCURRING DAYLENGTHS FOR RALEIGH, NC.....	21

NC State University Phytotron Procedural Manual

INTRODUCTION

The North Carolina State Phytotron, is a facility especially designed for research studies of the response of biological organisms to their environment. Within the Phytotron, there are more than 60 growth chambers, 4 greenhouses and a Biosafety Level 3 Lab with a greenhouse (Figs. 1-4). The high degree of control inside the growth chambers and greenhouses of the Phytotron makes it possible to duplicate any climate from tropical rain forests to arid desert. In the BSL3 Laboratory, it is also possible to study plant diseases and insects that are not found in the United States including those on the USDA select agent list. The NC State Phytotron concentrates on applied and basic research related to agricultural problems encountered in the southeastern United States. The ability to control all phases of the environment allows inclusion of research dealing with all aspects of plant science.

USE OF FACILITIES

A prospective user of Phytotron space needs to submit a resume of the proposed research to the Director of the Phytotron. The proposal form can be completed and submitted on line at https://harvest.cals.ncsu.edu/cfdocs/phytotron_prod/PhytotronLogin.cfm. The proposal is reviewed by the Phytotron Committee, and the Director uses the research outline to coordinate the space needs and environmental conditions desired into the overall program of the facility. Consequently, applications for Phytotron space should include the objectives of the study, a brief summary of the project and the proposed procedures including environmental conditions needed for the research.

As an example, a simple program might be:

<u>Day</u>	<u>Experimental Procedure</u>
1	Place seeds in germinators.
2	Transplant pre-germinated seeds to 10-cm containers filled with standard Phytotron substrate; place all containers in GH at 26/22°C day/night temperatures and long days.
20	Move plants to controlled-environment rooms and begin experimental conditions listed in the proposal.
90	Terminate experiment.

The operational schedule should include the days on which data are to be taken in order to reserve balances, leaf area instruments, ovens, freeze dryers and pertinent laboratory space and equipment. It might also include schedule changes in environmental conditions, the timing of non-routine operations, or off-schedule personnel needs to assure proper coordination of events.

REVIEW PROCESS

Applications for use of Phytotron space are reviewed by the Phytotron Committee composed of scientists experienced in the use of controlled environment facilities. The function of the review is to insure that Phytotron space is used effectively and to assign priorities when requests exceed the available space. The review process can be facilitated by discussing a preliminary draft of the proposal with the Director of the Phytotron. This initial discussion will enable the Phytotron staff to offer suggestions that will hasten approval of the program and allow them to provide possible starting dates and to estimate costs. *User Charges:* All projects are billed for the space used, and for supplemental services, such as use of Phytotron research assistants and construction of special equipment. Contact the Phytotron Director for pricing.

FACILITIES

The four-story Phytotron contains areas for general studies, plant transformation, and for research in plant pathology, and morphology (Figs. 1 to 4). The facility contains over 60 controlled-environment chambers of various sizes, plus a Biosafety Level 3 Lab with a greenhouse and 4 air-conditioned greenhouses. Temperatures in the 49.m², air-conditioned greenhouses (Fig. 5) can be maintained at any point between 8 and 40°C, but they are usually operated at 26/22C day/night temperatures.

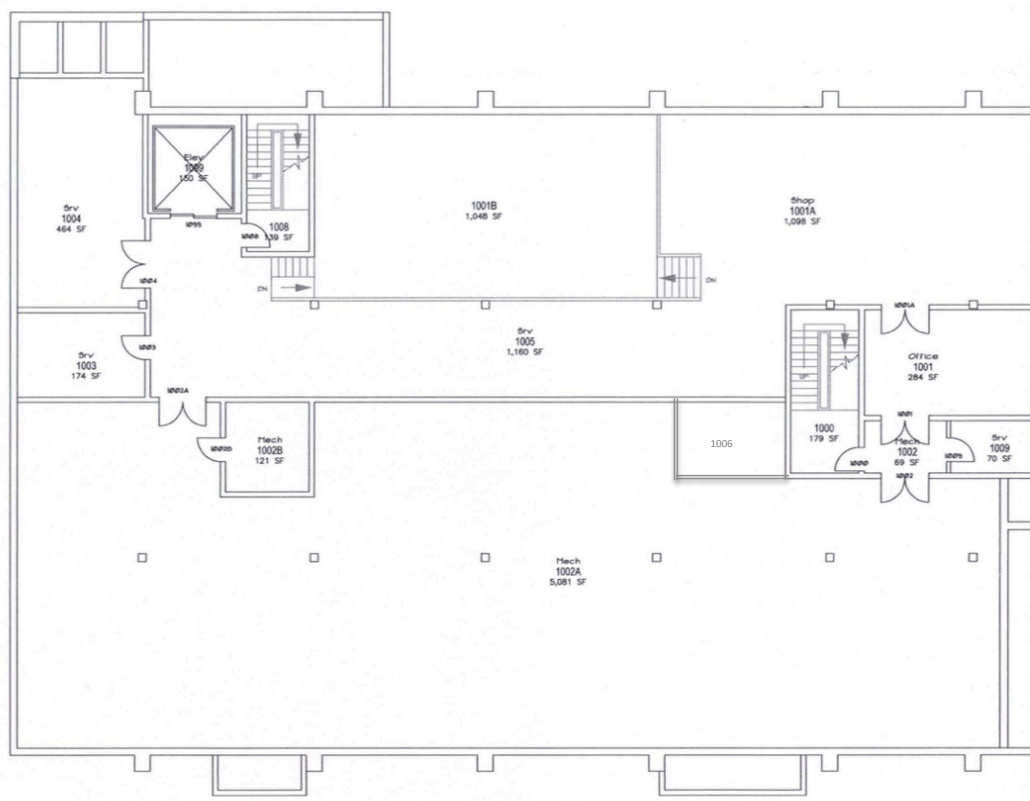
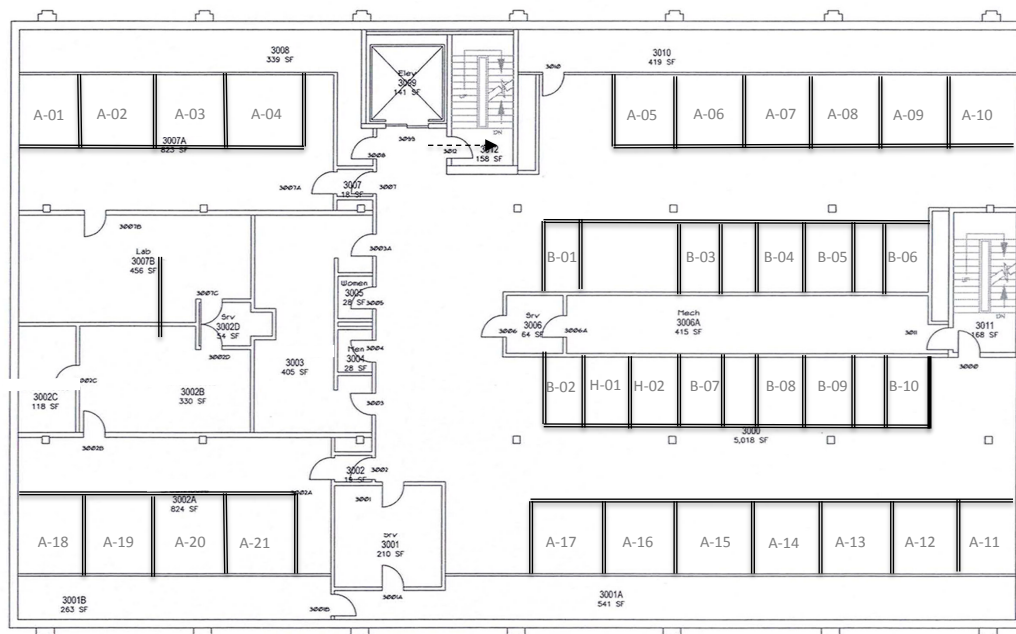
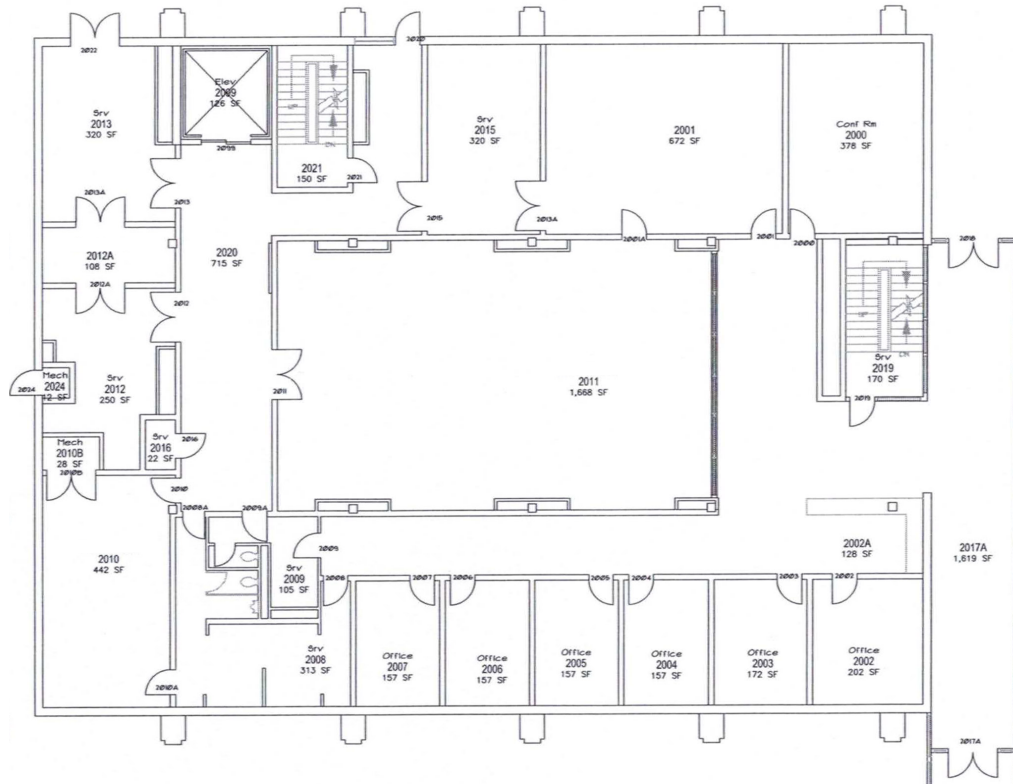


