

THESIS

CARBON DIOXIDE LEVELS IN THE PLANT MICROENVIRONMENT
AS INFLUENCED BY A POLY-COATED PAPER MULCH

Submitted by
Cheryl K. Tarter
Horticulture Department

In partial fulfillment of the requirements
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Fort Collins, Colorado
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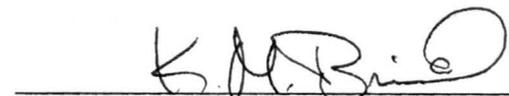
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Adviser


Department Head

ABSTRACT OF THESIS

CARBON DIOXIDE LEVELS IN THE PLANT MICROENVIRONMENT AS INFLUENCED BY A POLY-COATED PAPER MULCH

Effectiveness of carbon dioxide (CO_2) enrichment using a polyethylene coated black paper mulch, incorporated nitrogen and wheat straw particles, and field CO_2 release was investigated.

A mulch covering or mulch over incorporated plant residue, such as straw, has been suggested as a possible means of CO_2 enrichment which results from trapping the CO_2 evolved from the soil. This idea was tested using Great Lakes Mesa 659 lettuce seedlings in growth chambers and in an outdoor setting.

Carbon dioxide concentrations at the base of the plants and at a 5 cm depth in the root zone were greater in mulched and mulched straw treatments. Non-mulched straw did not increase surface CO_2 concentrations. Growth of mulched plants in the chambers was approximately 80% greater than that of non-mulched plants. Mulched plants in the outdoor study had a growth increase of about 13% when compared to non-mulched plants. Increases in growth of mulched plants were attributed to greater CO_2 levels, since soil moisture levels and temperatures were similar in all treatments. Straw suppressed growth and would not be recommended as a CO_2 source.

Mulch applied over CO_2 release lines in the field was found to be an effective means of CO_2 enrichment of a lettuce canopy by creating

a physical barrier to rapid air exchange, thereby concentrating released CO₂ under the mulch. Mulch over a release line more than tripled CO₂ concentrations near the soil surface when compared to CO₂ release with no mulch covering or CO₂ supplement. Significant enrichment levels were maintained to 25 cm above the soil surface on still days by means of a mulched CO₂ release line. Subsurface CO₂ concentrations were increased by the application of mulch and averaged 857 ppm which was considered non-phytotoxic. The effect on soil CO₂ levels from the release line was negligible. Inconclusive results in plant response suggest further study is warranted.

Cheryl K. Tarter
Horticulture Department
Colorado State University
Fort Collins, Colorado 80523
Spring, 1983

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INTRODUCTION

Well developed management practices combined with improved plant varieties through advanced breeding methods have been responsible for the tremendous yield increases of most of the world's major food crops in past years. Currently, yields of these crops have remained constant with significant increases in production uncommon (65).

Water, nutrients, insects, and disease are all factors recognized as limiting to plant growth. As all these factors are brought under control, maximum productivity may depend largely on the carbon dioxide (CO_2) concentration within the crop canopy. Enrichment of a plant microenvironment with CO_2 may increase the photosynthetic efficiency of crops which at maximum is only around 2 percent.

Vast yield increases of greenhouse crops in enriched atmospheres have been observed for some time. However, maintaining elevated CO_2 levels in a field situation is difficult and has been inefficient considering the low crop recovery rates of applied CO_2 . Mulch has been shown to increase the CO_2 concentration around mulched plants in the field. This effect on CO_2 levels has been suggested as the major reason for crop yield increases when opaque mulch is used as a cultural practice.

The objective of my research was to determine if concentrations of CO_2 sufficient to increase plant growth could be achieved and

ultimately applied in a field situation. It was assumed that CO₂ enrichment of the plant leaf microenvironment could be most easily achieved with a low growing species such as lettuce and that a C₃ species (lettuce) would benefit more from CO₂ enrichment than a C₄ species.

LITERATURE REVIEW

Ambient levels of CO₂ in the atmosphere are considered to be 330 to 340 ppm (2, 33, 67, 69). This level of available CO₂ can be considered suboptimal on the basis of greenhouse CO₂ enrichment studies where concentrations greater than 340 ppm generally cause significant yield increases in many crops (68). Furthermore, detection of CO₂ concentrations lower than ambient levels is common over field crops during calm, sunny days (10, 31, 37, 41, 63). Montieth et al. (41) predicted that when the atmosphere is stable and wind speed near the ground falls below 2 m sec⁻¹, turbulent mixing decreases causing CO₂ concentration at the crop surface to approach 250 ppm or less in bright sunshine. Carbon dioxide starvation may then be a common occurrence especially over irrigated crops in a dry environment. Kretchman and Howlett (34) stated that at normal concentrations a plant must 'process' quite a large volume of air in order to provide enough CO₂ for plant growth and development. Hence, early plant researchers theorized that crop plants could grow more rapidly and efficiently if the CO₂ content of the air was increased.

Normally, within concentrations between 300 and 500 ppm there appears to be an almost linear increase in the rate of net photosynthesis (13, 17). Also, a reduction in concentration from 300 to 200 ppm, which may occur over crop canopies, can reduce growth as much as 50% (17). On the whole, a general assumption is that the CO₂ content of the

atmosphere may be limiting photosynthesis at certain times when other factors are not limiting or are optimal.

