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PHYTOTRON PROCEDURAL MANUAL

For Controlled-Environment Research at the
Southeastern Plant Environment Laboratory

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On the Cover (from left and then down): Poppies(*Papaver nudicale*'Temptress'), Seabeach Amaranth(*Amaranthus pumilus*), Sunflower (*Helianthus annus*), Rhododendron (*Rhododendron catawbiense*), Snap Bean(*Phaseolus vulgargis*), Coneflower (*Echinacea sp.*), Delphinium(*Delphinium x cultorum* 'Magic Fountains'), Soybean (*Glycine max*), Bromeliad(*Aechmea fasciata variegata*)

Phytotron Procedural Manual

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For Controlled Environment Research at the Southeastern Plant Environment Laboratory

INTRODUCTION

The Southeastern Plant Environment Laboratory (SEPEL) is the official name of the Phytotron* at North Carolina State University. SEPEL is a regional laboratory available to any biologist requiring controlled-environment facilities. Laboratories, office space, and instrumentation are available to visiting scientists wishing to conduct extensive on-site work. Investigators also have access to excellent library facilities and complete computer services.

Many kinds of research conducted in the Phytotron do not require the daily presence of the investigator. In such cases, the scientist only needs to appear at critical times during the course of the study because daily care of the biological material and maintenance of the controlled-environments are managed by the Phytotron Staff.

Details of research activities in the Phytotron are summarized every year and presented informally in our Annual Phytotron Report.

USE OF FACILITIES

A prospective user of Phytotron space needs to submit a resume of the proposed research to the Director of the Phytotron (Appendix 1). The proposal form can be completed and submitted on line at http://www.ncsu.edu/phytotron/application.html. Paper copies are also available at the Phytotron. 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 and the proposed procedures. Experimental design guidelines are included in Appendix II and references for controlled environment usage and parameters are in Appendix III.

*A Phytotron is a collection of controlled-environment cabinets, rooms, and glasshouses organized in such a way that many combinations of environmental factors can be studied simultaneously.

As an example, a simple program might be:

Day	Experimental Procedure
1	Place seeds in germinators.
2	Transplant pregerminated seeds to 10-cm containers filled with standard Phytotron substrate; place all containers in GH at 26/22^{o}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 nonroutine operations, and 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 or Assistant 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