Figure 1. NCSU Phytotron First Floor



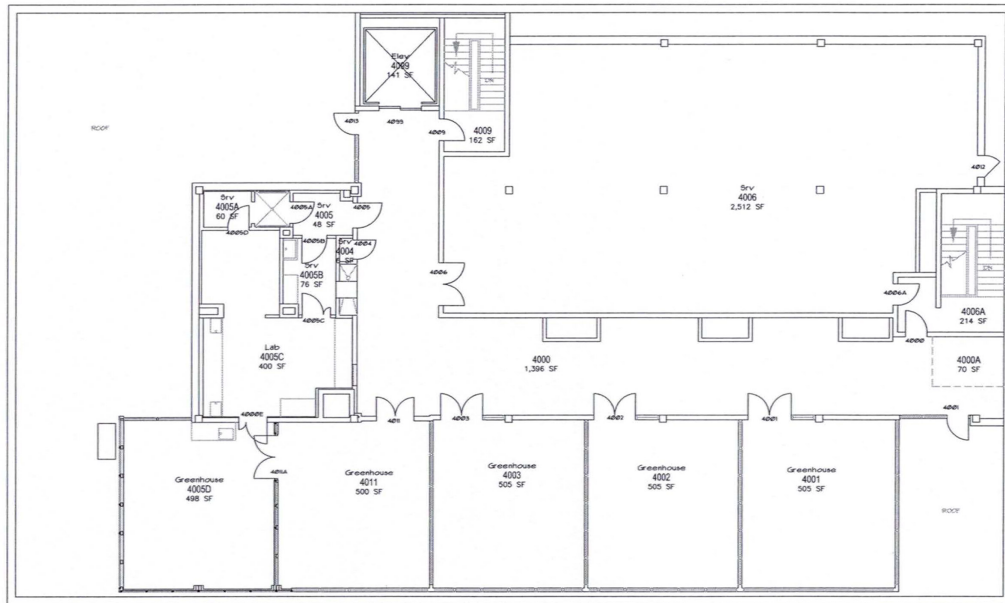


Figure 4. NCSU Phytotron Fourth Floor



Figure 5. Unshaded, temperature-controlled greenhouses.

Greenhouses

The Phytotron greenhouses are not whitewashed or otherwise shaded. As a result, the radiant flux density inside the greenhouses is 80 to 88% of natural-light conditions, depending on the solar azimuth (Fig. 6A). Although glazing is larger and structural members smaller and fewer than in conventional greenhouses, shadows from structural members still cause light reductions for relatively brief periods (Fig. 6B).