Carbon dioxide applications are normally most effective in early stages of plant development (17, 33). Seedlings exhibit the maximum response to elevated CO_2 because of the high demand for assimilate by actively growing tissues (15). Under lower assimilate demand, typical of older plants, this growth response often decreases or ceases. Photosynthesis under enriched conditions is normally increased, at least initially. However, after a few days, photosynthesis in plants exposed to higher CO_2 concentrations may approach or fall below plants exposed to ambient concentrations. Raper and Peedin (49), for example, grew two cultivars of tobacco (Nicotiana tabacum L.) at 400 and 1000 ppm CO_2 . Thirty-five days after transplanting, the rate of photosynthesis per unit of leaf area of the high CO_2 plants was only 70 to 80% of the rate of plants kept at 400 ppm. This lowering of photosynthesis rate may be caused by starch accumulation in the chloroplasts resulting from elevated CO_2 . Mauney et al. (39), for example, reported that accumulation of starch in cotton (Gossypium hirsutum L.) leaves reduced the rate of photosynthesis. This high starch content which could increase leaf thickness may explain the lower specific leaf areas found in enriched plants, particularly in C_3 plants (30, 47).

There are important differences among species in their photosynthetic response to enhanced CO_2 . Plants with the C_3 carbon pathway usually show greater increases in photosynthesis rate than plants with the C_4 carbon pathway (15, 33, 47, 69, 70). Most C_3 plants benefit from elevated CO_2 concentrations because of the increase in CO_2 in relation to O_2 reacting with ribulose biphosphate carboxylase/oxygenase,

thereby suppressing photorespiration and leading to higher rates of net photosynthesis. Patterson and Flint (47) presented net assimilation rates for four species grown for 45 days at 350, 600, and 1000 ppm CO₂. Increases in CO₂ concentration produced little change in net assimilation rates for the two C₄ plants, corn (Zea mays L.) and itchgrass (Rottboellia exaltata L. f.). In contrast, the assimilation rates of the two C₃ plants, soybean (Glycine max Merrill) and velvet-leaf (Abutilon theophrasti Medic.) were increased by as much as 35% at the two higher CO₂ concentrations with a corresponding increase in dry weight.

Exposure of plants to high concentrations of CO₂ can provide benefits beyond those caused directly by increased photosynthesis. An improvement in water use efficiency has been noted under enrichment conditions. Carbon dioxide levels greater than ambient concentrations around a leaf may cause stomatal aperture reduction which can account for decreases in transpiration (32, 42, 44, 67, 69, 70) while only marginally limiting carbon gain (21). Moss et al. (42) found a 57% reduction in stomatal apertures of leaves at the top of corn plants exposed to 575 ppm CO₂ compared to plant leaves exposed to 310 ppm CO₂. Thus, water stressed plants exposed to elevated CO₂ levels can maintain production levels equal to unstressed plants in many situations. For example, Sionit et al. (54) found water stressed wheat (Triticum aestivum L.) grown in 1000 ppm CO₂ had grain yields equal to unstressed plants grown at CO₂ concentrations near ambient. Other research has reported similar results (23).

Carbon dioxide, temperature, and light intensity interactions must be considered when analyzing total plant response to enrichment.

Krizek et al. (35) in greenhouse and growth chamber studies with various vegetables including lettuce, concluded that temperature was the most limiting factor for seedling growth. However, CO₂ concentration was more limiting than light intensity. Lettuce required all three factors to be elevated for maximum leaf number, a measure of plant development. Some conflicting reports concerning CO₂ enrichment could possibly be explained by the level of light energy experienced during the study. Generally, response to CO₂ fertilization is greater at high light intensities (9, 11, 13, 17, 42, 55). Low light levels lower the CO₂ saturation point of photosynthesis removing CO₂ as a limiting factor. Consequently, if low irradiances were employed during an experiment, any potential advantage from an elevated CO₂ level would probably be canceled; although some studies have shown a benefit from enrichment at illumination levels as low as 300 ft-c (29, 30). This may be caused by CO₂ concentrations sufficient to inhibit photorespiration, thereby preventing a carbohydrate drain on the plant when less light is available (24).

The value of CO₂ enrichment in greenhouses for increasing vegetative growth and enhancing reproductive development has been shown for a wide range of crops (12, 29, 34, 66, 68). Wittwer and Robb (68) cite early examples of this practice in Germany and England and the remarkable yield increases. They state that lettuce responds very markedly to CO₂ fertilization with maximum yield increases in greenhouses ranging from 30 to over 150% (34, 68). Allen (2) summarized considerable data on the effects of enhanced CO₂ concentrations on plant growth.

Knowing the successes of CO₂ enrichment of greenhouse atmospheres, it is surprising that relatively few attempts have been made to research field enrichment. Proposed models predicting the efficiency of CO₂ release in the field have been developed and studied for their practical potential (4, 19, 26, 41, 58). Most researchers note that field enrichment is difficult or impractical due to rapid gaseous exchange with the bulk atmosphere (2, 3, 4, 34, 35). Allen et al. (3) with a line source CO₂ release in the field using corn determined that CO₂ enrichment would not be practical during the daytime due to the thermally unstable air above a crop and the typically higher wind speeds at that time. They explain, however, that denser canopies might be more efficient in retaining released CO₂ because of lower eddy diffusivities. Furthermore, maximum efficiency of released CO₂ would more likely occur in C₃ plants than in C₄ plants. Harper (26) concluded from a similar experiment, however using cotton (C₃ plant), that CO₂ enrichment of a crop canopy could be practical in increasing crop yields if optimum management practices and proper crop selection, such as those with low, dense canopies, were used along with convenient, economical CO₂ sources. In his study, unexpectedly high CO₂ concentrations 4 m above the soil surface were encountered over cotton as well as Coastal bermudagrass due to vertical movement. His data suggest that a closed crop canopy should capture at least 33% of the released CO₂. Carbon dioxide release should coincide with times of high light intensity (midday) and low wind speed. Takami and VanBavel (58) in simulation studies with sorghum (Sorghum bicolor) predicted that at low irradiance levels, increases in released CO₂ uptake would be small, resulting in an equally small efficiency of 1.2 percent. An