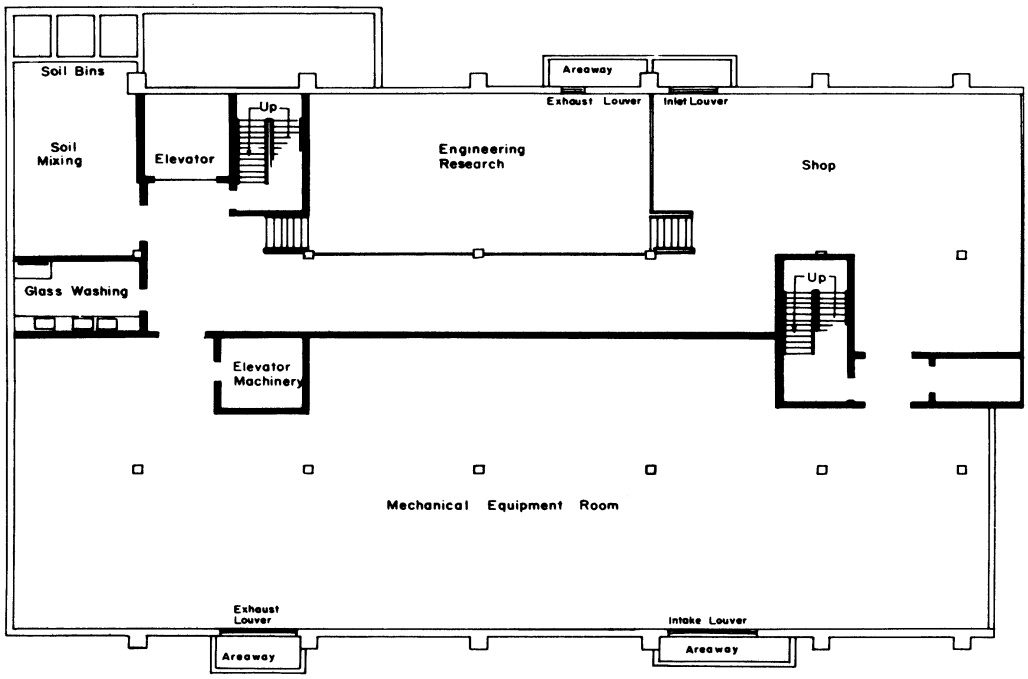

Figure 1. NCSU Phytotron - First Floor

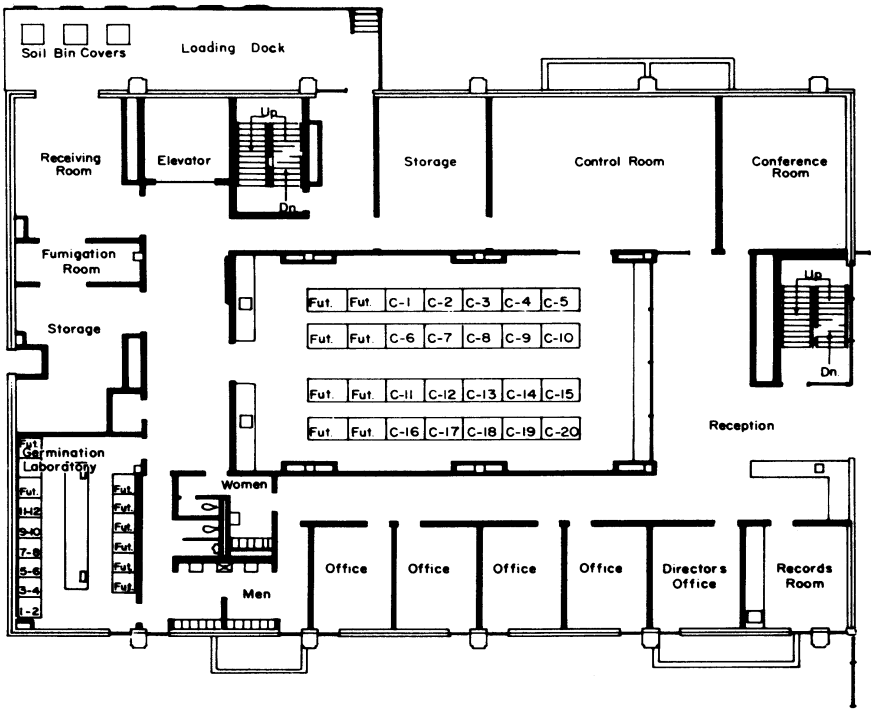

Figure 2. NCSU Phytotron - Second Floor

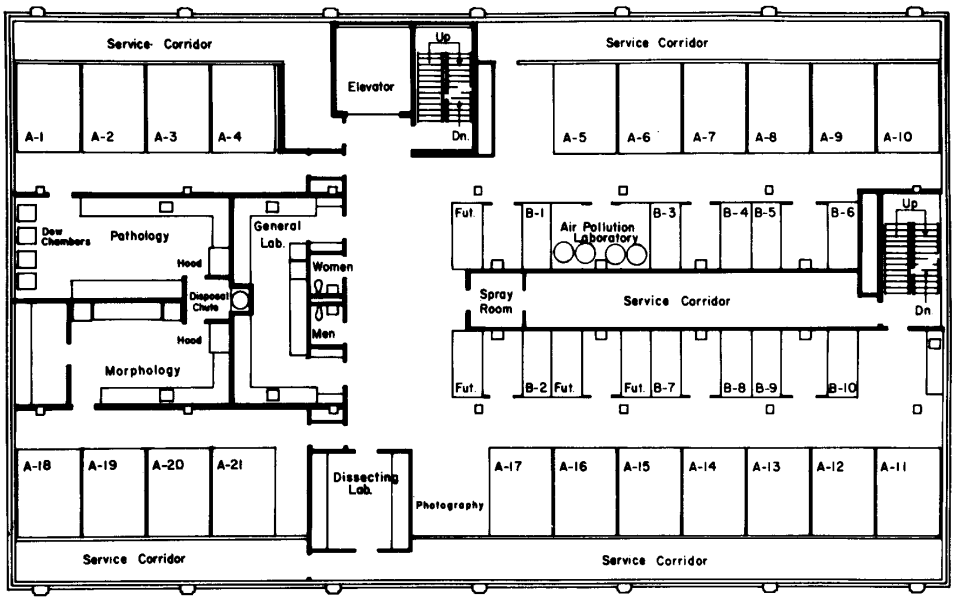

Figure 3. NCSU Phytotron - Third Floor

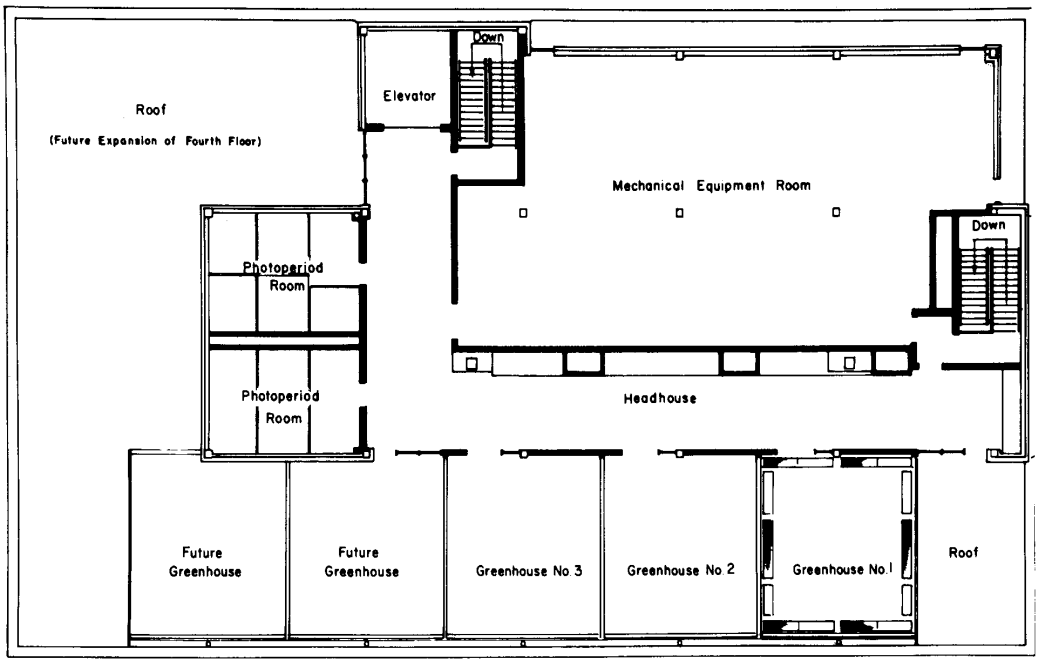

Figure 4. NCSU Phytotron - Fourth Floor

supplemental services, such as use of Phytotron research assistants and construction of special equipment. Costs for projects approved and funded by, or in cooperation with, the North Carolina Agricultural Research Service (NCARS) are paid by NCARS through the Phytotron operational budget. All other projects transfer funds to the budget line given on the quarterly invoice statement.

Table 1. Physical Characteristics of the Southeastern Plant Environment Laboratory.

Building Area	3922 m^2
Building Volume	18589 m^3
Electrical Supply 480/277 V.3 ph Y-connected	
Present connected load	2240 KVA
Possible connected load	3000 KVA
Refrigeration	
Chilled water	3720 MBH*
Ethylene glycol	3053 MBH
Controlled-Environment Chambers	
Greenhouses 3	49 m^2
Cooling	360 MBH
Heating, Steam	780 MBH
Light Incandescent	2700 W
A-Chambers 22	8.90 m^2
Cooling	87 MBH
Heating, Electrical	3000 W
Fluorescent lamps	18060 W
Incandescent lamps	4800 W
B-Chambers 10	3.15 m^2
Cooling	40 MBH
Heating, Electrical	1500 W
Fluorescent lamps	6020 W
Incandescent lamps	2400 W
C-Chambers 21	1.11 m^2
Cooling	6 to 9 MBH
Heat, Electrical	1000 W
Fluorescent lamps	1760 W
Incandescent lamps	600 W
Anterooms 4	3.8 m^2
Cooling	no load data
Heating, Electrical	750 W
Fluorescent lamps	320 W
Incandescent lamps	600 W
Seed Germinators 12	
Cooling	no load data
Heating, Electrical	
Incubators 2	
Cooling	6 MBH
Dew Chambers 4	
Cooling	6 MBH
Air Pollution Treatment Chambers 8	
Cooling, Heating	no load data
Light, Metal Halide	1000 W
Photoperiod rooms 9	3.5 to 6.5 m^2
Cooling, Heating	no load data
Fluorescent lamps	220 to 440 W
Incandescent lamps	900 to 1800 W

*MBH=Thousand British Thermal Units per hour. 1 MBH= 8.33 X 10^{-2} (U.S. Comm.) tons of refrigeration

FACILITIES

The four-story Phytotron contains areas for general studies, and for research in plant pathology, morphology, and seed germination. The facility contains *57* controlled-environment chambers of various sizes (Table 1), plus seed germinators, incubators, hydroponic units, photoperiod rooms and air pollution treatment chambers. Laboratories are available for anatomical analyses (Figs. 1, 2, 3, 4).

Greenhouses

Temperatures in the 49.m^2, air-conditioned greenhouses (Fig. *5)* can be maintained at any point between 8 and 40^0C, but they are usually operated at 30/26, 26/22, and $22/18^0$C day/night temperatures. The day/night temperatures are coordinated with the use of the photoperiod rooms and operate on a 9/15 hour cycle. Additional temperature regimes can be obtained by moving plants between greenhouses, and between greenhouses and photoperiod rooms.

Figure 5. Unshaded, temperature-controlled greenhouses.

The Phytotron greenhouses are not white-washed 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 considerable light reductions for relatively brief periods (Fig. 6B).

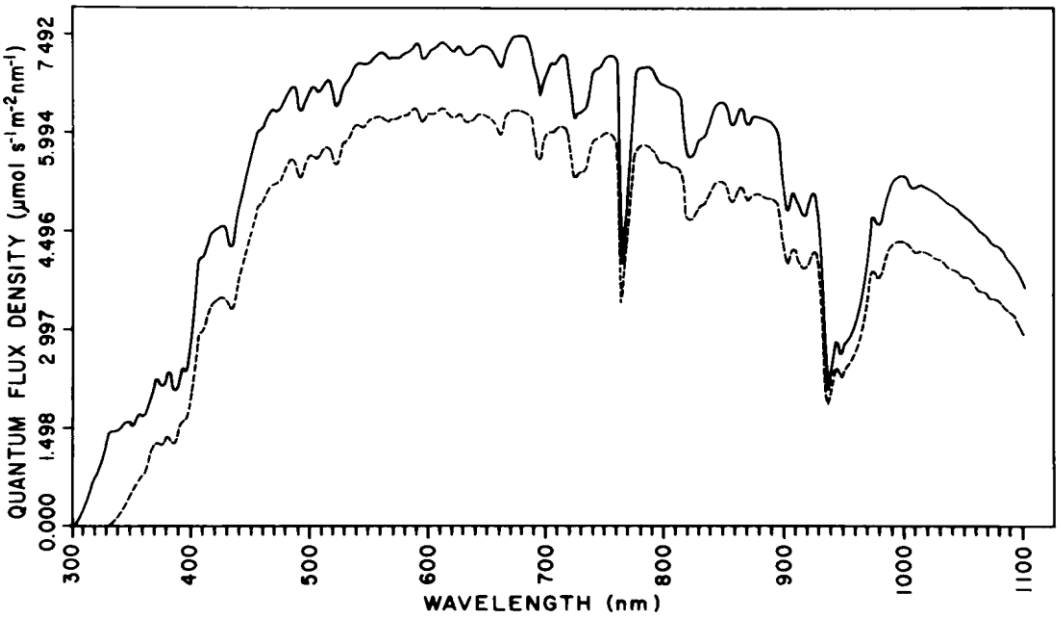

Figure 6A. Spectral energy distribution of sunshine outside and inside Phytotron greenhouse on a sunny day.