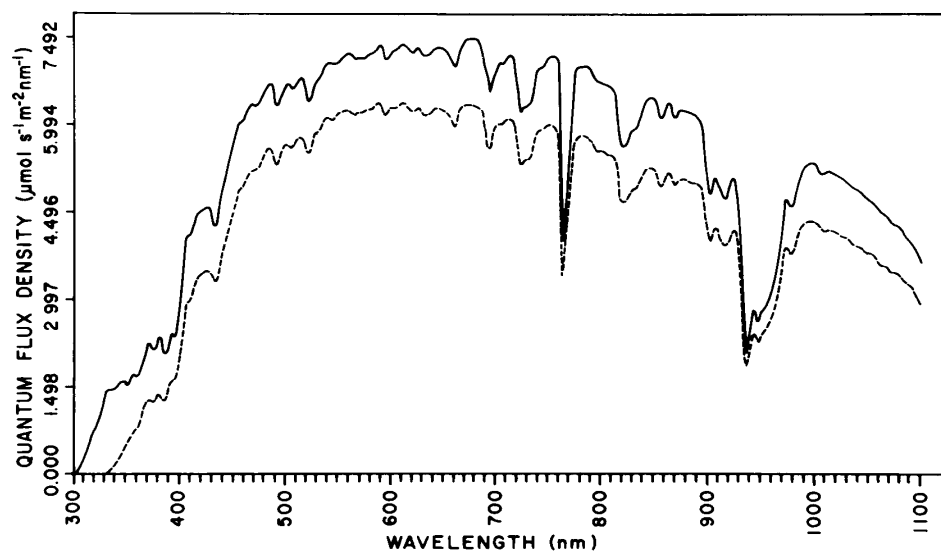


Figure 6A. Spectral energy distribution of sunshine outside and inside the Phytotron greenhouses on a sunny day.

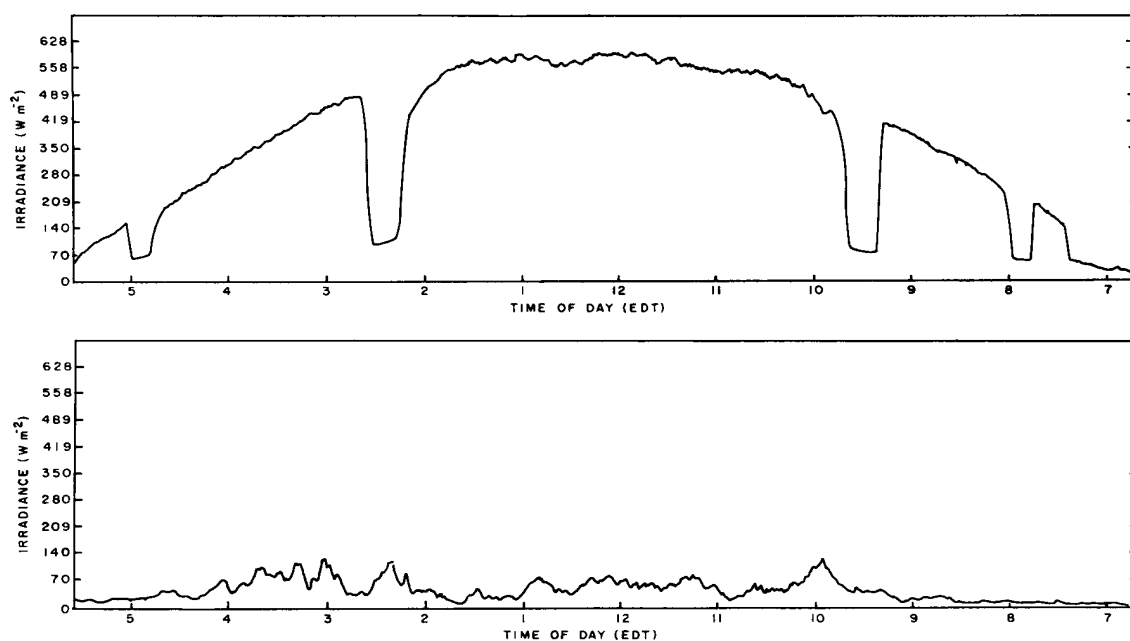


Figure 6B. Incident energy on clear (upper) and overcast (lower) days inside the Phytotron greenhouses (Downs, 1975). Note: pm on left, am on right.

A and B Chambers

The walk-in controlled-environment rooms provide growing areas of 9 and 3 m² respectively, with a vertical clearance of 2.13 m (Fig. 7).



Figure 7. Phytotron A-Chamber.

Temperature: Air temperatures can be selected over a range of 10° to 40°C. The variation about the set-point, measured with a type T (copper-constantan) thermocouple in a shielded, aspirated housing, is $\pm 0.25^\circ\text{C}$. If colder temperatures are needed, contact the Phytotron Director to discuss available options. The usual method of programming is to select a day and night temperature with sufficient differential to satisfy thermoperiod requirements. Additional diurnal temperature programs such as ramped or stepped regimes, however, can be programmed. *10 °C with the lights off.

Light: A combination of T-5, cool-white fluorescent (4100 Kelvin) and 60 W incandescent lamps are used results in the spectral energy distribution shown in Fig. 8. The lamps, separated from the growing area by a plexiglass barrier. Light levels are maintained within 12% of the initial level by a lamp-changing schedule. The vertical gradient of radiant flux density does not follow the inverse-square law, but varies as shown in Fig. 9.

The incandescent lamps may be programmed separately from the fluorescent in order to obtain different photoperiods with a minimum difference in photosynthetic photon flux density and long days can also be obtained by using a dark-period interruption from 11 p.m. to 2 a.m. with light from incandescent lamps only. In some chambers, reduced light levels can be obtained by reducing the number of operational lamps.

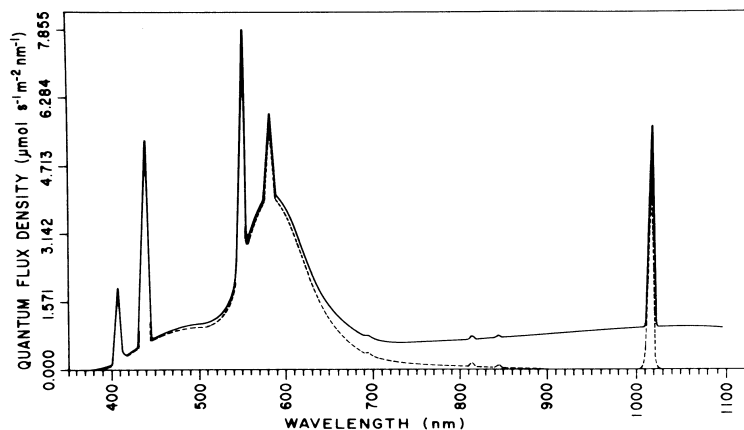


Figure 8. Spectral energy distribution in an A-type chamber fully lighted with 84 8-ft cool-white fluorescent lamps with and without 48 100-W incandescent lamps measured with a LI-COR 1800 spectroradiometer at truck level (113 cm from the barrier). This spectral energy produces 46 klx and a PPFD of 620 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

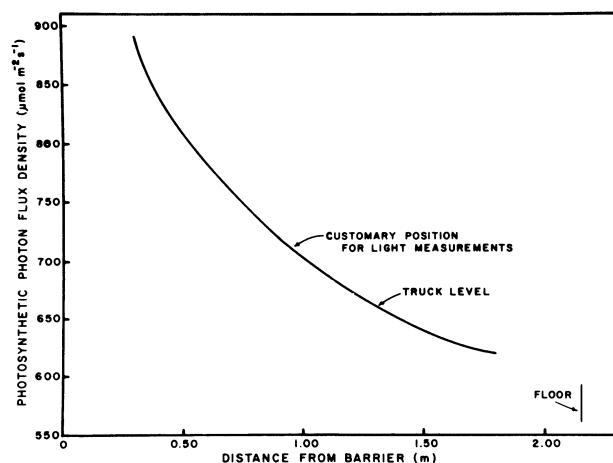


Figure 9. Vertical gradient of photosynthetic photon flux density in Phytotron artificially-lighted, controlled-environment rooms.