efficiency 7 times greater was predicted at high irradiance (1059 W m^{-2}) and the same wind speed (1.0 m sec^{-1}). The efficiency at high wind speeds (3.0 m sec^{-1}) was less than half that at low wind speeds (1.0 m sec^{-1}). These conflicting reports from field enrichment could be attributed to the different application methods and crop growth habits. Maximum responses from field enrichment normally occur with low, dense canopies that favor CO_2 concentration buildup as opposed to tall, sparse canopies when ground origin delivery sources are used.

One method of field enrichment originally suggested by Wittwer (66), but modified and carried out by Nakayama and Bucks (43) in 1980 was to mix CO_2 with water and convey it in buried trickle irrigation systems. Their preliminary results indicated a significant 20% increase in wheat yield. Takami (56), in his study with cotton, also used trickle tubing to transport CO_2 but did not bury the tubing and did not mix the CO_2 with water. By burying the tubes, rapid gas dissipation could be somewhat avoided by creating a physical barrier to gas movement. Pallas (45) suggested that if systems of CO_2 stagnation could be developed, then efficiency of CO_2 fertilization would be vastly improved by minimizing air exchange and turbulence. Also, by using a subsurface source, CO_2 introduction through the roots becomes a possibility.

It has been known for quite some time that some species are capable of absorbing and fixing CO_2 by their roots (7, 48, 50). Controversy exists, however, as to what percentage of CO_2 can be absorbed and supplied by roots over that furnished by leaves. Some investigators have shown that significant amounts of root absorbed CO_2 can be utilized. Studies with potato in solution culture have shown

that CO_2 applied to the root system can be transported to leaves and shoots for carbohydrate production and can enhance tuberization and stimulate photosynthesis rate while suppressing photorespiration by increasing the CO_2/O_2 ratio in the leaves (6). Arteca and Poovaiah (5) found that potato roots in solution culture exposed to $^{14}\text{CO}_2$ not only translocated CO_2 to the shoots, but also fixed CO_2 in the roots mainly in the form of malic acid. Their study suggested that CO_2 used in photosynthesis may be derived partially from CO_2 fixation via roots. Stolwijk and Thimann (56), however, suggested that the CO_2 content of most soils is already supra-optimal and that any soil enrichment would be unnecessary since plants take up so little CO_2 through their roots; although they did find a small but consistent stimulation of pea root growth when the root atmosphere contained 5000 ppm CO_2 . These conflicting reports suggest that utilization of root absorbed CO_2 may be species dependent. Also, different treatment durations and initial soil pH values may all influence CO_2 uptake by roots (22). Sub-surface CO_2 enrichment, in addition to supplying roots with greater concentrations, may cause lowering of soil pH which can be of major importance in calcareous soils. Nakayama and Bucks (43) temporarily decreased the pH of the calcareous soil used in their study by 1.5 units. This may have indirectly caused their observed yield increases by improving nutrient availability of phosphorus and minor elements.

Agricultural mulch (plastic or paper) has proven effective in increasing yields of various crops. Most of these increases have been largely attributed to increases in soil temperature (mostly from the use of clear plastic mulches), moisture retention, or weed control (1, 14, 16, 18, 20, 28, 52, 62). These differences in soil temperature and

moisture are often not great enough to explain the magnitude of yield response. Shelldrake (53) suggested as early as 1963 that elevated CO_2 concentrations due to mulch application might be partly responsible for yield increases. He described the mulch phenomenon on CO_2 concentration as a "chimney effect." Mulch provides a physical barrier to the upward flux of soil evolved CO_2 and funnels it out through the holes in the mulch provided for the plant. This creates an enrichment zone directly around the photosynthesizing leaves which could benefit crops, especially low growing species.

By increasing the soil organic content, an even greater flux of soil evolved CO_2 would be created from an increase in microbial activity. In fact, greater CO_2 concentrations found over organic muck soils compared to non-organic soils have been suggested as perhaps one reason why yields are increased in organic soils (31). Acting upon this theory, a non-crop study in the field combined incorporated straw in the soil as a CO_2 source with a mulch covering (36). Mulch was the key to raising CO_2 levels in that the mulched straw treatment, when compared to the non-mulched straw treatment or the bare soil control, increased the CO_2 level approximately 50% at the soil surface. Apparently straw, during its decomposition, created a CO_2 supplement which was pooled under the mulch covering and released directly through the mulch hole.

In summary, the benefits of increased CO_2 concentrations are well documented for enclosed crop environments. Conflicting reports on the feasibility of field enrichment has made it apparent that practical and economical methods need to be developed and studied. Thus, the purpose of this research was to determine if elevated CO_2 concentrations

can be achieved and maintained in a lettuce canopy by providing a physical barrier (mulch) to the CO_2 flux from the soil and evaluate the effect on plant response. Experiments 1 and 2 used mulch in conjunction with incorporated plant residue (wheat straw) as a potential CO_2 source. Experiment 3 was conducted in the field using a gaseous CO_2 source distributed through release lines into a lettuce canopy with or without a mulch barrier.

