

MORPHOLOGICAL AND PHYSIOLOGICAL ADAPTATIONS OF RED FESCUE  
(FESTUCA RUBRA, L.) SEEDLINGS TO SIMULATED SUNLIGHT,  
DIFFUSE SKYLIGHT, AND CANOPY SHADE

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## ABSTRACT

This research examined the physiological and morphological adaptations caused by two environmental parameters: light quality and two light intensities of simulated full sunlight, of skylight (neutral shade), and of canopy shade on one generation of red fescue plants. The plants were grown in a greenhouse using filtered sunlight and in a growth chamber using lamps and filters, and plant adaptations to these environments were followed during a 5-week growth period. Plants grown in sun-spectra simulations had the highest fresh weight, dry weight, number of leaves per plant, number of tillers per plant, and highest photosynthetic rate. These plants also had the lowest respiration rate, lowest compensation point and shortest leaf length. The plants had moderate chlorophyll content and a moderate number of stomates.

Plants grown in skylight simulations had a low fresh and dry weight, a moderate number of leaves and tillers, and the lowest photosynthetic rate. In both the high and low light experiments, skylight plants had the highest respiration rates and compensation points, very short leaves, the highest number of stomates and greatest stomatal area/100  $\mu^2$ . Skylight plants grown in the growth chamber had the greatest amount of total chlorophyll, chlorophylls a and b, and carotenoid per g dry top compared to the other treatments.

Canopy shade simulations produced plants that had low fresh and dry weights, highest fresh to dry weight ratio, lowest number of leaves and tillers and a low photosynthetic rate. Canopy shade plants had high respiration rates and compensation points, the greatest leaf length, but the lowest number of stomates and lowest total stomatal area.

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## INTRODUCTION

The ability of plants quickly to alter their morphology and to modify aspects of their physiology in response to different light regimes has been known for many years. For the most part, however, studies have been made on plants from different habitats or on taxonomically unrelated plants grown under ill-defined and ill-controlled conditions. This study attempts to avoid such complications by using a single cultivar of a plant, Festuca rubra L. "Golfrood", grown from seed under specified light regimes. Red fescue was chosen because of its known adaptability under different climatic and light regimes, its small size, rapid growth rates, reasonable genetic uniformity and high economic value for turf installations.

Plants were grown from seed under simulated light spectra representing full sunlight in the 400-750 nm range, noon sunlight filtered through deciduous leaves (canopy shade) and diffuse skylight. Two levels of light intensity were supplied for each spectrum. At intervals after seeding, adaptive response was determined by quantitative measurements of 9 parameters of growth and 6 parameters of the energy metabolism of the plants.

As compared with plants under full sun simulations, plants grown under both canopy shade and diffuse skylight conditions were photosynthetically less efficient during the early portion of their growth, but by five weeks from seeding, they were as efficient as were the full sun-grown plants. There were no

major differences in the concentration of photosynthetic pigment. The same trend was found for respiratory activity. When growth, measured by weight, tillering, and leaf number was used to evaluate adaptive capacity, it was found that adaptability was not apparent in that the canopy shade-grown plants and the diffuse skylight-grown plants were poorly developed and full sun-grown plants increased their biomass at a much faster rate. This apparent paradox, in which there was adaptation in the energy metabolism, but not in the growth of the plants, suggests that other facets of the interaction between the plant and the radiation regime determine morphological adaptation.

## LITERATURE REVIEW

In an extensive review, Daubenmire (1959) listed 31 morphological and physiological differences between shade and sun plants. It is, however, important to determine whether some or all of these differences represent structural and functional adaptations that permit or facilitate establishment and survival in a particular environment. The ecological classification of plants into ones that grow and develop best in full sunlight, called heliophytes by Haberlandt (1914), and sciophytes, that do best in lower light intensities, suggests that at least some of the observed differences do represent adaptations (Levitt, 1972).

It is difficult categorically to define adaptation. Adaptation is any characteristic that aids an individual organism or the species to survive and reproduce in the environment it inhabits. Adaptation may be genetically simple, controlled by only one or a few genes, or may be complex and controlled by large numbers of genes. Although the effectiveness of structural or functional adaptations is tested in terms of individual survival and prolificacy, this capability depends on effective adaptation at every level of bio-organization, from molecule to ecosystem. Not all individuals are equally adaptable because the total amount of genetic material is limited and vascular plants, in contrast to some microorganisms and animals, cannot quickly change their form and function to cope with extremely diverse and rapidly-changing environments.