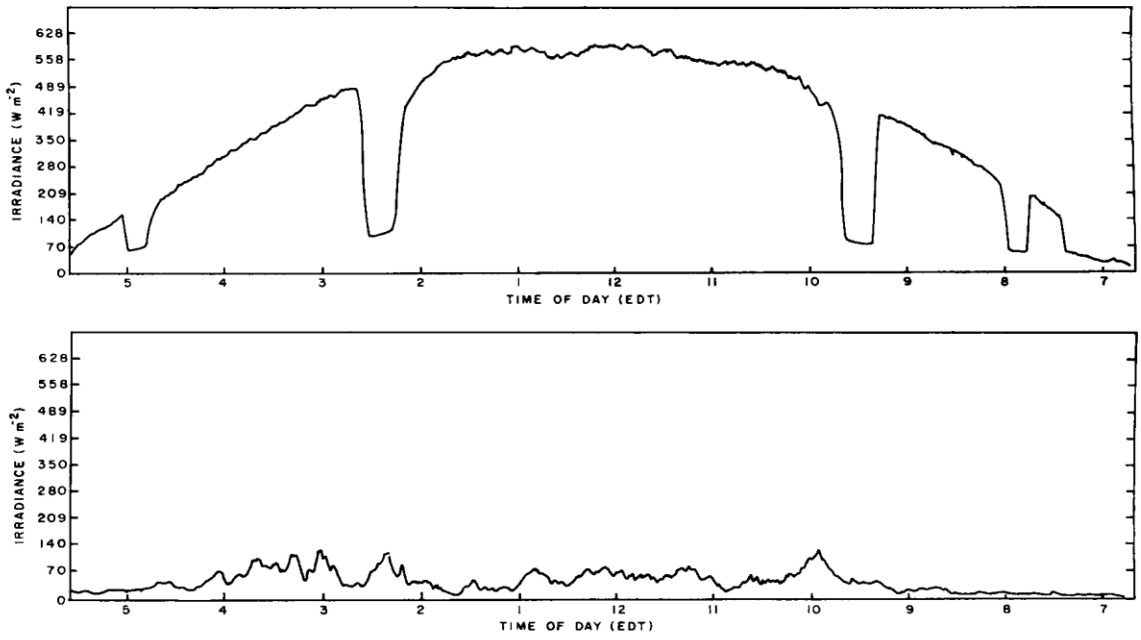

Figure 6B. Incident energy on clear (upper) and overcast (lower) days inside the Phytotron greenhouses (Downs, 1975). Note: p.m. on left, a.m. on right.

Table 2. Photoperiod room radiation data from various light sources compared to red and far-red luminaires.

	Illum. (klx)	PPFD 400-700nm	BLUE 400-500nm	RED 600-700nm	FARRED 700-800nm	R/FR*
		(μmol $s^{-1}m^{-2}$)				
Red Luminaire	-	15	0	15	2.0	7.307
Red Fluorescent	0.2	11	0	11	1.6	6.684
CWFL	3.0	37	6.8	10	0.1	5.395
CWFL, Red Filter	0.1	5	0.1	5	1.0	4.756
CWFL, Blue Filter	1.1	15	4.2	3	0.8	2.596
Blue Fluorescent	1.6	32	19.0	2	0.7	2.189
CWFL + Incandescent	4.4	66	8.4	30	35.0	0.838
100 W Incandescent	1.5	30	1.7	20	34.7	0.675
60 W Blue Incandescent	0.02	1	0.2	1	13.5	0.022
Far Red Luminaire	-	-	0	0.3	164.5	0.006

*655-665/725-735 based on μmol m^{-2} s^{-1}

Plants remaining in the greenhouses at night are subjected to long-day photoperiods by interrupting the dark period from 11 p.m. to 2 a.m. with 11-12 µmol s^1m^{-2} from incandescent-filament lamps. The effectiveness of the 3-hour dark-period interruption in controlling flowering of a wide range of short-day and long-day plants was established by H.A. Borthwick's Laboratory at Beltsville, MD. However, a few plants, some varieties of chrysanthemum and kalanchoe for example, are known to require longer night breaks. When such varieties are being studied, the interruption period is increased by moving the plants to a photoperiod room.

Photoperiod Rooms

Photoperiod room temperatures can be maintained at any point between 10 and 35°C. Photoperiods of all durations can be provided, including the natural progression of daylengths at any latitude. Each room is equipped with cool-white fluorescent and incandescent lamps (Table 2) that can be programmed separately, together, or alternately on any cycle from a few seconds per minute to continuous light. Phytochrome photoequilibrium can be altered by filters and different kinds of fluorescent and incandescent lamps (Table 2).

A and B Chambers

These walk-in controlled-environment rooms provide growing areas of 9 and 3 m^2 respectively, with a vertical clearance of 2.13 m (Fig. 7).

Temperature: Air temperatures can be selected over a range of 5 to 40°C. The variation about the set-point, measured with a type T (copper-constantan) thermocouple in a shielded, aspirated housing, is ± 0.25C. Two of the B-chambers are equipped with two defrosting systems that allow temperatures of 4°C with the lights on and 0°C in the dark.. 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.

Light: The combination of T-12, 1500 ma, cool-white fluorescent and 100 W incandescent lamps results in the spectral energy distribution shown in Fig. 8. These lamps, separated from the growing area by a plexiglass barrier, provide the radiant flux densities presented in Table 3. 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. In some chambers, reduced light levels can be obtained by reducing the number of operational lamps. It also is possible in some chambers to simulate the sunrise to

Figure 7. Phytotron A-Chamber.

sunset progression of radiant flux density by programming an increase and decrease through seven levels of light. Additional increments are not possible without disrupting the uniformity of the light over the plant growing area.

Relative Humidity: Normally, relative humidity levels are kept above 70% at 22°C (a vapor pressure of 1.82 kPa) by a spray injection system (Fig. 10). Saturation levels that maintain a film of water on the leaves are available in the plant pathology chambers. Relative humidities below 70% can be obtained by installing a dehumidifier in the chamber. For example, in a B-chamber, 30% RH at 18C can be obtained with the additional dehumidifier provided a collection system for pot drainage water is installed.