Relative Humidity: High humidity carts are available upon request and humidity levels can be increased. Relative humidities below 70% can be obtained by installing a dehumidifier in the chamber. For example, in a B-chamber, 30% RH at 18°C can be obtained with the additional dehumidifier provided a collection system for pot drainage water is installed. (Fig. 10).

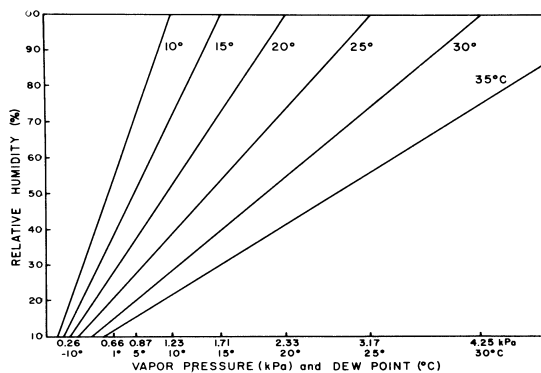


Figure 10. Relationship of relative humidity, vapor pressure and dew point temperature at different dry-bulb air temperatures.

Carbon Dioxide: CO₂ concentration is maintained between 400-450 ppm by controlled injection of commercial grade gas. This system also allows enhanced CO₂ levels of up to 2000 ppm.

C-Chambers

The reach-in C-chambers have a growing space of 0.91 x 1.22 m with a vertical clearance of 1.22 m (Fig. 11). Temperatures can be controlled between 10 and 40°C with $\pm 0.5^\circ\text{C}$ variation about the set point. C-chamber lighting is normally attained from T-5, cool-white fluorescent 4100 Kelvin) and 60 W incandescent lamps, so the spectral energy distribution (SED) is about the same as in the A and B chambers. Several light levels can be

provided by manual switching of lamp pairs. As different numbers of lamps are used, the SED is altered, primarily by changes in the ratio of photosynthetic photon flux density to photomorphogenic radiation (.



Figure 11. Phytotron C-Chambers

Relative humidity in the C-chambers normally fluctuates between 40 and 70%, but can be controlled between 30 and 85% in some chambers.

H-Chambers

The H-Chambers are walk-in rooms with a growing area of about 4 m² and a vertical clearance of 2.5 m, and are equipped with a 1:1 ratio of metal halide and high pressure sodium lamps plus incandescent lamps to provide a PPFD of 600-650 $\mu\text{mol s}^{-1} \text{m}^{-2}$.

Miscellaneous Chambers

Numerous reach-in chambers of different sizes and models are also located in the Phytotron. See the Phytotron Director for information about the capabilities and sizes that are available.

Pathology (BSL-3)

A BSL-3 laboratory with a greenhouse is located on the 4th floor and isolated from the rest of the Phytotron with unique and secure engineering and design features to function as a separate entity to provide for analyses and research with approved plant pathogen select agents. Access to the anteroom is through a card access reader. The anteroom serves as a changing room. After PPE is donned, entry through a second locked door leads to the Autoclave Room. The Autoclave Room houses the pass-through autoclave, and leads to the Main Laboratory which houses a Class II A-2 biosafety cabinet, sinks and drains connected to the effluent decon system, HEPA filtered room exhaust, and locking freezers. Opposite the entry through the main lab is access to the greenhouse where plants can be grown. To the right of the entry is the exit door through which PPE doffing and shower facilities lead back to the Anteroom. Researchers must study and follow biosafety guidelines in order to use the BSL-3 facility (Adair, D. and R. Irwin. 2008)

Greenhouse air is locally recirculated to meet temperature tolerance requirements. Air is between the Greenhouse and the Lab and is eventually fully exhausted through banks of HEPA filters on the rooftop. All liquid effluent from the containment facility is piped to basement Effluent Decon System where it is heat sterilized and pH balanced prior to sewer release.

Utilities

Hot and cold city water, nutrient solution and reverse osmosis (RO) purified water are available in or near all plant growing areas. Resin filtered water is available in some locations.

Three-wire, grounded, 120 V duplex receptacles are located in every controlled-environment space.

CULTURAL PRACTICES

Plant Density

The various controlled-environment chambers hold a certain optimum number of trucks. As the plants grow, the number of plants per truck may need to be reduced to prevent mutual shading. Some experimental procedures may allow an interim harvest to reduce the population. In other studies, the container size may need to be increased with a corresponding increase in the number of trucks used to carry the experiment. Since pot number and size requirements need to be decided during the planning stage of the study, pertinent information is included in Table 1.

Maximum loading of the A-chambers and greenhouses, rather than the optimum loading, may lead to overcrowding as the plants grow. However, the most serious problem encountered with maximum loading is that nondestructive measurements such as stem length, anthesis and fruit development, as well as many phases of the daily plant-care routines, will require some of the plants to be removed from the controlled environment.

Table 1. Truck and pot capacity of each type of controlled-environment chamber.

Unit	Usable Area (m ²)	No. of Trucks	Styrofoam Cups (225 ml)	Number of Pots			
				114 mm Dia. (660 ml)	152 mm Dia. (1650 ml)	203 mm Dia. (4000 ml)	254 mm Dia. (6000 ml)
Truck	0.37	1	36	16	9	5	4
A-Chamber							
Maximum	8.90	24	864	384	216	120	96
Optimum	5.57	16	576	256	144	80	64
B-Chamber							
Maximum	2.97	8	288	128	72	40	32
Optimum	2.97	8	288	128	72	40	32
C-Chamber*							
Maximum	1.11	*	192	90	40	28	16
Optimum	1.11	*	135	60	40	24	12
Greenhouses							
Maximum	33.00	88	3168	1408	792	440	352
Optimum	25.5	60	2160	960	540	300	240

•Reach in unit

Nutrition and Substrate

A standard nutrient solution (Table 2) of defined analysis (Table 3) and a standard substrate composed of gravel and peat-lite have been developed for use in the Phytotron. The steam-sterilized and washed gravel is #16 construction grade. The peat-lite is commercial mixture (Redi-Earth, Sun Gro Horticulture) of peat moss and vermiculite based on the original "Cornell Mix" (Boodley and Sheldrake, 1972).

The Phytotron also stocks other substrates, such as vermiculite and river-bottom sand. Pine bark, soil and other growing media can be used, but they will need to be purchased, and brought to the Phytotron receiving room by the investigator. Please see Phytotron Director to make arrangements.