It is equally difficult categorically to designate a par-

ticular plant or population of plants as either a sun or shade species, for this species may have diverse ecotypes. Moreover, sun and shade environments are a complex of environmental factors including altered quality and quantity of solar radiation, temperature, relative humidity, soil moisture and wind. The relative success of heliophytes or sciophytes in sun and shade is determined by an array of concomitant variables operating on a series of interdependent plant functions (Daubenmire, 1959).

Although the problem is enormous, researchers have attempted to analyze the array of concomitant environmental variables singly and in combination and relate them to the series of interdependent plant functions and the resulting plant adaptations. In the shade complex, light is perhaps the most ecologically significant factor, for it is consistently different from sun to shade environments even though other conditions may be the same. In general terms, there are four types of natural light regimes: 1) full sun where all wavelengths of visible light are present in defined proportions and intensity; 2) neutral shade where sunlight is reduced in intensity but the proportions of wavelengths are unaltered from full sun conditions; 3) deciduous canopy shade where full sunlight is filtered through deciduous leaves and proportions of various wavelengths relative to full sun are altered; 4) diffuse skylight where both the intensity and the relative proportions of wavelengths of full sunlight are altered by diffusion, reflection and selective absorption.

Although plants respond to wavelengths above and below the

visible range, the greatest plant response is to sunlight which is composed of approximately 10% ultraviolet, 25% blue, 25% green, 23% red and 18% far-red light, i.e., fairly well balanced among wavelength bands (Vezina and Boulter, 1966; Henderson, 1970).

When sunlight is filtered through cloud cover, neutral screening such as cheese cloth, hardware cloth, or saran cloth, or through light to moderate coniferous canopies, only the intensity of the sunlight is reduced (Vezina and Boulter, 1966; Atzet and Waring, 1970). However, when sunlight is filtered through deciduous plant canopies, sunlight is not only reduced in intensity, but the transmitted light is then composed of 8% ultraviolet, 12% blue, 18% green, 10% red, and 51% far-red light, i.e., rich in green and far red light (Vezina and Boulter, 1966; Coombe, 1957). This spectral composition is fairly constant, except when distorted by sun flecks, or the occasional spots of full or weakly-filtered sunlight. These flecks, caused by "holes" in the canopy, have been shown to be physiologically important, but are too variable to be included in a general description of canopy shade (Evans, 1966; Anderson, 1966; Pollard, 1970).

The spectral quality of sunlight is also altered in the shade of a building, under a dense coniferous plant canopy, or on a heavily overcast day with the resultant spectra called diffuse skylight. Diffuse skylight has not only a lower intensity, but is composed of 18% ultraviolet, 23% blue, 21% green, 14% red and 27% far-red, i.e., rich in blue and poor in red wavelengths (Vezina and Boulter, 1966).

Previous research has demonstrated that the structure and function of plants varies greatly with the nature of the light regime. There are several good literature reviews on plant responses to light (Shirley, 1935; Crocker, 1936; Duggar, 1936; Wassink and Stolwijk, 1956; Went, 1957; Geise, 1964; Daubenmire, 1959; Bainbridge, Evans and Rackham, 1966), but none provide information on simulating natural light environments, nor do they provide data dealing with specific adaptations of plants of similar genetic composition to these environments. In general, the standard for adaptability has been the response to a light regime different from full sunlight. An examination of pertinent literature will form a base for evaluating the present study.

### Neutral Shade

Korstian (1925) grew various coniferous nursery stock in beds under 0%, 25%, 50%, 75% lath shade and found the highest germination and survival occurred under 50-75% shade and the lowest in the open. He also found that seedlings grown under neutral shade had lower osmotic pressures, thinner epidermis and cuticle, more spongy tissue, and a tighter cell arrangement. Stomata were less deeply depressed than were those in plants grown in full sunlight. In this instance, the adaptive modifications made by the sun-grown plants aid in surviving the greater dessication threat of the full light environment.

Logan and Krotkov (1969) grew sugar maple seedlings for 3



years under lathing shades of 13, 25, 45, and 100% of full sunlight. They also measured  $\text{CO}_2$  uptake as a measure of photosynthesis and concluded that plants grown in the lower light intensity utilized light more efficiently per unit of leaf area than did those grown in full light. However, on a per seedling basis, the full light plants had the highest apparent photosynthetic rate. There was a compensating effect of low light plants having the lowest leaf area but the greatest photosynthetic rate/leaf area. They concluded that the photosynthetic apparatus of shade-tolerant sugar maple seedlings is better adapted to various shaded habitats than non shade-tolerant plants that experience only a reduction in leaf area when grown in the shade. Logan and Krotkov believe that the difference in photosynthetic response between shade-adaptable and non-adaptable plants is due to the photochemical efficiency and enzymic differences. Later, Logan (1970) conducted similar studies with shade-intolerant yellow birch seedlings and found none of the adaptations made by the shade-tolerant sugar maple seedlings.