MATERIALS AND METHODS

The mulch used in these experiments was a black paper with a 0.25 mm layer of polyethylene on each side.

Experiment 1 - Low Irradiance Growth Chamber Study

This experiment consisted of 4 treatments: (1) a control - bare soil, (2) a mulch covering over bare soil, (3) straw incorporated into the soil mix, and (4) a mulch covering over the straw incorporated soil mix (mulched straw). The purpose of the straw was to generate CO₂ during its decomposition.

Twenty-five 8.5 x 11.5 cm white cylindrical containers (#202 cans) having a volume of 600 ml were prepared for each treatment.

Media and Container Preparation

A mixture of 1 kg screened sandy clay loam soil, 15.5 g of screened peat moss, 145 g deionized water, and Ca(NO₃)₂ at 160 mg N was prepared. These components were thoroughly mixed together to ensure uniform distribution. Wheat straw particles consisted of fines up to approximately 2.5 cm long. These were produced with a hammer-mill and used at 13 g kg⁻¹ of soil mix for treatments involving straw. The C:N ratio of the straw was 123:1. The amount of added nitrogen reduced the C:N ratio enough to allow immediate planting without competition for nitrogen between the crop and microorganisms (60, 61).

Containers were either filled with 780 g of the soil mixture or 700 g of the soil-straw mixture. Fifty containers of each type were prepared. An open-ended metal sleeve was placed over a container to prevent spillage during the filling process.

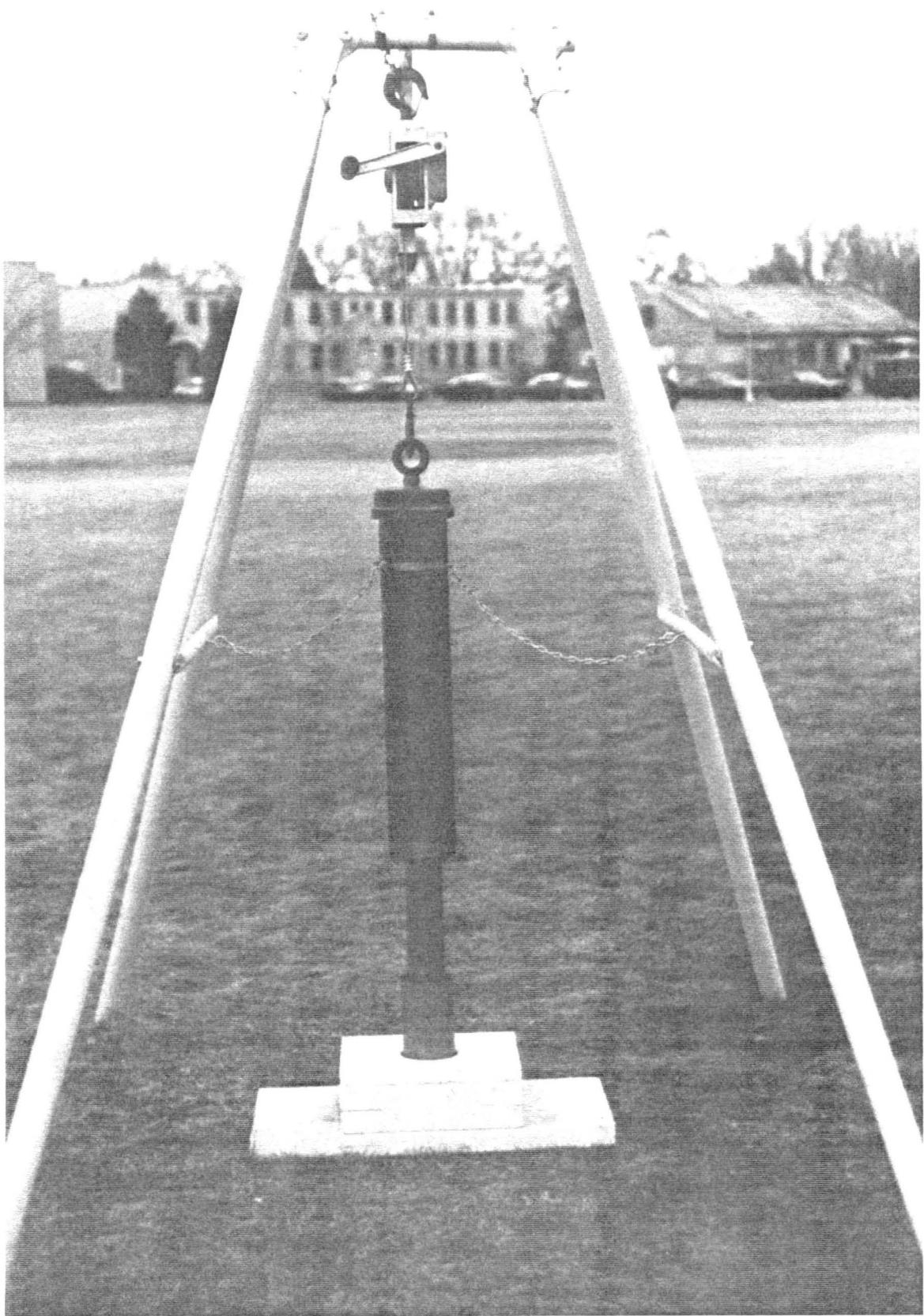
A wooden disk with a 1.2 cm diameter and 2.0 cm long dowel stick attached to the center of the soil-facing side was placed on top of a container after filling. This apparatus created a punch hole on the soil surface after placement under a compaction device employing a weight of 47.8 kg which was lowered on top of the disk as shown in the photograph (Fig. 1). (Calibration curve is in the Appendix.)