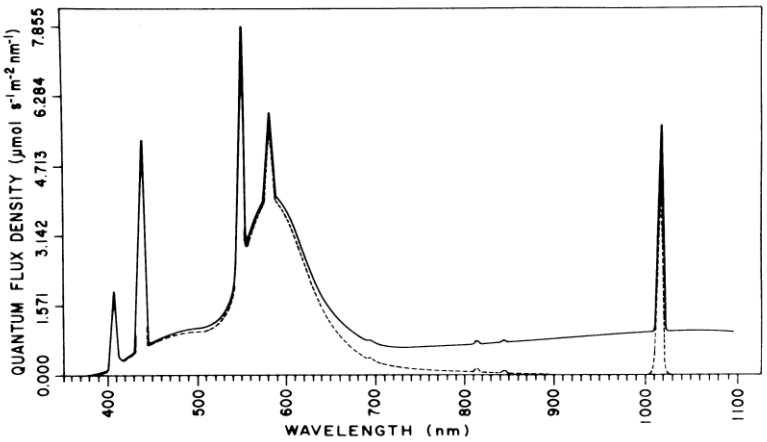

Figure 8. Spectral energy distribution in an A-type chamber fully lighted with 84 8-ft cool-white fluorescent lamps with and without 48 100-W incandescent lamps measured with a LI-COR 1800 spectroradiometer at truck level (113 cm from the barrier). This spectral energy produces 46 klx and a PPFD of 620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

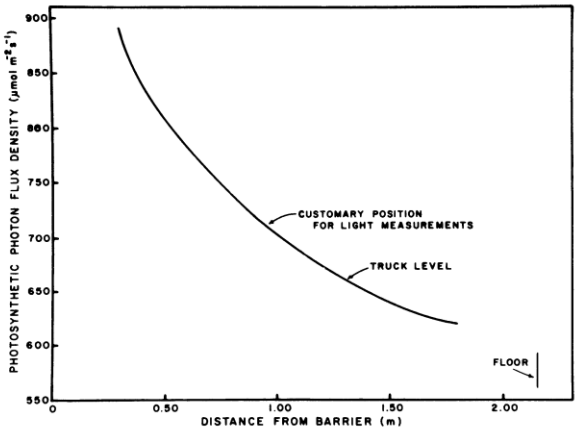

Figure 9. Vertical gradient of photosynthetic photon flux density in Phytotron artificially-lighted, controlled-environment rooms.

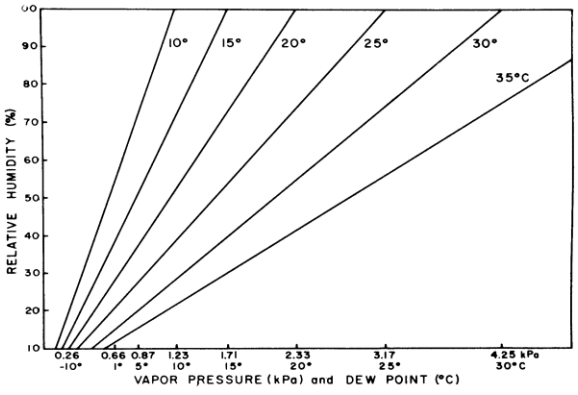

Figure 10. Relationship of relative humidity, vapor pressure and dew point temperature at different dry-bulb air temperatures.

Carbon Dioxide: CO_2 concentration is maintained between 300-400 ppm by controlled injection of commercial grade gas. This system also allows enhanced CO_2 levels of up to 2000 ppm.

Scheduling: Two-thirds of the A-chambers and all of the B-chambers are assigned to single investigators whenever environmental requirements, plant size, or populations require it. However, efficiency of space utilization often is increased by combining several projects when environmental and space requirements permit.

A standard set of A-chambers with day/night temperatures of 30/26, 26/22, 22/18 and 18/14°C are available for preliminary studies or for experiments that do not require all the space in a unit. Additional warmer or cooler chambers are included in the series whenever further increases in range are desirable. Two combinations of day/night temperatures are used, one in conjunction with short days and one with long days. Both temperature regimes coordinate the day temperature with 9 hours of high-intensity light. The long days are obtained by using a dark-period interruption from 11 p.m. to 2 a.m. with light from incandescent lamps.

Table 3. Typical radiant energy values 95 cm below the barrier in A and B chambers.

	A-Chamber		B-Chamber	
	Fluor	Inc	Fluor	Inc
Installed watts				
Total	18060	4800	6020	2400
Per square meter	2024	538	2026	806
Illuminance (klx)	49	1.8	48.1	2.4
Photosynthetic photon flux density* (μmol m^{-2} s^{-1})	600	39	600	49
Photomorphogenic flux density** (μmol m^{-2} s^{-1})	15.4	58	13.6	72

*Photosynthetically active radiation, 400-700 nm
**Photomorphogenic radiation, 700-800 nm

Anterooms

Anterooms, located between pairs of B-chambers, act as dark rooms to allow access to the biological material during the dark period. The anterooms are equipped with fluorescent and incandescent lamps and often serve as photoperiod rooms. These spaces also are used as exposure rooms for irradiations with special, monochromatic light sources. Temperature is controlled over a range of 5 to 40°C, but the variation about the set point is greater than in the adjacent chambers.

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 7 and 40°C with ± 0.5°C variation about the set point. C-chamber lighting is normally attained from 1500 ma, cool-white fluorescent and incandescent lamps, so the spectral energy distribution (SED) is about the same as in the A and B chambers. However, the SED can be altered easily by using other kinds of fluorescent lamps, such as Vitalite, Gro Lux and Agrolite, and broad-band monochromatic light can be obtained by replacing the transparent barrier with filters.

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 (Table 4).

Table 4. Average light levels with various numbers of lamps 84 cm from the barrier in C-chambers.

Number of lamps		Illuminance	PPFD*	PI**
Fluor	Inc	(klx)	μmol $m^{-2}s^{-1}$	W m^{-2}
16	6	31.6	411	7.35
16	3	31.0	400	5.22
16	0	29.9	379	1.66
14	6	28.2	370	7.06
14	3	27.5	358	4.91
14	0	26.5	336	1.4
12	6	24.4	322	6.83
12	3	23.8	311	4.66
12	0	22.7	288	1.15
10	6	20.8	276	6.58
10	3	20.1	265	4.42
10	0	19.0	246	1.08
8	6	17.2	229	6.38
8	3	16.6	218	4.16
8	0	15.4	195	0.74
6	6	13.8	189	6.18
6	3	13.0	174	3.97
6	0	11.8	151	.58
4	6	9.8	139	6.01
4	3	9.0	122	3.72
4	0	7.8	100	0.38
0	6	2.0	40	5.65
0	3	1.1	24	3.35

*PPFD - Photosynthetic photo flux density, 400-700 nm.
**PI – Photomorphogenic irradiance, 700-850 nm.

Figure 11. Phytotron C-chambers

Relative humidity in the C-chambers normally fluctuates between 60 and 70%, but can be controlled between 20 and 98% in some chambers. Chemical driers must be used for lower humidities.

H-Chambers

Four of the C-chambers have been converted from fluorescent to high intensity discharge lamps. These chambers are equipped with 1:1 ratio of metal halide and high pressure sodium lamps plus incandescent lamps to provide a PPFD of 1200 μmol s^{-1} m^2.

In addition, two walk-in chambers have been constructed to provide high intensity light. These chambers, with a growing area of about 4 m^{-2} and a vertical clearance of *2.5* m, are equipped with a 1:1 ratio of metal halide and high pressure sodium lamps plus incandescent lamps to provide a PPFD of 1500 μmol m^2 s^{-1}

Pathology

A semi-isolated area with four A-chambers, is available for experiments involving plant pathogens. All Phytotron facilities are available for preinoculation environments of pathology experiments. Post-inoculation conditions, however, are confined to the isolated areas, except when very specific organisms, such as rusts, are studied. Compressed-air-driven mist nozzles installed in the A-chambers deliver the 100% RH required to maintain a film of water on the leaves that facilitates the infection process.

Contamination of other areas of the Phytotron is reduced by operating the isolated areas at a lower air pressure than the other sections. Personnel movements into the pathology controlled-environment rooms are reduced by using automatic watering and during some kinds of studies only authorized persons are allowed into the area. Viewing ports placed in the doors of controlled-environment rooms in the pathology area provide visual access to material within the rooms, thereby lessening contamination by reducing the frequency of door opening. Release of spores to the outside is further reduced by electrostatic precipitators, and spore trapping filters are installed in the exhaust air system.