The pigment contents of plants grown in various light regimes is modified and is assumed to be of adaptive significance by permitting the plant to utilize the available light better. Logan and Krotkov (1969) found that chlorophyll content declined with increasing light intensity with seedlings from the 13% light treatment, having 5 times more chlorophyll on a fresh weight

basis than did full sunlight plants. The chlorophyll a/b ratio increased with light intensity, suggesting that chlorophyll b was either broken down or not produced in high light treatments.

Stomatal density and opening constitute the major control of CO<sub>2</sub> entry into the plant and thus govern the rate of photosynthesis. Gast (1930) postulated that some species grow best in sunny situations because they require high light intensities to open the stomates, and Loehwing (1930) observed that when shade-loving plants were transplanted to full sunlight, stomates close, thus causing photosynthesis to almost stop. Using golden-rod taken from sun and shade environments, Holmgren (1968) measured CO<sub>2</sub> uptake. He found a low correlation between stomatal conduction and CO<sub>2</sub> uptake, but a high correlation between CO<sub>2</sub> uptake and mesophyll conductance, suggesting the great importance of CO<sub>2</sub> pathways in the mesophyll from cell surfaces and stomates to CO<sub>2</sub>-fixation sites. However, Holmgren used ecotypes and not clonal material, and his results must be carefully evaluated because of the different gene pools and the fact that some plants were probably fully adapted.

It can be concluded that neutral shade-grown plants have lower light saturation points, lower compensation points, and are more efficient users of weak light than are full light-grown plants. As the above research has demonstrated, reducing only the intensity of light, as in neutral shading, caused profound adaptations. Plant adaptations to deciduous canopy shade are equally dramatic.

### Deciduous Canopy Shade

As early as 1927, Klugh measured the spectral quality of forest light and compared it to sunlight. He found a swamp forest canopy to transmit 0.036% in the orange region, 0.33% in the green, and 3.05% in the blue-violet. Vezina and Boulter (1966) and Federer and Tanner (1966) verified that under a deciduous canopy far-red made up the greatest percentage of light with decreasing amounts of green, blue, red and ultraviolet wavelength bands. In comparison with the open on clear days, the deciduous forest light is low in blue, high in green, low in red, and exceptionally rich in far-red.

Daubenmire (1959) felt that such spectral variations were not great enough to be critical as an environmental factor and that light intensity is of greater importance. But Vezina and Boulter (1966) suggest that under a forest canopy where light is often 10% or less of the open, the quality of light may significantly affect the rate of photosynthesis and the germination of seeds. This is supported by the work of Wood (1969), who grew turfgrass species under a natural deciduous canopy and found large differences. Red fescue produced the most dry matter, bluegrasses produced less, with bentgrass producing the least dry matter. There were also differences between cultivars, indicating that some were more adaptable to spectral shade than others that performed similarly in full sun. Because of uncontrollable variations in natural spectral shade, laboratory techniques were developed to alter spectra and

to measure plant response. Some of the earliest and most extensive research on spectral effects was conducted by H. W. Popp and co-workers. Popp grew different plants in greenhouses fitted with glass filters, made observations on vegetative growth and vigor, on flowering and fruiting, and did chemical and micro-chemical analyses of plant tissue. In some cases, anatomical changes were also followed.

Popp (1926) found that the most striking results in growth and development were obtained in those houses where the blue-violet end of the spectrum ( $< 529$  nm) was eliminated, providing essentially a deciduous canopy shade. Plants in these houses were morphologically etiolated and succulent with long slender internodes and few branches, but they showed good green color. Their cells were thin-walled and loosely organized with little differentiation. Strengthening tissue was weakly developed so that after 2 or 3 week's growth the plants had to be supported. Leaves were of normal size but tended to roll.

Popp's findings and studies of leaf morphology of sun and shade leaves have been reviewed and confirmed by Isanogle (1944), Hughes (1966), and Lewis (1972). Popp also found that fresh and dry weights of entire plants were reduced, and flowering and fruiting of some species were markedly retarded. Surprisingly, these plants also had the highest percentages of total nitrogen and lowest percentage of total carbohydrates. In most instances, soluble nitrogen compounds were particularly elevated while starch and other acid-hydrolyzable carbohydrates were reduced.

Because of the many observed morphological adaptations of spectral shade-grown plants, it seemed reasonable there must be equally dramatic physiological adaptations, and they are inter-related. However, isolation and measurement of the physiological modifications is difficult.