Daily care of the plant material will be performed by the Phytotron staff, provided one of the watering schedules shown in Table 4 can be used. All other schedules, and applications of any special nutrient solution, are to be performed by the investigator.

Table 2. NCSU Phytotron Nutrient Solution

Stock Solution (g)	Formula Weight	Grams/liter of stock solution
"A"		
Magnesium nitrate $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	256.41	26.0
Calcium nitrate $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	236.15	64.0
Sequestrene 330 Fe 10% Fe		10.0
"B"		
Potassium nitrate KNO_3	101.11	40.44
Ammonium nitrate NH_4NO_3	80.04	16.00
Potassium phosphate mono KH_2PO_4	136.09	4.80
Potassium phosphate dibasic K_2HPO_4	174.18	5.60
Potassium sulfate K_2SO_4	174.27	6.00
Sodium sulfate Na_2SO_4	142.04	6.80
Boric acid H_3BO_3	61.83	0.28
Molybdic acid $\text{MoO}_3 \cdot 2\text{H}_2\text{O}$	179.97	0.002
Zinc sulfate $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	287.54	0.011
Manganous chloride $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	197.9	0.0816
Copper sulfate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	249.7	0.004
Cobalt chloride $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	237.9	0.00024
Uranine		0.10

1. The compounds containing the minor elements are dissolved together before being added to the "B" stock tank.
2. Stock solutions are proportioned at the rate of 1 ml "A" + 1 ml "B" per 200 ml RO purified H_2O .
3. Phytotron nutrient pH values are:
RO purified H_2O 6.20

Stock Solution "A" 2.60
Stock Solution "B" 6.25
Nutrient Solution 6.25

4. Uranine (sodium fluorescein) is added to Stock "B" to give the nutrient solution a green color so that it may be distinguished from H_2O .











*While usage of the unit 'grams/liter' is common laboratory practice, the correct but less familiar SI units would be kg m^{-3} or mg m^{-3}

Table 3. Analysis of the Phytotron Nutrient Solution.

Element	Symbol	Source	Total ppm in the solution*
Nitrogen	N	$\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ NH_4NO_3 KNO_3	106.23
Phosphorus	P	KH_2PO_4 , K_2HPO_4	10.41
Potassium	K	KH_2PO_4 , K_2HPO_4 K_2SO_4 , KNO_3	111.03
Calcium	Ca	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	54.40
Magnesium	Mg	$\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	12.40
Iron	Fe	Sequestrene 330	5.00
Sulfur	S	K_2SO_4 , Na_2SO_4	13.19
Manganese	Mn	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.113
Boron	B	H_3BO_3	0.24
Zinc	Zn	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.013
Copper	Cu	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.005
Cobalt	Co	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.00003
Molybdenum	Mo	$\text{MoO}_3 \cdot 2\text{H}_2\text{O}$	0.005
Sodium	Na	Na_2SO_4	11.04

*While usage of the unit "ppm" is common laboratory practice, the correct but less familiar SI unit would be mol m^{-3} .

Table 4. Watering schedules maintained by the Phytotron staff

WATERING & LABELING SCHEDULE			
Label	Color	Morning	Afternoon
	Blue	Nutrient	Nutrient
	Green	Nutrient	None
	Green & White	Deionized H ₂ O	Nutrient
	Yellow	Deionized H ₂ O	None
	Yellow & White	Deionized H ₂ O	Deionized H ₂ O
	Red	None	None
	Orange	Nutrient 1X Mon Wed Fri	
	Pink	Deionized H ₂ O 1X Mon Wed Fri	
	Wood	Naming & Numbering of Plants	
	White		

OPERATIONAL PROCEDURES

Pest Control

We work to keep the Phytotron pest-free and prefer that plants are started from seed in the Phytotron instead of grown elsewhere brought to the Phytotron. If you have a reason for growing your plants for Phytotron experiments at another location, contact the Phytotron Director to discuss your options.

All supplies, equipment, plants and substrates enter and leave the Phytotron via the receiving room. Most items are treated before transfer to the Phytotron interior. Sterile cultures, insect research material and analytical instrumentation are usually exempted. Soils and other non-Phytotron substrates must be steam pasteurized elsewhere by the investigator so only the substrate container is involved in the fumigation process.

We ask researchers to come to the Phytotron before going to other greenhouses or out to the field to prevent insect infestations in the Phytotron. Insect and disease problems result primarily from investigators bringing contaminants into the Phytotron on their persons. The resulting infestations as a rule, first affect the plant material of the person that carried the contaminant. Obviously, it is to everyone's advantage to observe conscientiously the clean-up procedures and to attend first to Phytotron work before visiting other greenhouses or field locations.

If you observe insects on your plant material, contact the Phytotron Research Operations Manager for treatment.

Responsibility of the Investigator

The project leader is expected to use clean seeds, free of latent diseases and insect problems. The investigator is responsible for planting the seeds and will arrange for all experimental treatments and special watering programs, as well as proper labeling, staking, pruning, pinching, and sucker removal. Well-trained personnel can be hired through the Phytotron Director as hourly assistants to aid in initiating experiments, assigning or administering experimental treatments, and taking data.

The project leader is expected to assume responsibility for the safety training (i.e. equipment operation, handling chemicals, laboratory safety) for all members of the research group.

Special care should be taken to identify the desired watering schedule following the color code in Table 4. *Any trucks without labels will be watered twice daily with Phytotron nutrient solution.*

Since the investigator establishes the routine of the research program, it seems reasonable to expect the schedule to be followed. If the schedule is not followed and the assigned chambers remain empty for as much as 5 days, we assume the study has terminated and the space is assigned to others. These problems can be avoided by informing the Director of the Phytotron whenever unforeseen circumstances force an alteration of the initial schedule of research events.

Reserving Phytotron Equipment

You may reserve Phytotron equipment such as the Leaf Area Meter, Seed Cleaner or Seed Sorter on the Phytotron website. Please make arrangements at least 24 hours before equipment is needed and ask Phytotron staff for assistance if you have not used our equipment previously. You can also request to have plant material freeze dried using the website. A staff member will contact you to make arrangements.

Role of the Phytotron Staff

The Phytotron staff sets up and maintains the environmental conditions. Any suspicion of an off-normal situation should be reported immediately to Phytotron Staff. The Phytotron staff prepares substrates and will provide the investigator with the correct number of trucks, racks and pots of the specified size. On the scheduled planting date, the staff may be available to help fill the pots with the specified substrate. This assistance is not automatic and arrangements should be made in advance. Availability of staff will depend on staff work schedule at the time and availability of students.

Phytotron Operating Schedule

Initiation of a study and the establishment of a new set of environmental conditions on a Friday is not recommended. Therefore, with few exceptions, environmental conditions are established early in the week and allowed to proceed for a 24-hour period before experiments are initiated. Once the experiment is started, the chambers are not to be opened by anyone, including the investigator, when the dark-condition, red-light indicator is on. Only by following this rule can everyone be assured of photoperiod integrity.