Figure 12. Air pollution roomettes.

Root Environment Research

Uniform root environments are obtained by growing plants in a continuous flow liquid culture with automatic pH and temperature control. The continuously flowing nutrient solution avoids the temperature gradient problems encountered with sand and soil substrates, so root zone temperatures can be maintained between 10 and 35°C at any ambient temperature over the same range.

Air Pollution Research

Research on the effects of phytotoxic gases on biological organisms is done in cooperation with the USDA Air Pollution Research group headed by Dr. Kent Burkey. Four roomettes (Fig. 12) designed specifically for chronic exposure studies, operate within an A-chamber. Equipped with automatic watering, these units allow continuously chronic exposures without interruption of the environment. with associated monitoring and control instrumentation for carbon monoxide, ozone, nitrous oxides and sulfur dioxide. Air temperature, CO_2 concentration, dew point temperature and PPFD are monitored and controlled continuously.

Utilities

Hot and cold city water, compressed air, nutrient solution and reverse osmosis (RO) purified water are available in or near all plant growing areas.

The nature of reverse osmosis membranes requires the presence of chlorine to preserve them so the effluent contains 0.1 μmol m^{-3} of chlorine and 0.04 /μmol m^{-3} of fluorine. Additional purification can be obtained by passing the RO water through a mixed-bed deionizing column.

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

Animals

Although the controlled-environment facilities were designed for plant research, many kinds of animals, vertebrate and invertebrate, can be accommodated. The researcher is responsible for the feeding and watering of animals studied using

Phytotron facilities. The Phytotron staff works with the zoologist to develop programs and adapt cages and containers to the controlled-environment chambers.

The fumigation procedures discussed later are likely to be of major concern to the animal scientist, so it should be emphasized that these policies are altered to protect whatever animal population is being investigated.

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 5.

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.

Nutrition and Substrate

A standard nutrient solution (Table 6) of defined analysis (Table 7) and a standard substrate composed of gravel and peat-lite have been developed. The steam-sterilized and washed gravel is #16 construction grade. The peat-lite is commercial mixture (Redi Earth, W.R. Grace Co.) of peat moss and vermiculite based on the original "Cornell Mix" (Boodley and Sheldrake, 1972). An indication of volume-weight and water-holding capacity are presented in Table 8.

Other substrates, such as vermiculite and river-bottom sand, also are stocked by the Phytotron. Pine bark, soil and other media can be used, but they will have to be obtained, sterilized, and brought to the Phytotron receiving room by the investigator.

Daily care of the plant material will be performed by the Phytotron staff, provided one of the watering schedules shown in Table 10 can be used. All other schedules, and applications of any special nutrient solution, are performed by the investigator.

Automatic Watering

Most of the controlled-environment space can be programmed to provide automatic watering with Phytotron nutrient solution and water. A choice of several "on" times can be used at frequencies ranging from every minute to once each 24 hours. Different flow rates can be obtained for each frequency and "on" period by altering the number of emitters per pot.

OPERATIONAL PROCEDURES

Fumigation

All supplies, equipment, plants and substrates enter and leave the Phytotron via the receiving room. Most items are fumigated before transfer to the Phytotron interior. Sterile cultures, animals, insect research material and analytical instrumentation are usually exempted. When plants are involved, choice of fumigant is based on discussions with the investigator.

Table 5. Truck and pot capacity of each type of contolled-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	15	540	240	135	75	60
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	31.58	80	2880	1280	720	400	320

•Reach in unit

Fumigations usually are made on Tuesday night, and all fumigated material is then moved into the Phytotron Wednesday morning. 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.

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.

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 9. *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.

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 filled with 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 will depend on the work schedule at the time.

Table 6. NCSU Phytotron Nutrient Solution.

Stock Solution (g)	Formula Weight	Grams/liter of stock solution
" A"		
Magnesium nitrate $Mg(NO_3)_2.6H_2O$	256.41	26.0
Calcium nitrate $Ca(NO_3)_2.4H_2O$	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 diabasic 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 $MoO_3.2H_2O$	179.97	0.002
Zinc sulfate $ZnSO_4.7H_2O$	287.54	0.011
Manganous chloride $MnCl_2.4H_2O$	197.9	0.0816
Copper sulfate $CuSO_4$ $5H_2O$	249.7	0.004
Cobalt chloride $CoCl_2$ $6H_2O$	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 1 ml "A" + 1ml "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 useage 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 7. Analysis of the Phytotron Nutrient Solution.

Element	Symbol	Source	Total ppm in the solution*
Nitrogen	N	$Mg(NO_3)_2.6H_2O$ $Ca(NO_3)_2.4H_2O$ NH_4NO_3 KNO_3	106.23
Phosphorus	P	KH_2PO_4, $K2HPO_3$	10.41
Potassium	K	KH2PO4, K2HPO4 K_2SO_4, KNO3	111.03
Calcium	Ca	$Ca(NO_3)_2.4H_2O$	54.40
Magnesium	Mg	$Mg(NO_3)_2.6H_2O$	12.40
Iron	Fe	Sequestrene 330	5.00
Sulfur	S	K_2SO_4, Na_2SO_4	13.19
Manganese	Mn	$MnCl_2.4H_2O$	0.113
Boron	B	H_3BO_3	0.24
Zinc	Zn	$ZnSO_4.7H_2O$	0.013
Copper	Cu	$CuSO_4.5H_2O$	0.005
Cobalt	Co	$CoCl_2.6H_2O$	0.00003
Molybdinum	Mo	$MoO_3.2H_2O$	0.005
Sodium	Na	$Na2SO_4$	11.04

*While useage of the unit "ppm" is common laboratory practice, the corect but less familiar SI unit would be mol m_{-3}.

Table 8. Waterholding capacity for selected values of substrate moisture tension for a typical batch of standard Phytotron substrate.* Bulk density is about 1.2 g/cm^3.

Substrate Moisture Tension		Water Content	
mm Hg	kPa	Weight Basis %	Volume Basis %
0	0	34.4	41.4
3.04	0 405	26.7	32.1
10.64	1 418	17 1	20.5
18.24	2 431	13.9	16.7
21.28	2.837	12 4	14.9
40.28	5.369	11.3	13.5
62.32	8.307	10.3	12.4
79.04	10.536	9.4	11.3
760.00	101.308	4.8	5.8
1140 00	1519 620	3 1	3.7

*Determined by D.K. Cassel, Department of Soil Science, NCSU.

Table 9. Watering schedules maintained by the Phytotron staff

WATERING & LABELING SCHEDULE

Label	Color	Morning	Afternoon
	Blue	Nutrient	Nutrient
	Green	Nutrient	None
	Green & White	Deionized H_2O	Nutrient
	Yellow	Deionized H_2O	None
	Yellow & White	Deionized H_2O	Deionized H_2O
	Red	None	None
	Orange	Nutrient 1X Mon Wed Fri	
	Pink	Deionized H_2O 1X Mon Wed Fri	
	Wood White	Naming & Numbering of Plants	

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 vast majority of Phytotron activity takes place between 7:30 am. and 4:30 p.m. When investigators need to work at other hours or on weekends, the Phytotron Director should be notified and a key will be issued or arrangements made to enter the building. For example, those who need to work infrequently on weekends or holidays can make arrangements in advance for the person on duty to permit entrance and exit.

Campus security demands that the Phytotron be locked at all hours other than the normal working hours. Therefore, delivery or removal of equipment, supplies, and new or harvested plant materials must occur between 7:30 am. and 4:30 p.m., Monday through Friday, unless special arrangements are made.