After all containers had been prepared, 25 from each of the 2 media mixtures were covered with a piece of poly-coated paper mulch. A 4 cm diameter hole had been pre-cut in the center of the mulch for the plant.

Seeding and Harvesting

On 21 March 1981, 3 to 4 Mesa 659 lettuce seeds were dropped in the punch hole of each container. Each container was considered an experimental unit. Thirty-two containers, 8 replicates per treatment, were placed in each of three growth chambers in a randomized complete block design. Prior to emergence, the chambers were not operating in order to keep the seeds in the dark and prevent excess evaporation. A 12-hour day length with a 22/15° C day/night temperature was used once emergence had begun. Ten days after planting, the seedlings were thinned to 1 plant per container.

Fig. 1. Compaction device for punch planting.



Lighting in the chambers was provided by four, 60 watt incandescent bulbs and twelve, 120 cm cool white fluorescent tubes. Irradiance was approximately $60 \text{ cal cm}^{-2} \text{ day}^{-1}$.

The containers were individually bottom watered by hand using individual watering mats. A moisture probe (Instamatic[®]) was inserted into the containers before watering to ensure that moisture levels in the 4 treatments would be similarly maintained.

After 45 days, all containers were harvested and leaf areas were determined for each plant by a LI-COR[®] area meter, model LI-3100. Plant tissue was then placed in a 70° C forced air drying oven for 48 hours and dry weights for each plant were determined using a 160 g capacity Mettler P163 balance.

Air Sampling Procedures

One container from each treatment was used in the air and soil-air sample collections for CO₂ analysis. Air sample differences with regard to treatments were found to be more distinct without the presence of the plant. In this way, no contribution or interference from the plant itself would be detected in the measured CO₂ levels. Consequently, reported surface CO₂ levels were those measured after plant removal. Samples were taken at 1 cm above the soil surface or mulch hole on April 23-25, and at 5 cm below the soil surface 25 to 29 days after planting.

Air samples were taken simultaneously from each container for determination of surface CO₂ concentrations. Labeled 10 ml Plastipak[®] syringes with hypodermic needles were used as the sampling device. Two Sage model 352 pumps with 2 syringes each were used in the

sampling procedure. The tips of the syringe needles were positioned over the soil surface or mulch hole at the specified height and the pump slowly withdrew the plungers collecting a 6 to 7 ml sample at a constant speed over a period of approximately 30 minutes (Fig. 2). Needles were then sealed with neoprene stoppers and the syringes removed from the pumps. All samples were analyzed within 2 hours.

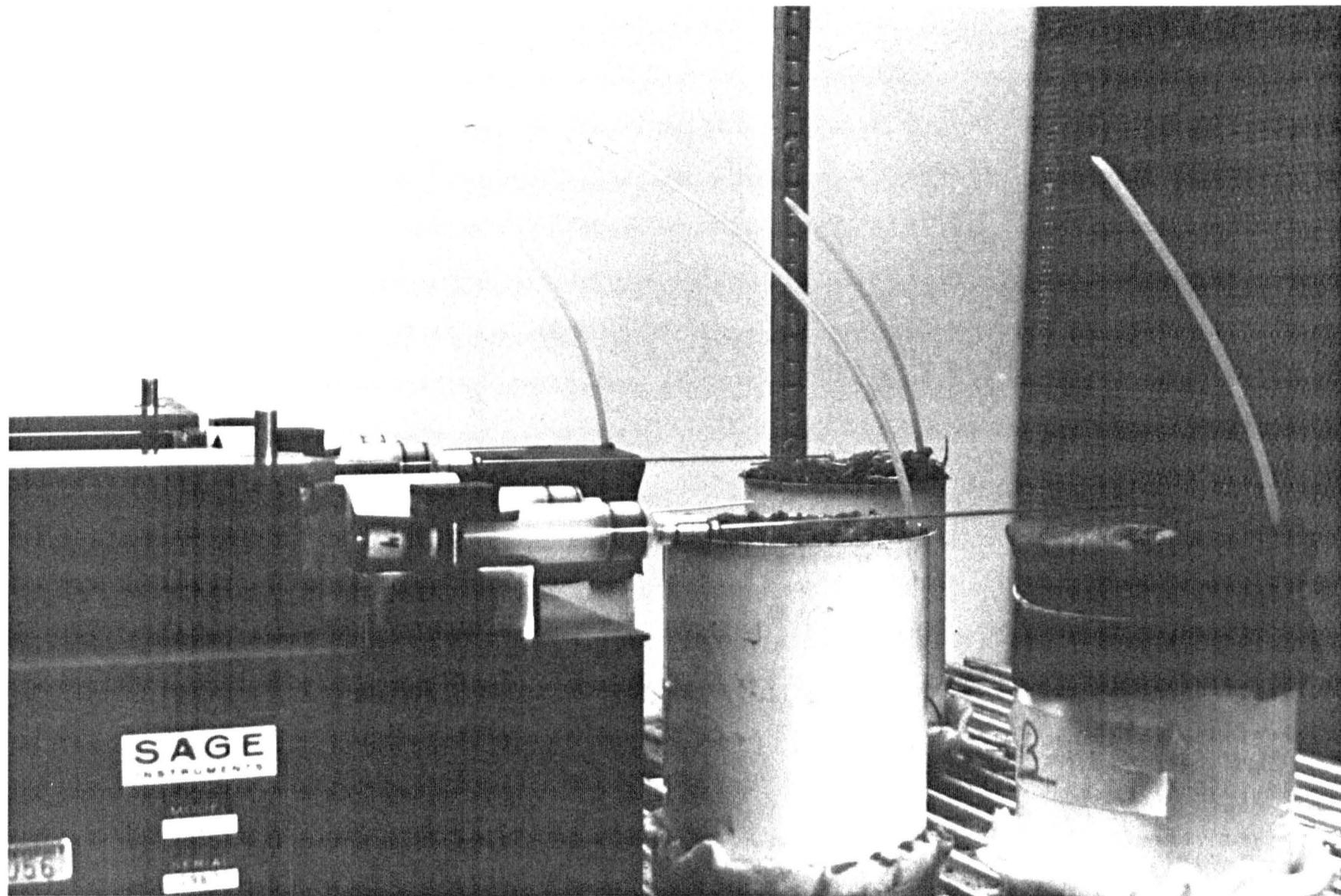
Soil-air samples were collected from the 5 cm depth using a method described by Hanan (25). Four cm lengths of 1 cm I.D. glass tubing were constructed and sealed at one end with a one-hole stopper. One end of a 25 cm length of 1 mm I.D. poly-tubing was inserted through the stopper. The glass tubes, one per container, had been previously buried horizontally at the designated depth with the poly-tubing extending above the soil surface. The exposed end of the tube was heat sealed to allow the tube-air to equilibrate with the soil-air. The sealed end of the tube was snipped off and a needle attached to a 10 ml Plastipak[®] syringe was inserted into the poly-tube for sample collections. A 10 ml sample was withdrawn and the needle capped. The exposed end of the poly-tube was resealed after each sampling.