The Phytotron staff are between 7:30 am and 4:30 pm. Therefore, delivery or removal of equipment, supplies, and new or harvested plant materials should occur between 7:30 am and 4:30 pm, Monday through Friday, unless arrangements are made with the staff.

Program Changes

Chamber program adjustments, replacement or new cart requests can be made on the Phytotron website or the iPad in the lobby of the Phytotron. We require that requests are made in writing to avoid misunderstandings. The investigator should be aware that changes may not be feasible because of space limitations and that extensions of time may not be possible because of conflict with subsequent space assignments.

Project Termination

You must notify the Phytotron Director in writing when you terminate your experiment. There is a form of the website that can be submitted or you can send an email. There is also a form on the website to make arrangements to have your plant material devitalized.

Visitors

Members of the Phytotron staff are always available to discuss the facility with visitors. Phytotron users also may conduct visitors through the facility. The visitors should be asked to record their names and organizations in the guest book located in the conference room. Advance notice of visitors will keep disruptions of the working schedule to a minimum and will insure availability of the necessary clean-room clothing.

Standard Methods of Reporting Environmental Conditions

The NCERA-101 Committee on Controlled Environment and Technology and Use have worked with their international partners to develop International Controlled Environment Guidelines. The Guidelines for reporting environmental conditions used in biological research can be found on their website (<https://www.controlledenvironments.org/international-controlled-env-guidelines/>). In addition, the Growth Chamber Manual edited by Langhans, and Tibbits, 1997 can also be found on the NCERA-101 website (<https://www.controlledenvironments.org/growth-chamber-handbook/>). These guidelines should be followed where applicable. The descriptive information suggested in the guidelines can be summarized by using this manual as a cited reference for the Phytotron nutrient solution and details of the pertinent features of the controlled-environment rooms, in the methods section along with the environmental conditions. The recommended standards are listed in Table 4 and should be used without exception.

Table 4. Guidelines for Measuring and Reporting the Environment for Plant studies

Parameter	Typically Used Unit	Measurements		
		Where to take	When to take	What to report
Radiation:				
PAR (Photosyn- thetically active radiation)				
a) Photosynthetic photon flux density (PPFD) 400-700 nm with cosine correction	$\mu\text{mol s}^{-1} \text{m}^{-2}$	At top of plant canopy. Obtain average over plant growing area.	At start and finish of each study and bi- weekly if studies extend beyond 14 days.	Average over containers at start of study. Decrease or fluctuation from average over course of study. Wavebands measured.
b) Photosynthetic Irradiance 400-700 nm with cosine correction	W m^{-2}	(Same as PPFD)	(Same as PPFD)	(Same as PPFD)
Total irradiance with cosine correction indicate bandwidth.	W m^{-2}	(Same as PPFD)	At start of each study.	Average over containers at start of study. Wavebands measured.
Spectral irradiance 250-850 nm in 20 nm bandwidths with cosine correction.	$\text{W m}^{-2} \text{nm}^{-1}$ or $\mu\text{mol s}^{-1} \text{m}^{-2} \text{nm}^{-1}$	At top of plant canopy in center of growing area.	At start of each study.	Graph or table of irradiance for separate wavebands.
Illuminance*** 380-780 nm with cosine correction	klx	(Same as PPFD)	At start of each study.	(Same as total irradiance)
Carbon dioxide:	mmol m^{-3}	At top of plant canopy.	Hourly over the period of the study.	Average of hourly average readings and range of daily average readings over the period of the study
Watering:	liter (ℓ or L)	---	At times of additions.	Frequency of watering. Amount of water added per day and/or range in soil moisture content between waterings.
Substrate:	---	---	---	Type of soil and amendments. Components of soilless substrate. Container dimensions.
Nutrition:	Solid media: mol kg^{-1} or mol m^{-3}	---	At times of nutrient additions	Nutrients added to solid media. Concentration of nutrients in liquid additions and solution culture. Amount and frequency of solution addition and renewal.
pH.	pH units	In saturated media, extract from media, or solution of liquid culture.	Start and end of stu- dies in solid media Daily in liquid culture and before each pH adjustment.	Mode and range during study.
Temperature:				
Air Shielded and aspirated (3 m sec ⁻¹) device	$^{\circ}\text{C}$	At top of plant canopy. Obtain average over plant growing area.	Hourly over the period of the study (con- tinuous measurement advisable).	Average of hourly average values for light and dark periods of study with range of variation over growing area.
Soil or liquid	$^{\circ}\text{C}$	In center of container.	Hourly during the first 24 hr of the study. Start immediately after watering (monitoring over the course of the study advisable).	Average of hourly average values for the light and dark periods for the first day or over entire period of the study if taken. Location of measurement.

Table 4. Continued.

Parameter	Typically Used Unit	Measurements		
		Where to take	When to take	What to report
Atmospheric moisture: Shielded and aspirated (3 m sec ⁻¹) psychrometer, dewpoint sensor or infrared analyzer	% RH, dewpoint temperature, or g m ⁻³	At top of plant canopy in center of plant growing area.	Once during each light and dark period taken at least 1 hr after light changes. Monitoring over the course of study advisable.	Average of once daily readings for both light and dark periods with range of diurnal variation over the period of the study (or average of hourly values if taken).
Air velocity:	m s ⁻¹	At top of plant canopy. Obtain maximum and minimum readings over plant growing area.	At start and end of studies. Take 10 successive readings at each location and average.	Average and range of readings over containers at start and end of study.
Electrical conductivity:	dS m ⁻¹ **** (decisiemens per meter)	In saturated media, extracted from media or solution of liquid culture.	Start and end of studies in solid media. Daily in liquid culture.	Average and range during study.

*Proposed by the North Central Regional Committee (NCR-101) on growth chamber use.

**This is preferred because it follows the SI convention. However, since 1 Einstein = 1 mol of photon, the values are equivalent. It is inaccurate to report that "radiation values are XX.X $\mu\text{mol s}^{-1}\text{m}^{-2}$ ". This is wrong for the same reason that reporting mol kg⁻¹ is without associating that value and units with the element (i.e. K was 300 mol kg⁻¹). Thus, "the PPFD was 320 $\mu\text{mol s}^{-1}\text{m}^{-2}$ " is correct since it specifically associates a definition (i.e. photons with a certain waveband) with the value and units.