Program Changes

After a research program is approved and given initiation and termination dates, all subsequent changes should be in writing to avoid misunderstandings. Every effort will be made to accommodate modifications that do not significantly alter the approved version of the proposal. 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.

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.

Procedural Summary

1. All materials and equipment enter and leave the Phytotron through the receiving room between 7:30 am. and 4:30 p.m., Monday through Friday.

2. Fumigation takes place each Tuesday.

3. New environmental conditions are set up Monday through Wednesday.

4. Clean-up procedures described above should be followed rigorously.

5. Controlled-environment rooms are not to be entered during dark periods.

6. The investigator is responsible for building security when entering or leaving the Phytotron after normal working hours, or on weekends and holidays.

ENVIRONMENTAL CONDITIONS

Discussion of environmental measurements and instrumentation in this handbook will be confined to methods and systems of measurement now used in the Phytotron, and, where pertinent, to interactions between the measured factors and other aspects of the environment. Manufacturers of instruments used in the Phytotron are noted primarily because published measurement guidelines (Krizek, 1970; Tibbitts and Kozlowski, 1979) recommend it; but, this does not suggest that the product is preferred or superior to other comparable equipment.

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. The temperature controls are adjusted until the thermocouple output, measured with an expanded scale, Leeds and Northrup potentiometric recorder, or a digital read-out device, coincides with the temperature conditions desired by the investigator.

Aspirating the temperature sensor results in a room averaging effect and avoids the error (up to 13°C) that can occur with nonaspirated 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. 13).

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. 14). 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.

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.

Radiant Energy

The physical aspects of radiant energy have been standardized and unambiguous definitions of terms can be found in a number of references (APPENDIX IV).

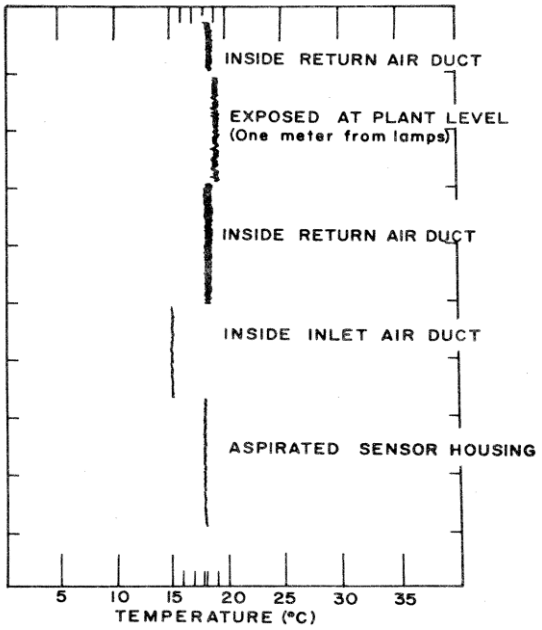

Figure 13. Temperatures indicated by #24 thermocouples at various locations in a single controlled-environment room (Downs, 1975).

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. (Table 11, APPENDIX V, VI). 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. 15). 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.

Illuminance measurements have very little real meaning in plant science because of the great difference between the spectral sensitivities of the human eye and the plant photochemical systems. Illuminance measurements, however, are useful for comparing various amounts of light received from the same kinds of light sources. It would be legitimate, for example, to use illuminance measurements ~o represent the relative energy received from different numbers of cool-white fluorescent lamps.

The most familiar instrument for measuring illuminance is the Weston foot-candle meter, although photometers made by General Electric, Gamma Scientific and a number of others are also available. Most of the Phytotron light measurements are made with a Li-Cor combination quantum/radiometer/photometer.

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 rim (Fig. 16) and the units are $\mu molm^{-2}s^{-1}$. 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 m^1s^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 $\mu mol \cdot m^{-2}s^{-1}$ (Table 10).

Although PPFD is the best measure of photosynthetically active radiation (PAR McCree, 1972), photosynthetic irradiance as $W \cdot m^{-2}$ may also be used. It is not correct, however, to use PAR as a synonym for PPFD.

Photomorphogenic Radiation (PR): The radiant flux density of photomorphogenic radiation defines the energy in the far-red region of the spectrum in terms of $W \cdot m^{-2}$. 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: Radiant flux density, or irradiance, is often called total energy. This is obviously not true, because most of the instruments used to measure irradiance have a limited spectral sensitivity. The spectral range of thermopile-based pyranometers, for example, is limited by the protective transparent dome. Radiometers that use a silicon photodiode, not only have distinct wavelength limits, but also are spectrally sensitive over the range of wavelengths they detect. Silicon photodiode instruments usually are calibrated for solar radiation and will give erroneous readings under most artificial light sources.

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.

Long wavelength radiation does have an effect on plant growth and Cathey et al., (1982) discuss thermally-sensitive and insensitive species. Measurement techniques for detecting very long wavelengths are being studied* and when proper methods are recommended, they will be incorporated into Phytotron instrumentation.

Cosine Correction: Radiant energy measurements, typically, are intended to represent the radiation incident on a horizontal surface. The illumination of a surface, however, is proportional to the cosine of the angle of the incidence of the light beam. Energy incident at a large angle from the normal, therefore, will be measured inaccurately unless some method of cosine correction is applied. The most common method of correction is to place a diffusing disc over the sensor so that the energy received at large angles of incidence will be refracted onto the sensor. The mere presence of a diffusing disc on a light sensor does not guarantee adequate cosine correction. It may only mean that some improvement is attained. The degree of correction, however, can be measured and should not allow more than *5%* error at an 80^o angle of incidence. Phytotron instruments are cosine corrected to within this limit of error.

Table 10. Proposed Guidelines for Measuring and Reporting the Environment for Plant studies*

Parameter	Typically Used Unit	Measurements: Where to take	Measurements: When to take	Measurements: What to report
Radiation:				
PAR (Photosynthetically active radiation)				
a) Photosynthetic photon flux density (PPFD) 400-700 nm with cosine correction	μmol s^{-1} 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.	W m^{-2} nm^{-1} or μmol $\cdot s^{-1} m^{-2}$ 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 studies 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	°C	At top of plant canopy. Obtain average over plant growing area.	Hourly over the period of the study (continuous measurement advisable).	Average of hourly average values for light and dark periods of study with range of variation over growing area.
Soil or liquid	°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 10 continued.

Parameter	Typically Used Unit	Measurements: Where to take	Measurements: When to take	Measurements: 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:	$m \cdot s^{-1}$	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:	$dS \cdot m^{-1}$**** (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 $\mu mol\ s^{-1}m^{-2}$". 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 $\mu mol\ s^{-1}m^{-2}$" 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.

****$dS \cdot m^{-1} = mho \cdot cm^{-1}$

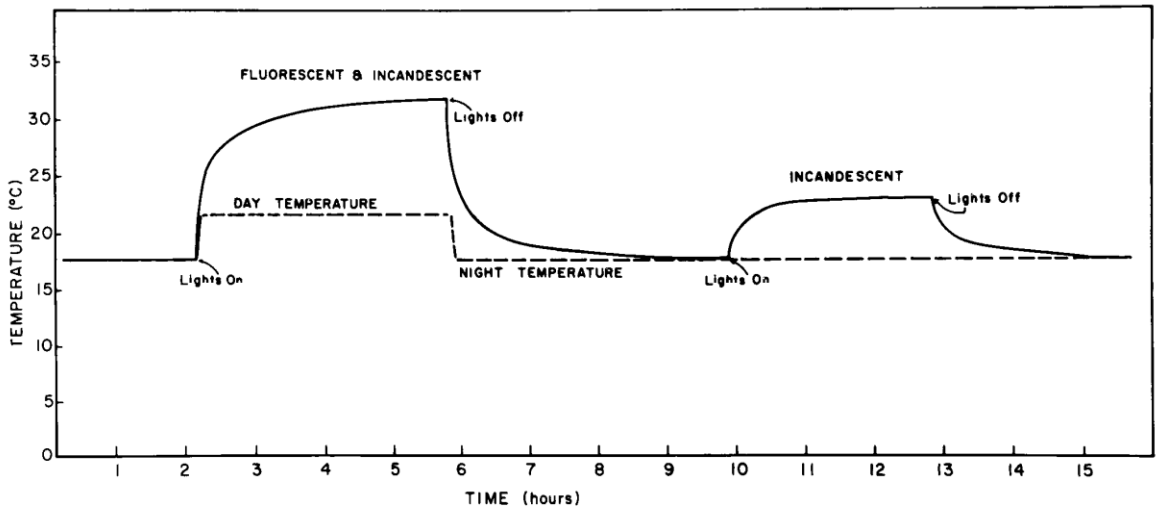

Figure 14. Temperature inside a cotton-stoppered Ehrlenmeyer flask (solid line) in a controlled-environment room with a 22/18°C day/night air temperature (dashed line). Fluorescent and incandescent lamps produce an illuminance of 43 klx and the incandescent lamps 3.4 klx.