Each collection time was considered a replicate for both air and soil-air samples. Samples were analyzed for CO₂ content with a Beckman 865 infrared gas analyzer connected to a strip chart recorder.

Soil Temperature

The soil temperatures of 5 containers from each treatment were monitored in the chambers after harvest for three 24-hour periods. Copper-constantan thermocouples were placed at a 5 cm depth in each container. Treatment temperature means were reported.

Fig. 2. Experiment 1. Air sampling in the growth chamber.



Experiment 2 - High Irradiance Outdoor Study

This phase of the study was performed in an outdoor situation to take advantage of the greater light intensities, and by so doing perhaps increase the magnitude of plant response. Rootview boxes were used as containers. The boxes were 48.8 cm long, 42.5 cm tall, and 25 cm wide across the top with a volume of 19.2 liters. The clear, plexiglass sides sloped inward forming a 15° angle with the base. The base width was approximately 2 cm from side to side. The transparent sides allowed root length to be measured; however, preliminary analysis indicated variability among boxes in root length was so great that no measurable differences could be found.

The same 4 treatments as in the growth chamber experiment were compared. Twenty of these boxes, 5 per treatment, were prepared.

Media and Container Preparation

A mixture of 10:10:1 by weight peat moss, vermiculite, and sandy clay loam soil was prepared and moistened. The soil was added as an inoculum to ensure microbial activity. Ten boxes were packed lightly with this mixture. The straw treatments contained the same type and amount of straw per kg of mix as in the previous experiment. Calcium nitrate at 160 mg N was added to prevent nitrogen immobilization. Ten boxes of the soil-straw mixture were prepared. After all the boxes were filled with their respective mixes, 2000 ml of water were added to each container.

Each box had buried at 5 cm depths, 2 soil-air sampling devices identical to those in the previous experiment. These devices were positioned midway between 2 proposed plants.

A pre-punched piece of mulch was placed over 10 boxes (5 for the mulch covered soil and 5 for the mulch covered soil plus straw treatments) and secured with adhesive tape for the mulch treatments.

On 10 August 1981, the boxes were planted with Mesa 659 lettuce seeds. The boxes were kept indoors in the dark until germination. After germination they were moved to an outdoor area and each box was thinned to 2 staggered rows of 6 plants on one side and 5 plants on the opposite side for a total of 11 plants per box. Each row was 2.7 cm from the side of the container. Cardboard or plywood panels were placed over the 2 transparent sides of each box to prevent root illumination.

Watering was done by hand. A moisture probe (Instamatic[®]) inserted into the box determined when water should be applied.

Environmental Parameters

Air temperature and irradiance were monitored during the experiment. A hygro-thermograph was placed at the experiment site at approximately 45 cm above the ground surface. Global solar radiation was measured with a Belfort[®] pyranograph also placed at 45 cm. Hourly and daily irradiance was recorded.

Soil temperatures at a 5 cm depth were monitored after plant harvest. Twelve boxes, 3 per treatment, were used in the soil temperature determinations. Two thermocouples per box were placed at the designated depth and a recorder identical to the one used in Experiment 1 recorded the temperatures. Average hourly temperatures were reported.

Harvesting Procedures

A randomized complete block design was used for leaf area and dry weight data collection. Each box was an experimental unit and there were 5 boxes per treatment. Two sampling units or 2 harvests were taken within each experimental unit. An average leaf area and corresponding dry weight for each treatment replicate was reported.

On 19 September, 40 days after planting, 5 plants per box for a total of 100 plants were harvested. Leaf areas were measured with a LI-COR[®] area meter (Mod. LI-3100) and plant tissue dried in a forced draft oven at 70° C for 48 hours. Dry weights were determined with a Mettler P163 balance. On 27 September, 47 days after planting, this same procedure was repeated with 5 more plants per box.