Bohning and Burnside (1956) measured the apparent photosynthesis of leaves from eight sun species such as beans and tobacco and five shade species including ferns and Oxalis. They found that light saturation was at about 2,000-2,500 ft-c and the compensation points for the sun species were at 100-150 ft-c. For shade species, light saturation was reached at 400-1,000 ft-c and the compensation point was approximately 50 ft-c. Gauhl (1969), measuring the light-saturated CO<sub>2</sub> uptake of clones of nightshade isolated from sun and shade environments, obtained similar results.

The adaptation of citrus trees to light similar to that under a canopy of trees was of interest to Erner, Goren and Monselise (1972). They used different types of celluloid sheets to filter sunlight and grew sour orange seedlings for 6 months. Their closest simulation of canopy shade was an elimination of the wavelength band between 560-700 nm. They found that this treatment enhanced seedling length and elevated the protein, chlorophyll, and peroxidase activity of the leaves.

Erez and Kadman-Zahavi (1972) studied the growth of peach plants under conditions nearly identical to those of Erner et al. (1972). They found that the same blue plus far-red light re-

gime suppressed growth rate, leaf size and number, rate of spring bud opening, and secondary branching compared to either blue light or neutral shade. However, the intensities of light in their treatments were not equal and could account for these differences.

In comparison to a full-light-grown plant, a spectral-shade-grown plant generally has less dry weight, is tall and thin with long internodes and few branches, cells are thin-walled and poorly differentiated, have high moisture content and a lower percentage of total carbohydrates. Spectral-shade-grown plants have lower light-saturated photosynthesis and lower compensation points than full light plants. However, because no work has been done using carefully controlled conditions in natural or simulated deciduous canopy shade, these conclusions may not apply to canopy-shade-grown plants.

### Diffuse Skylight

As stated earlier, diffuse skylight is a product of the diffusion, reflection, and selective absorption of sunlight and is therefore variable in its intensities and proportion of wavelengths. In general, diffuse skylight was found to have its maximum intensity in the blue-violet region of the spectrum, while the maximum energy of sunlight is in the yellow-green region (Kimball, 1924; Vezina and Boulter, 1966). Although large numbers of plants grow in diffuse skylight, there is almost no published research data on plant responses to diffuse skylight with

the exception of Popp (1926) and Shirley (1929) whose work will be discussed later.

### Goals of this Research

Although differences in plant response between full sunlight, neutral shade, deciduous canopy shade, and diffuse skylight shade have been examined, no adaptation trends can be traced. This is due to the complexity of the experimental environments studied in regard to temperature, moisture, wind and relative humidity, so that light might not be the only response initiator. Even when the light factor has been isolated, measurements of that light regime have been inadequate, so that different researches cannot be compared. No research has adequately simulated spectra of sunlight, diffuse skylight, and canopy shade or compared the plant adaptations made in response to these spectra when light is the only major environmental variable.

This research will measure the morphological and physiological adaptations made by a single cultivar grown from seed as a function of spectral simulations of full sunlight, deciduous canopy shade, and diffuse skylight at 2 levels of intensity. Such comparative studies will permit an evaluation of the capacity of a plant to adapt rapidly to differences in light environments.

corrected light spectra, and the effect of these natural spectra will be compared in a growth chamber.

Greenhouse. In a growth chamber.

## MATERIALS AND METHODS

### Plants

Red fescue (Festuca rubra L. "Golfrood") was selected because of its ability to grow in both sunny and shaded sites (Wood, 1969). Sixty seeds were planted in each square plastic pot (6.5 x 6.5 x 7.0 cm) containing 500 g of sterilized and amended sandy loam soil. The pots were placed under mist for 6 days, e.g., just prior to seedling emergence, and then randomly assigned and moved to the light treatments. To insure a homogenous population, pots were selectively thinned 14 days after seeding to obtain 54 plants. Pots were sub-irrigated daily with distilled water and their positions randomly rotated daily in each light treatment. All plants were sprayed with Benlate (Du Pont) at 200 ppm on days 7 and 14 to control a Cylindrocladium spp. infection.

### Light Treatments

Spectral distribution of natural environments of open sunlight, north building shade, and under-tree-canopy shade were monitored at noon of 8 August 1969 with an ISCO model SR spectroradiometer, YSI model 65 radiometer, and a Weston model 756 cosine-corrected light meter. From these data, simulations of these natural spectra were made in a greenhouse and a growth chamber.

Greenhouse. In a south-facing greenhouse room, light-



filtering units were constructed to provide spectral simulations of sunlight, canopy shade, and skylight shade (Figs. 1, 2, 3). On a clear summer day at noon, total spectral energy between 400-750 nm was equalized at  $44,000 \mu\text{W}/\text{cm}^2 \pm 3\%$ , or approximately 10% of full sunlight ( $420,000 \mu\text{W}/\text{cm}^2$ ).