Spectral Energy Distribution (SED): A grap representation of the radiant flux density at wavelength is provided by SED curves (Fig. Lamp manufacturers provide SED data for eac their lamp types. These data are useful, but dc always coincide with the spectral irradiance bec of reflectance and transmittance effects encount in controlled-environment chambers.

Selection of light sources for plant gr should be based on spectral irradiance between and 1100 nm or more. Unfortunately, the SED cu provided by manufacturers rarely exceed 380 to nm and spectroradiometers designed to mea spectral irradiance are priced beyond the reac most investigators. At present the best spe irradiance information is obtained from Lc Campbell's USDA laboratory at Beltsville, Mary (see Campbell et al., 1975; Thimijan and H 1982).

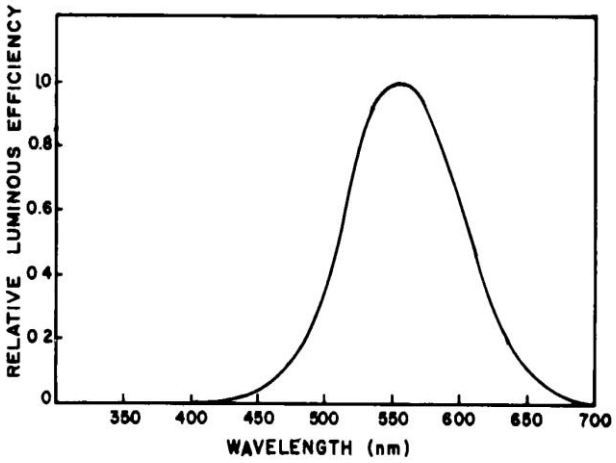

Figure 15. Spectral sensitivity of the human eye.

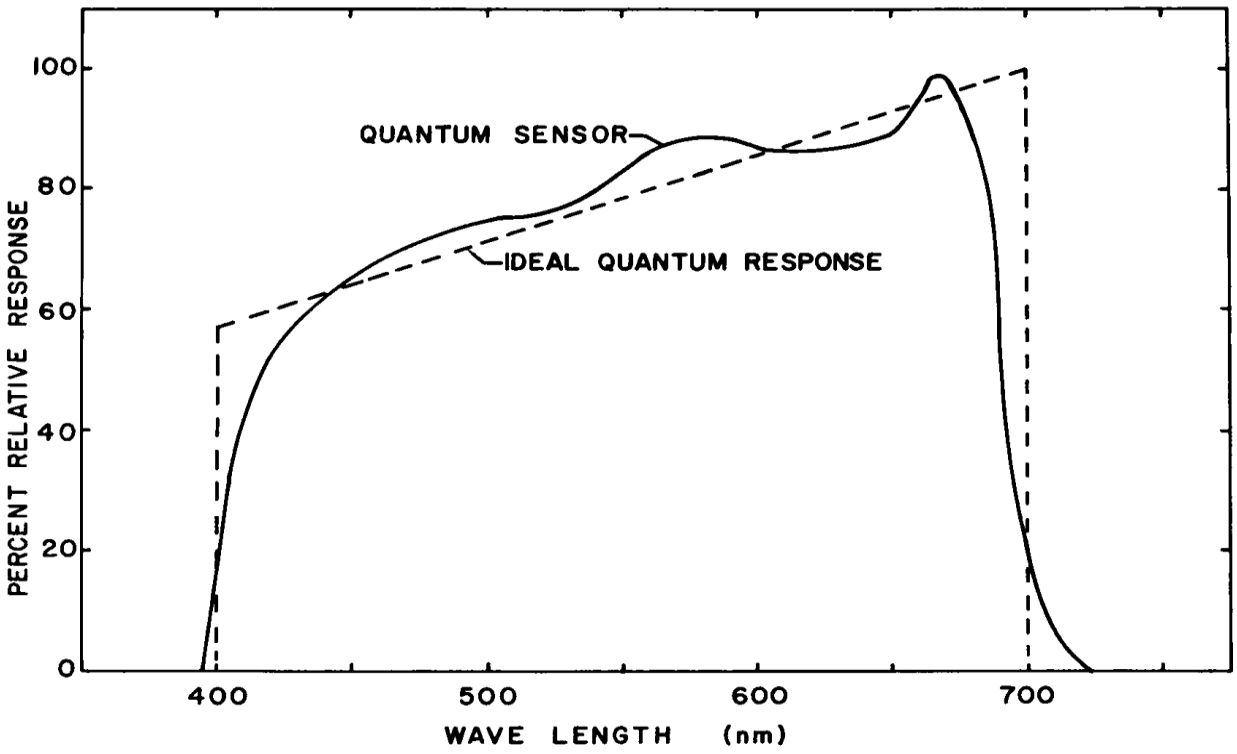

Figure 16. Spectral sensitivity of the Li-Cor photosynthetically active radiation sensor (Biggs et al., 1971).

Spectral Energy Distribution (SED): A graphical representation of the radiant flux density at each wavelength is provided by SED curves (Fig. 8). Lamp manufacturers provide SED data for each of their lamp types. These data are useful, but do not always coincide with the spectral irradiance because of reflectance and transmittance effects encountered

in controlled-environment chambers.

Selection of light sources for plant growth should be based on spectral irradiance between 300 and 1100 nm or more. Unfortunately, the SED curves provided by manufacturers rarely exceed 380 to 700 nm and spectroradiometers designed to measure spectral irradiance are priced beyond the reach of most investigators. At present the best spectral irradiance information is obtained from Lowell Campbell's USDA laboratory at Beltsville, Maryland (see Campbell et al., 1975*;* Thimijan and Hems, 1982).

Photosynthetic Photon Flux Fluence Rate (PPFFR): 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 photons between 400-700 nm incident for a unit time on the surface of a sphere divided by the cross sectional area of the sphere. Therefore, in a perfectly diffuse radiation environment, PPFFR would be about 4 times greater than photosynthetic photon flux density. In a typical Phytotron growth chamber, PPFFR is 2.08 times the PPFD. Obviously PPFD, which represents the energy falling on a horizontal surface, cannot be converted to PPFFR by simply changing the name. PPFFR can be measured, however, using Li-Cor spherical quantum sensor.