Air Sampling Procedures

Air samples for CO₂ analysis were collected at the 1 cm height after harvest on September 27-29. Samples were taken during the day and night hours. Night measurements were reported because of the more thermally stable conditions and lower wind speeds prevalent at that time. These concentrations would then represent the maximum CO₂ concentration buildup over the respective treatments.

Ten ml Plastipak[®] syringes were used for sample collection. However, the plungers were withdrawn by hand without the aid of pumps. The needle tips were placed 1 cm over where a plant had been and the plungers pulled back until at least 7 ml of air had been collected. An average of five samples from each treatment collected at each sampling time was reported. Air samples were collected from the air layer between

the soil and mulch surface on September 27-29. A 15 cm long needle on a 10 ml syringe was placed under the mulch via the mulch hole and a sample was drawn. These samples were taken during the night hours.

Soil-air samples were collected September 10-25, between 1400 and 1500 MDT. The same sampling procedure as in the previous experiment was followed. All air and soil-air samples were analyzed with a Beckman 865 infrared gas analyzer.

Experiment 3 - Field Study

Carbon dioxide was measured in a lettuce canopy to evaluate the feasibility of CO₂ fertilization under field conditions in conjunction with 4 treatments: (1) mulch covered soil, (2) CO₂ release line, (3) CO₂ release line under a mulch covering (mulched CO₂ line), and (4) control - no means of enrichment.

The lettuce was grown at the Horticulture Research Center, 7 miles northeast of Colorado State University. Field preparation of the clay loam soil included incorporation of 300 kg ha⁻¹ of triple superphosphate.

The crop was seeded on 10 June 1982, in north-south oriented double row beds on 100 cm center. There were 20 plants in each row. The plot area, 200.7 m², was divided into 33 beds. Between and within row spacing was 30 cm on each bed. The 2 outside rows, east and west and the 2 plants on the north and south ends of all rows were treated as border rows and plants and were eliminated from data collection. Also, between each treatment bed, a non-treatment bed was seeded for a barrier between treatments. This made a total of 16 treatment beds, 4 per treatment, and 17 guard or border beds.

A 1 m width of poly-coated paper mulch was cut into 7.5 m lengths prior to placement on the mulched beds. Holes for the plants were pre-punched with the sharpened end of a 4 cm I.D. pipe. These mulch pieces were then placed over the beds and anchored at the sides and ends with soil.

Irrigation System

The plots were drip irrigated. There were separate systems for the mulch and non-mulch beds, because mulch conserves soil moisture by preventing excess evaporation from the soil surface. Hence, different irrigation schedules were required. One flexible polyethylene bi-wall drip irrigation hose extended the length of each row and was sealed at one end. The opposite end of the drip hose was connected to one of two 1.88 cm I.D. polyethylene main supply lines. These 2 main lines were connected to 2 flowmeters (Rockwell Mfg. Co., 5/8" connections) that monitored the amount of water applied through each line at each irrigation time. The flowmeters were connected by a 2.5 cm I.D. plastic pipe to the water supply. At this connection a drip irrigation flow rate control device (Watts No. IR56, 3/4" connection, 10-60 p.s.i.) was inserted. The drip tubes for the mulched beds were placed directly under the poly-coated paper mulch. Tensiometers placed in the soil at 25 cm depths were used to determine when irrigation was required. A treatment was irrigated when the average of 4 tensiometers reached a tension of 0.5 bar.