Treatment 1, termed greenhouse sun (G/S), simulated a solar spectrum by filtering sunlight through 2 layers of Cinemoid #30 (clear), 8 layers of white tissue paper, and 1 layer of Cinemoid #29 (heavy frost) (Fig. 1). These filters reduced the light intensity to equalize total energy between units without greatly altering the solar spectrum in the visible wavelength range. Treatment 2, designated greenhouse canopy shade (G/CS), simulated a deciduous tree canopy spectrum by filtering sunlight through 1 sheet each of Cinemoid #4 (medium amber) and Cinemoid #61 (slate blue) (Fig. 2). Light treatment 3, designated greenhouse skylight (G/SL), simulated building shade by filtering sunlight through one layer of Cinemoid #17 (steel blue) and 2 cm of 0.625%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  solution contained in a 6 cm Plexiglas box, plus 6 layers of 60 mesh white cheesecloth (Fig. 3).

Each unit was 57 cm x 120 cm with a 45° sloping front containing the filters. The sides were constructed of cardboard faced with aluminum foil with baffles to allow air movement. A manifold system joined each light treatment to a 30 cm exhaust fan to allow fairly uniform relative humidity, air circulation, and cooling. The average temperature in all units was 24° day and 18° night. On hot days, air temperatures reached 35-40° and relative humidity dropped to as low as 30%

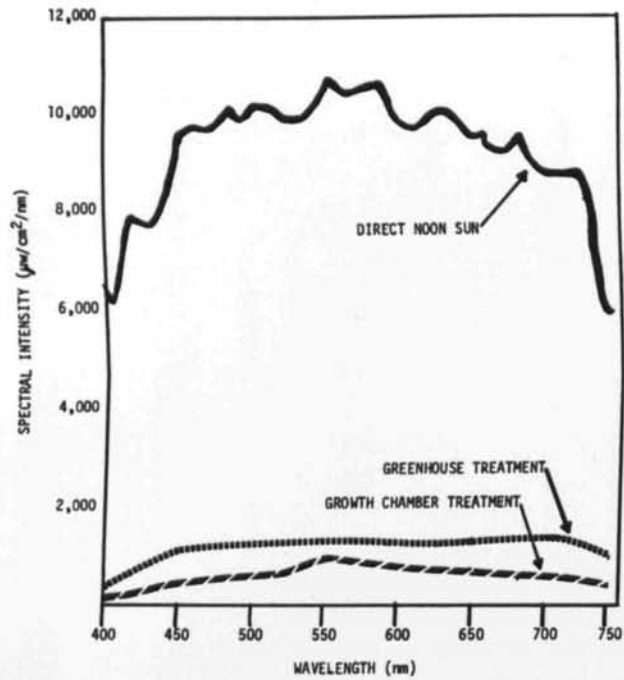


Fig. 1. Comparison of spectra of direct noon sun, greenhouse simulation, and growth chamber simulation.

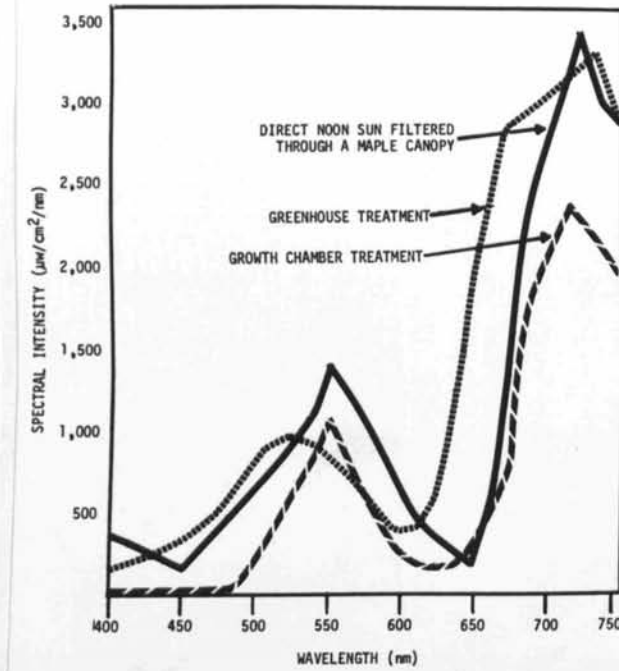


Fig. 2. Comparison of spectra of direct noon sun filtered through a maple canopy, greenhouse simulation, and growth chamber simulation.