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 Phvtotron provides instruments to measure relative humidity and dew point. Although atmospheric moisture is often expressed as relative humidity, vapor pressure deficit, or more correctly 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.
[1]

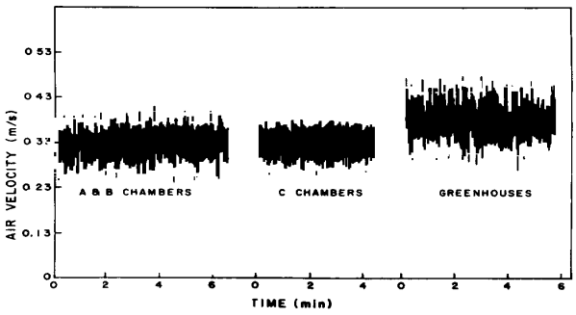

Figure 17. Air velocity in the Phytotron controlled-environment chambers.

Standard Methods of Reporting

Guidelines for reporting environmental conditions used in biological research have been published (Krizek, 1970; Downs, 1975; Langhans, and Tibbits, 1997). 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, such as specular walls, size, and top-to-bottom air flow, in the methods section along with the environmental conditions.

Terminology and measurement standards also have been discussed (Shibles, 1976; Tibbitts and Kozlowski, 1979; Vorst et al., 1981, Langhans, and Tibbits, 1997). The recommended standards (Table 11) should be used without exception.

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APPENDIX I

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 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.

APPENDIX II

EXPERIMENTAL DESIGN

The environment in plant growth chambers is much more uniform, and can be duplicated with greater precision, than in other research locations. However, environmental factors, such as temperature, light and air velocity, are not perfectly uniform in the plant growth chamber, nor are environmental conditions in two or more physically similar chambers exactly the same. Generally a significant difference in plant behavior between chambers or between trials can be traced to improperly setting one or more environmental conditions. Nevertheless, relatively small environmental variations can, under the proper set of circumstances, alter population uniformity within chambers, between chambers and between trials in the same chamber.

The following view of experimental design in controlled-environment rooms is presented by John 0. Rawlings:

Experiments conducted in growth chambers frequently are deficient with respect to one or more aspects of experimental design, primarily, proper use of randomization and replication. Uniformity studies in the NCSU Phytotron (Lee and Rawlings, 1982) confirmed that the basic principles of experimental design are as important in growth chamber experiments as in greenhouse and field experiments. Patterned variation within chambers and differences between chambers, or over time for the same chamber, make it essential that true replication and proper randomization be used to avoid biases in the estimation of treatment effects and serious underestimation of experimental error. The high cost of growth chamber studies makes it even more important that proper designs be used.

For "between-chamber" experiments, those in which all plants within a chamber are given the same treatment, such as temperature or day-length, plant-to-plant variation within a chamber is never a proper estimate of experimental error. The chamber is the experimental unit and true replication requires using more than one chamber for each treatment or, even better, replicating the experiment over time. For "within-chamber" experiments, those in which the treatments can be applied to different plants within a chamber, plant-to-plant variation is an estimate of experimental error only if the individual plant is the experimental unit and randomization has been properly used. This includes random assignment of treatments to the individual plants and random placement of these plants within the chamber consistent with the constraints of the experimental design being used. Even in the case of ~within-chambers~ experiments, repetition of the experiment over chambers and/or time is desirable to confirm that the results obtained are not an artifact of the specific chamber-time episode.

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APPENDIX III

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APPENDIX IV

RULES AND GUIDELINES OF LE SYSTEM INTERNATIONAL (SI) D'UNITES

All units refer to only seven, dimensionally independent, base elements and two supplemental elements.

Physical Quantity	Unit	Symbol
Base Units		
Length	meter	m
Mass	kilogram	kg
Time	second	s
Electrical Current	ampere	A
Thermodynamic Temperature	kelvin	K
Amount of Substance	mole	mol
Luminous Intensity	candela	cd
Supplemental Units		
Plane Angle	radian	rad
Solid Angle	steradian	sr

APPENDIX IV (continued)

Derived Units

Derived units are obtained by combining two or more of the base units.

Some derived units have special names.

Physical Quantity	Unit	Symbol	Derivation
absorbed dose	gray	Gy	J/kg
capacitance	farad	F	A.s/V
conductance	siemens	S	A/V
disintegration rate	becquerel	Bq	l/s
electrical charger	coulomb	C	A/s
electrical potential	volt	V	W/A
energy	joule	J	N.m
force	newton	N	$kg.m/s^2$
inductance	henry	H	V.s/A
illumination	lux	lx	lm/m^2
luminous flux	lumen	lm	cd.sr
magnetic flux	weber	Wb	V.s
magnetic flux density	tesla	T	Wb/m^2
pressure	pascal	Pa	N/m^2
power	watt	W	J/s
resistance	ohm		V/A
volume	liter	liter*	dm^3

*liter may be abbreviated using a script "ℓ" or an upper case L.

APPENDIX IV (continued)

Prefixes

A series of prefixes providing a range of unit magnitudes of 10^{18} to 10^{-18} are an integral part of SI.

Multiplication Factor	Prefix	Symbol	Multiplication Factor	Prefix	Symbol
10^{18}	exa	E	10^{-18}	atto	a
10^{15}	peta	P	10^{-15}	femto	f
10^{12}	tera	T	10^{-12}	pico	p
10^{9}	giga	G	10^{-9}	nano	n
10^{6}	mega	M	10^{-6}	micro	μ
10^{3}	kilo	k	10^{-3}	milli	m
10^{2}	hecto	h	10^{-2}	centi	c
10^{1}	deka	da	10^{-1}	deci	d

Non-SI Units

A few units with special names that have been in use for a long time are accepted for use with SI although they are not part of the system.

Name	Symbol	Value
nautical mile	-	1852 m
knot	-	1.852 km/h
hectare	ha	10^{4} m^{2}
millibar	mbar	10^{2} Pa
curie	Ci	37 GBq
roentgen	R	2.58 x 10^{-4} Ci/kg
ton	t	10^{3} kg

APPENDIX IV (continued)

Rules of SI

Proper use of SI units can be assured by following a few simple rules:

1. Units must be written in full or denoted by correct symbols. Velocity, for example, would be written as meters per second, m/s or m s^{-1}, but not as meters/sec.

2. All units, except Celsius (C), when written in full, use lower case letters.

3. Symbols also are written in lower case letters except those derived from proper names and for powers greater that 10^3. Thus, megajoules would use the symbols MJ and kilograms per cubic meter would be denoted as kg/m^3 or kg m^3. The prefix is always attached to the unit

4. Symbols are never pluralized, but unit names follow grammatical rules. The only unit names never changed to plural are siemens, lux and hertz.

5. The product of two units is indicated by a space or dot, such as N m, N.m or N·m.

6. Division of one unit or combination by another can be expressed by a solidus, J/s, or by a negative index J s^{-1}. Only one solidus is allowed so $W/m^2/sr$ is incorrect and should be written $W/m^2 \cdot sr$ or W $m^{-2}s^{-1}$.

7. A prefix symbol is considered to be combined with the unit symbol to which it is attacked. Thus, 1 mm^3 is the same as $(10^{-3}m)^3$ or $10^{-9}m^3$, but 1 mm^3 does not equal 10^3m^3.

8. Compound prefixes must not be used. For example, the old use of micromicrofarad, μμf is replaced by the picofarad pF.

9. Only one (1) prefix can be used in forming a decimal multiple. The frequent use of uW/cm^2 is, therefore, incorrect since both micro, μ, and centi, c, are prefixes. Moreover, the prefix should be attached to the numerator unit so mW/rn^2 or W/m^2 would be used instead of $\mu LW/cm^2$. About the only exception to this rule would be the use of kilograms, as GBq/kg, because kilograms is a base unit.

APPENDIX V

CONVERSION FACTORS

To Convert From	To	Multiply By
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)	μmol $m^{-2}s^{-1}$(400-700)	0.0134*
lux (incandescent)	" "	0.02*

*Thimijan and Heins (1983)

APPENDIX VI

NATURALLY-OCCURRING DAYLENGTHS AT RALEIGH, N.C.

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.)