***Report with PAR reading ONLY for historical comparison.

****dS m⁻¹ = mho cm⁻¹

MEASUREMENT OF ENVIRONMENTAL CONDITIONS

Temperature

Temperature in the Phytotron refers to air temperature unless specifically stated otherwise. Generally, air temperature is sensed by a type T (copper-constantan), 24 gauge (0.51 mm), welded thermocouple mounted in a shielded, aspirated housing. The control sensor is usually a resistance element, also mounted in the aspirated housing. Two or three additional sensors may be placed in strategic locations to act as lead-lag elements for the room sensor. Aspirating the temperature sensor results in a room averaging effect and avoids the error (up to 13°C) that can occur with non-aspirated sensors. This error occurs because most thermal elements have heat capacities that enable them to detect radiant energy. Consequently, if the thermocouple is placed in some location other than the shielded, aspirated housing, the temperature reading may not represent the air temperature at plant level, and the variation about the set point may be different (Fig. 12).

Research material enclosed within a container such as a flask, test tube, plastic bag, or Petri dish will be at a higher temperature than the ambient air during the light period because of the "greenhouse effect" and the very low air movement. The differential between the temperature inside and outside the container, therefore, depends on the amount of radiant energy and spectral distribution of the light source as well as the kind of container and the way it is closed. Because of the thermal inertia of the container, temperature fluctuations are smaller than those of the air. Moreover, the contents of the container will respond more slowly to a temperature change, and more time will be required to reach alternating day/night levels (Fig. 13). Air temperature is adjusted by the Phytotron staff to whatever level is necessary to produce the desired temperature inside the container and several 24-hour records are obtained to describe the interior temperature conditions.

In addition, each controlled-environment room is equipped with a Partlow, round-chart recorder operated from a

hydraulic temperature sensor located in the return air duct. The round-chart recorders serve as trend indicators for troubleshooting and early detection of set point drift and variation. They are *not* used to set or measure temperature, because while these instruments are calibrated to read air temperature, they also sense temperature artifacts. Moreover, due to a narrow scale span calibration is much less precise.

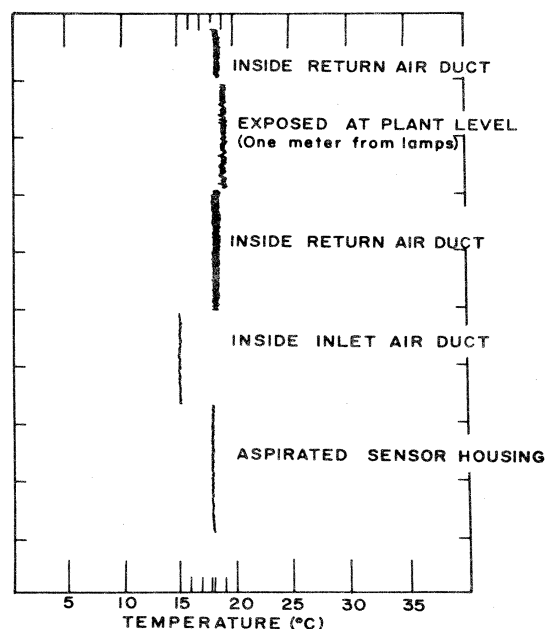


Figure 12. Temperatures indicated by #24 thermocouples at various locations in a single controlled-environment.

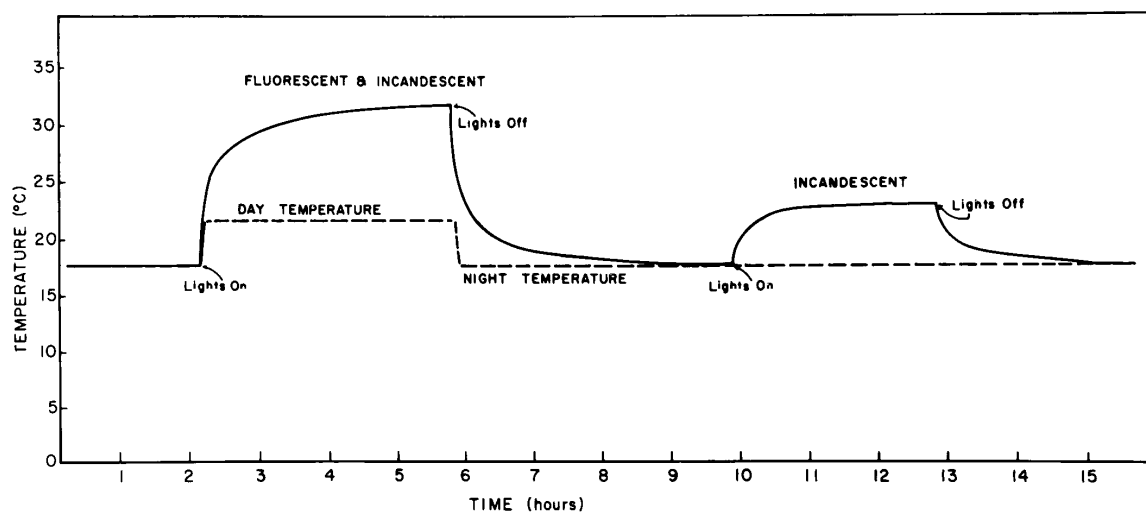


Figure 13. Temperature inside a cotton stoppered Erlenmeyer flask (solid line) in controlled-environment room with a 22/18°C day/night air temperature (dashed line).

Radiant Energy

The physical aspects of radiant energy have been standardized and unambiguous definitions of terms can be found in a number of references. Radiant energy in the Phytotron is measured as illuminance, photosynthetic photon flux density (PPFD) (Downs, 1988a), flux density of photomorphogenic radiation (PR) and occasionally as irradiance and photon fluence rate. This enables the investigator to relate a familiar unit of measurement to current standards. The spectral energy distribution of the light sources is available on request.

Illuminance: Illuminance, like irradiance, is the process of interception of energy. Also called luminous flux density, illuminance is simply the amount of light falling on a unit area. Thus, a foot-candle represents the amount of light incident upon one square foot of surface (a lumen per square foot). The meter-candle, properly called a lux (Ix), indicates luminous flux per square meter. The foot-candle or lux is *not* a measure of intensity. Intensity refers to the light source and provides little information about the amount of light received by the plants.

Light is visually evaluated radiant energy where the evaluation is made by multiplying the energy at each wavelength by the spectral luminous efficiency for photopic vision at each wavelength (Fig. 14). The resulting measurement, therefore, is spectrally sensitive and considerably influenced by the spectral energy distribution (color) of the light source. A green lamp, for example, may produce more lux than a white or red lamp, even though the total radiant emittance of the green lamp may be less.

Photosynthetic Photon Flux Density (PPFD): Since photosynthesis is a quantum process, the most realistic measurement of the radiation used by the photosynthetic system is the number of photons within a specific waveband falling on a unit area per unit time. Usually the waveband is 400-700 nm and the units are $\mu\text{mol m}^{-2}\text{s}^{-1}$. The mole is simply the amount of some substance. The substance must be identified to make the unit complete.

Measurement units in biology now follow the rules of the International System of Units (SI, Downs, 1988b), so technically PPFD should be reported as moles per unit area in a unit of time. SI rules, however, do not allow the inclusion of a qualifier, such as mol quanta $\text{m}^{-2}\text{s}^{-1}$, to describe what the moles are; thus, the unit must be preceded or followed by a descriptor. Current practice is to describe the measurement as a photosynthetic photon flux density, or PPFD, of some number of $\mu\text{mol m}^{-2}\text{s}^{-1}$ (Table 10).