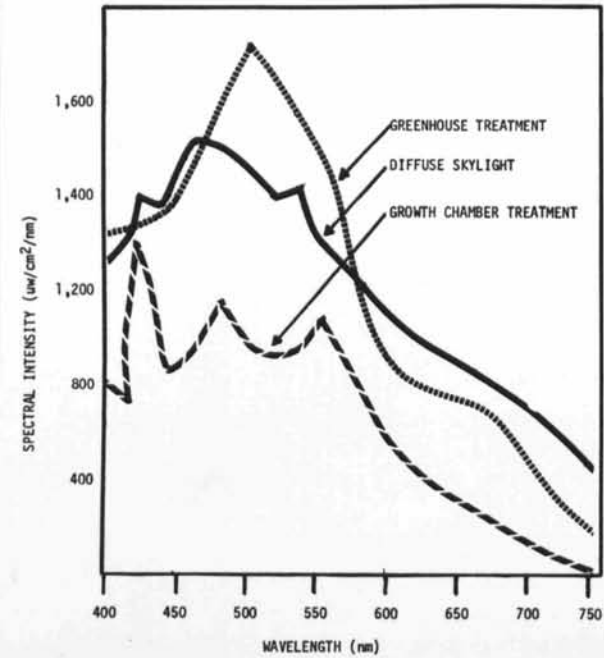


Fig. 3. Comparison of spectra of diffuse skylight, greenhouse simulation, and growth chamber simulation.

Natural light environments were measured by an ISCO spectroradiometer and simulated in a greenhouse by filtering sunlight, and simulated in a growth chamber by filtering electric lamps.

for a few hours. The natural photoperiod was 14.5 hours at the beginning of the experiments and 15.3 hours at the end.

Growth chamber. The primary luminaire in a modified Percival PGC-78 growth chamber was an alternating sequence of blue F72T12/B/HO and "Vitalite" F72T12/HO fluorescent lamps. The chamber was divided into 3 equal sections of 60 x 75 cm to accommodate 3 areas simulating sunlight, canopy shade, and building shade (Figs. 1, 2, 3). In all treatments, total spectral energy between 400-750 nm was equalized near  $22,000 \mu\text{W}/\text{cm}^2 \pm 5\%$ , half that of the greenhouse systems.

The simulated solar spectrum, termed chamber sun (C/S) (Fig. 1) was obtained by supplementing the fluorescent lamps with 200-watt incandescent lamps plus 1 layer each of Cinemoid #50 (pale yellow), Cinemoid #52 (pale gold), and Cinemoid #29 (heavy frost). Light treatment 2, designated chamber canopy shade (C/CS) simulated a deciduous tree canopy (Fig. 2) spectrum by using a dual filter system. The first consisted of 1 layer of Cinemoid #23 (light green), placed directly below the fluorescent lamps. Through this filter barrier protruded 4 GE R-40 150-watt green reflector flood incandescent lamps. Below both the fluorescent and green lamps was a second filter system, consisting of 1 layer of Cinemoid #4 (medium amber) and 4 layers of Cinemoid #29 (heavy frost) placed directly under the R-40's serving as a diffusing screen and to eliminate bright spots. Light treatment 3, designated chamber skylight (C/SL), was made to simulate building shade (Fig. 3) by filtering the

fluorescent light through 1 layer each of Cinemoid #36 (pale lavender), and Cinemoid #50 (pale yellow), plus 1.25 cm of 1.25%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  solution contained in a 6 cm Plexiglas box. The light units were separated by cardboard dividers covered with aluminum foil. Air temperatures were stable, averaging  $24^\circ$  day and  $18^\circ$  night. The photoperiod was adjusted every second day to match the natural photoperiod.

### Plant Testing

To determine the extent that various plant responses changed with age over time, plants were measured 3, 4, and 5 weeks after seeding. At each collection period, 3 pots from each treatment were randomly selected, watered to maximum retention capacity, and the soil surface of each pot was sealed to prevent evaporation or gas exchange by soil organisms or roots. The sealant was a 1:1 mixture (m.p.  $44^\circ$ ) of Tissuemat paraffin (m.p.  $52.5^\circ$ ) and paraffin oil (viscosity 335/350). The wax was heated, cooled to approximately  $45^\circ$ , and poured on the soil surface. No adverse effects of this warm wax on seedlings were noted. The bottom of the pot was sealed with 2 layers of "Saran Wrap" (Dow Chemical Co.) held by an elastic band. Pots were sealed 24 hours before testing and were kept in darkness until placed into a photosynthesis chamber.

Photosynthesis chamber. The system (Fig. 4) used to measure transpiration and photosynthesis (Gates, 1969; Pratt, 1971) consisted of a plant chamber, a humidity-temperature recorder, a

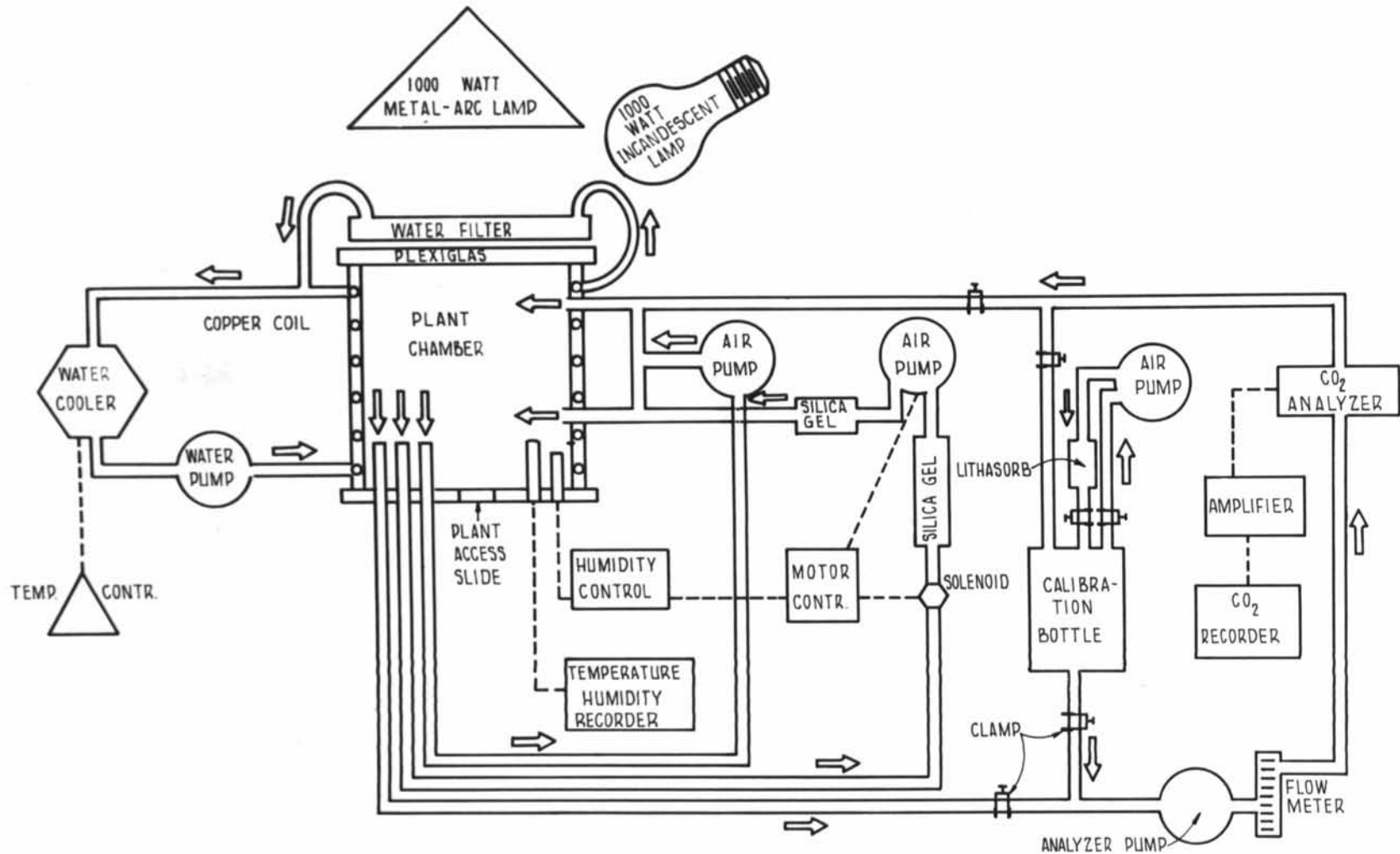


Fig. 4. System used to measure plant respiration, transpiration, and photosynthesis. This system consisted of a plant chamber, a humidity-temperature recorder, a humidity control, a CO<sub>2</sub> analyzer and recorder, a water cooling system, 2 light sources, and a calibration unit.

humidity control, a CO<sub>2</sub> analyzer and recorder, a water cooling system, a light source, and a calibration unit. The chamber was 25 cm high with an inside diameter of 25 cm. The hollow wall contained copper tubing through which water at 14° was continuously circulated from a water cooler by a pump. The bottom and top of the chamber were enclosed by 11 mm thick Plexiglas plates. The bottom plate was fitted with a slide, through which the plants were placed into the chamber. Silicon grease was used to prevent air leaks.