Photomorphogenic Radiation (PR): The radiant flux density of photomorphogenic radiation defines the energy in the far-red region of the spectrum in terms of W m^{-2} . Other wavelength regions also induce photomorphogenic effects, but in practice the amount of far-red relative to the photosynthetic photon flux density seems to exert the greatest influence on morphogenesis. A specially-calibrated sensor is used with the Li-Cor meter to measure the PR flux density between 700-850 nm or the 700-850 nm radiation is calculated from the spectral energy distribution data. This waveband usually will provide an excellent estimate of the photomorphogenic effects of various light sources. However, if the light source has a strong emission line between 800-850 nm, the measurement will indicate a higher PR level than demonstrated by the plant response.

Irradiance: Irradiance measurements do not relate well to plant growth because such a large portion of the measured energy is beyond the range of the photosynthetic and photomorphogenic photoreactions. As a result, irradiance measurements in the Phytotron usually are confined to the greenhouses and are used only to relate to meteorological data.

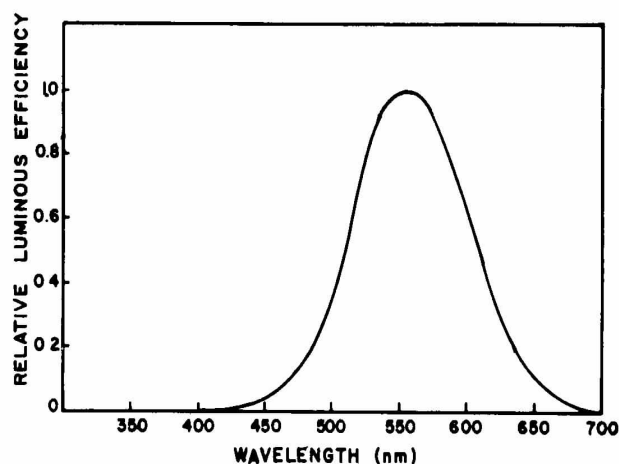


Figure 14. Spectral sensitivity of the human eye.

Air Velocity

Air flows through controlled-environment rooms of the Phytotron from top to bottom. This type of flow results in a certain amount of turbulence (Fig. 17). The average air velocity is 0.46 m s^{-1} in the A-chambers, 0.5 m s^{-1} in the B-chambers, 0.4 m s^{-1} in the C-chambers and 0.85 m s^{-1} in the glasshouses.

Relative Humidity

The Phytotron provides instruments to measure relative humidity and dew point. Although atmospheric moisture is often expressed as relative humidity, vapor pressure deficit, or more correctly
The photobiologist often uses the term fluence rate to describe the radiation field because it more precisely describes the radiation falling on the plant than does flux density. PPFFR is defined as the number of saturation deficit, is preferable when related to biological research (see water relations references). Dew point is the temperature at which the water vapor in an air sample will condense. If the air temperature is known, dew point temperatures offer ready access to saturation deficits. For example, the saturation pressure at a 14°C dew point is 1.60 kPa (16.0 mb) and at 25°C air temperature is 3.17 kPa (31.671 mb). By subtraction the saturation deficit is 1.57 kPa (15.671 mb), and the ratio $1.60/3.17$ (100) provides the relative humidity 50.4% (Fig. 10). A dew point temperature of 14°C represents 10 grams of water per 1000 grams of dry air. The relative humidity produced by this moisture content depends on the air temperature, for example, 94% at 15°C , or 50% at 25°C . Obviously, relative humidity values have little meaning unless air temperature, dew point or vapor measure also is reported; whereas dew point temperature provides immediate access to pertinent atmospheric moisture information.

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PHYTOTRON STAFF DUTIES

Director provides consultation concerning environmental, biological and cultural phases of the research programs.

Research Unit Manger assists the investigators in initiating their studies, including programming the initial environmental conditions. Most of the problems that might arise during the course of a study can be brought directly to the Research Unit Manger for action.

Phytotron Research Technicians carry out the daily care of the plant material, controlled-environment rooms and building, including preparation of substrate, trucks and plant containers at the start of the program and their disposal at the close of the study.

Mechanical and Electronics Personnel- keep the Phytotron operating and, when time permits and requested through the director, may assist investigators in developing special equipment and instrumentation.

Part-time Technicians are available to assist investigators during the course of their program, providing that arrangements are made at the time the space request is approved.

CONVERSION FACTORS

<u>To Convert From</u>	<u>To</u>	<u>Multiply By</u>
Acres	hectares (ha)	0.404
Cubic feet/min (cfm)	liters/s	0.472
	m^3/s^{-1}	1.698
Cubic inch	m^3	1.639×10^{-5}
	mm^3	16.39
Cubic foot	m^3	0.0283
Cubic yard	m^3	0.765
Foot	m	0.3048
Feet/min (fpm)	m/s	0.0051
Firkin	liters	34.1
Foot-candle	lux	10.48
Furlong	m	201.2
gallons/min (gpm)	liters s^{-1}	0.063
hectare	m^2	10,000
inch	mm	25.4
Langley/min	W/m^2	0.0698
kg/cm^2	kPa	98
miles per hour	km/h	1.6
"	m/s	1608
mm Hg	Pa	133.3
ounce (avdp)	g	28.35
ounce (U.S. Fluid)	ml	29.57
pound (avdp)	g	453.6
pounds/ in^2	g/mm^2	0.703
pounds/ ft^2	kg/m^2	4.88
pounds/ ft^3	k/m^3	16
quart (U.S. Fluid)	liters	0.946
scruple	g	1.3
square foot	m^2	0.0929
square yard	m^2	0.836
Ton of refrigeration (U.S. Comm.)	BTU/h	12,000
	horsepower	4.72
	MJ/s	12.65
metric ton	kg	1000
short ton	kg	907.18
yard	m	0.914
lux (cool-white Fluorescent)	$\mu\text{mol m}^{-2}\text{s}^{-1}$ (400-700)	0.0134*
lux (incandescent)	" "	0.02*

NATURALLY-OCCURRING DAYLENGTHS FOR RALEIGH, NC

Sunrise and sunset are considered to occur when the upper edge of the solar disc appears exactly on the horizon. Civil twilight ends or begins when the center of the sun is 6° below the horizon. Nautical twilight ends or begins when the center of the sun is 12° below the horizon. The upper limit of twilight equals sunrise or sunset, and the illuminance based on the unobscured sun would be 420 lux. At the lower limit the civil twilight illuminance would be 3.2 lux and the nautical twilight illuminance 0.009 lux. (Tables of Sunrise, Sunset and Twilight. Supplement to the American Ephemeris, Nautical Almanac Office, U.S. Naval Observatory, 1946.)