The light sources for the chamber were a 1,000-watt Sylvania Metalarc continuous spectrum lamp, plus a 1,000-watt reflector flood lamp. Light intensity in the chamber was controlled by varying distance and by Saran shade neutral density filters. A 10 cm thick plastic-box water filter was placed between the lamps and the chamber to eliminate radiation above 1,100 nm (Withrow and Price, 1953). The filter was cooled with an internal plastic manifold through which water at 14° was circulated. Temperature and humidity within the chamber were constantly monitored with a wide range humidity-thermister sensor and a Hydrodynamics humidity-temperature recorder (model 15-4050).

Changes in CO<sub>2</sub> concentration in the air were measured with a Beckman Infra-red Analyzer Model IR-15A connected through an amplifier to a Bausch and Lomb strip chart recorder. Air flow through the analyzer was regulated by a flow meter positioned between the air pump and the analyzer. An event recorder mounted on the recorder registered major power interruptions

such as starting and stopping of the dehumidifying system. The event recorder's power supply was maintained at 6 volts by a Kepco (Flushing, N.Y.) model 800B Power Supply Voltage Regulator.

The CO<sub>2</sub> system was calibrated each day. After removing all CO<sub>2</sub> from the calibration unit by circulating the air through Lithosorb, known increments of CO<sub>2</sub> were introduced into the system. The CO<sub>2</sub> was allowed to equilibrate, and readings were then plotted against cc CO<sub>2</sub> injected; a straight line could be drawn through the points. The operating range ( $\pm 5\%$  of 300 ppm) was determined by locating the points of intercept of 5.7 and 6.3 cc CO<sub>2</sub> injected. Radiation was monitored as mW/cm<sup>2</sup> by a YSI model 65 radiometer with the sensor placed in a glass tube fitted into the chamber side at plant height.

Operation. In the dark, plants were moved from the holding box and were placed in the photosynthesis chamber. CO<sub>2</sub> evolution, representing the apparent dark respiration, was measured. The Metalarc lamp was then turned on and the light intensity at the compensation point was determined.

Immediately after determining compensation point, shade screens were removed, the reflector flood lamp was switched on, and the radiation intensity in the chamber adjusted to 40 mW/cm<sup>2</sup>. This was termed high light photosynthesis. The operation was replicated once.

After the plants were removed from the chamber, leaves were removed at the waxed pot surface, counted, weighed, dried in



vacuum at 60° for 1 hour, and reweighed. Pigments were extracted from the dry material in boiling methanol and read in a Spectronic 20 at wavelengths specified by Weybrew (1957). Total chlorophyll, chlorophylls a and b, total carotenoid, and  $\beta$  carotene were calculated. Since Weybrew's equation was based on ether extractions, a series of correction factors were determined by extracting and making readings in methanol, then transferring the pigment sample to ether and making readings.

CO<sub>2</sub> calculations. Apparent respiration and photosynthesis were calculated as the time required for each pot of plants to absorb or release 30 ppm of CO<sub>2</sub> (315-285 ppm CO<sub>2</sub>) and expressed as g CO<sub>2</sub>/g dry top wt/hr.

Additional plant measurements. Length of first leaf on each tiller, number of tillers per plant, and stomate size and number of six-week-old seedlings were measured. Stomate size and number were determined from leaf impressions made with acrylic resin (Brown and Rosenberg, 1970).

Additional pots of Golfrood seedlings were allowed to grow for six weeks in the greenhouse in full sunlight and are referred to as unfiltered sunlight plants. Although these plants were not statistically analyzed against the other treatments, their results are included for their comparison value.

Statistical considerations. The basic design was a completely randomized experiment with a  $3^2 = 9$  factorial array of treatments assigned at random to 27 pots (each treatment combination was assigned at random to 3 pots). Hence, pot to pot



variation within a treatment combination was used as a measure of random variation, since all pots were assumed to be homogeneous. The 2 factors are time at 3 levels (3, 4, and 5 weeks) and treatments at 3 levels (sunlight, canopy shade, and skylight simulations). This basic experiment was replicated once in time. Each replicate was analyzed separately and then replicates were pooled for each of 10 response variables to give an estimate of the error variance with 36 degrees of freedom. Due to highly significant interactions between treatments and time for nearly all response variables, Scheffé's procedure was employed within each time period to separate treatment means at the .01 level of probability.

Plants were actually yellow-green in appearance.

Plants of the 3:4:5 sunlight-grown plants were between those described above. These plants had an intermediate number of tillers and leaves per plant, were moderately upright in growth, and their leaves were shaped as a semi-open fan. Plants under growth chamber conditions were the shortest of the experiment and were also the darkest green, whereas greenhouse-grown leaves were as long as sun leaves and only slightly darker green. The plants were sufficiently different in growth habit from each other so that even an inexperienced observer could have correctly identified a plant's growth environment, given the above information.

Although the growth chamber treatments only had one-half-