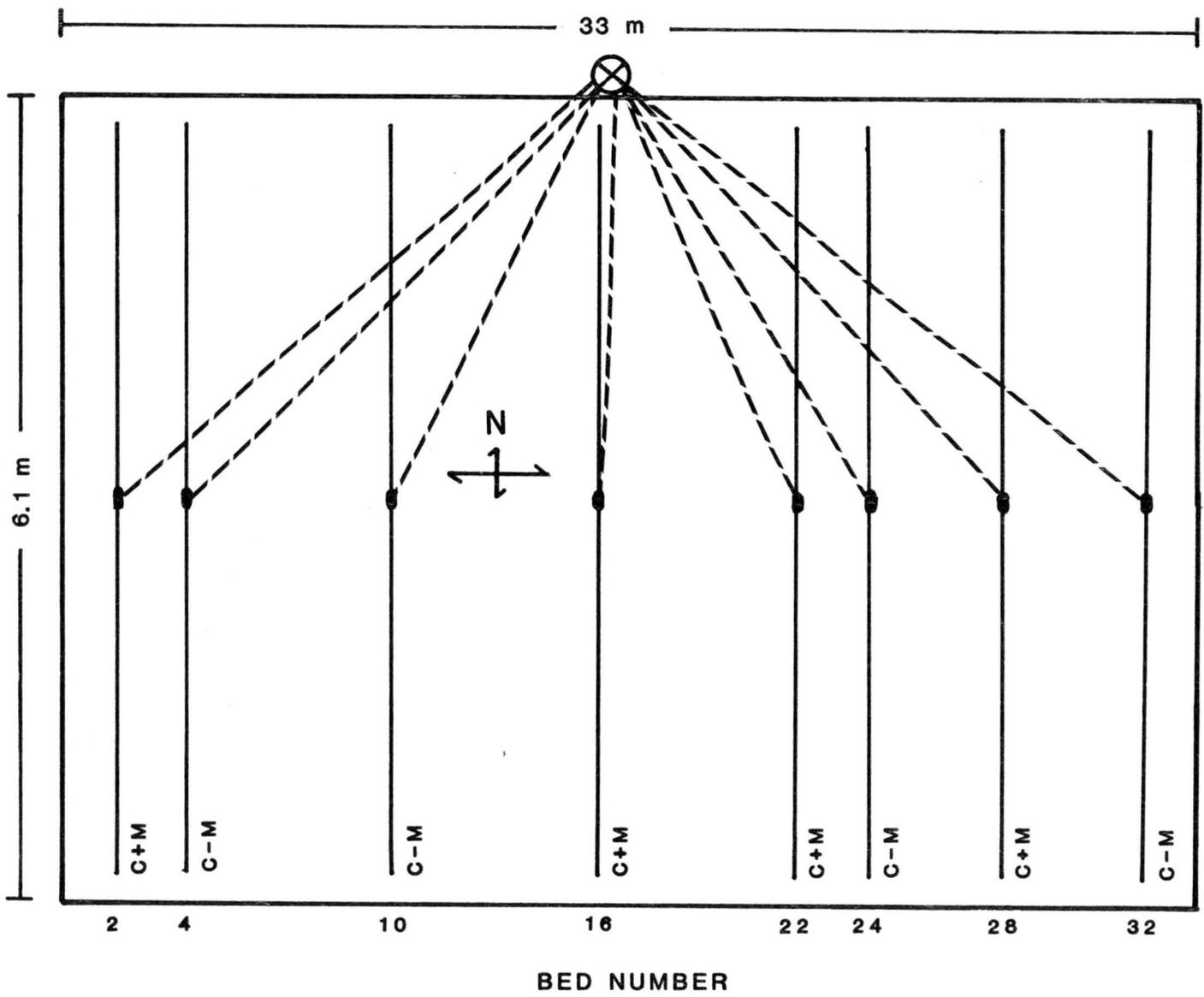
Carbon Dioxide Release System

The release system was designed to simulate a multiple line source, releasing CO₂ at ground level at a constant rate. A diagram of the plots and the CO₂ release system is presented in Figure 3.

A 6.1 m long bi-wall drip irrigation line was laid on the soil surface midway between 2 rows on a treatment bed and the 2 ends sealed. The outer wall of the drip line contained 0.6 mm holes, spaced 0.3 m apart. For the mulched line treatment, the line was placed directly under the mulch along with the regular drip irrigation line used for irrigation purposes. There were 8 hoses in all, 4 for the mulch covered lines and 4 without a mulch covering. A sealed 5 mm I.D. rigid plastic tube extended the entire length of each line used for CO₂ release to keep the drip lines from becoming kinked and thereby prevent CO₂ flow. In this way, a uniform discharge was maintained throughout the line.

The CO₂ source consisted of a standard CO₂ cylinder with a standard regulator. The cylinder was centered at the north end of the test plots. Carbon dioxide gas flowed from the regulator into two, 4-valve manifolds through 1 cm I.D. tygon tubing. Eight 15 m lengths of 5 mm I.D. tygon tubing, connected to the 2 manifolds, transported the CO₂ to the drip lines on the treatment beds. Each tygon tubing was inserted into a 1 cm by 1.2 cm by 1.2 cm glass T-joint which had been placed midway between the ends of each CO₂ release line. This divided a 6.1 m run into approximately two 3 m intervals, thus decreasing the length of CO₂ release and the hazard of pressure drop along the line.

Fig. 3. Diagram of CO₂ release system. ⊗ = CO₂ cylinder; ■ = T-joint connection; — = Tygon tubes (15 m lengths); C+M = CO₂ line (drip tube) plus mulch; C-M = CO₂ line minus mulch. A randomized block design was used with mulched and non-mulched beds as well as the CO₂ beds randomized in an east-west direction with 4 replicates per treatment. Beds were 100 cm from center to center and buffer beds were provided between each treatment bed. A single CO₂ release line was centered on the beds. Only beds with CO₂ release lines are shown.



A 12 liter min^{-1} capacity flow meter, inserted into the 1 cm I.D. tygon tube directly before the 2 manifolds, monitored the flow rate into the 5 mm I.D. tygon tube. The CO_2 flow rate in liters per minute was determined from an equation (below) given by Takami (57) which takes into account the total release area (A , m^2). His calculations indicated that a release rate of $0.01 \text{ g m}^{-2} \text{ s}^{-1}$ of CO_2 should produce about 1000 ppm CO_2 near the bottom of a canopy at a windspeed of 1 m s^{-1} , depending on the type of crop canopy. This rate was used as an approximation in this study. The entire release area in this study was approximately 15 m^2 . The area was not continuous in that non-release or non- CO_2 treatments interrupted this area. Therefore, calculated flow rates were tested by air sampling to ensure that enrichment was indeed occurring in all eight CO_2 treatment beds.

The calculated final flow rate (FR) in liters min^{-1} was determined by:

$$\text{FR} = (A/D_c) \times 0.01 \text{ g m}^{-2} \text{ s}^{-1} \times 60,000^1$$

where D_c is the density of carbon dioxide gas and A is the release area:

$$A(\text{m}^2) = 8(\text{beds}) \times 0.305 \text{ m} \times 6.1 \text{ m} = 14.9 \text{ or } 15 \text{ m}^2.$$

Therefore,

$$\text{FR} = (15/1800) \times 0.01 \times 60,000 = 5.0 \text{ liters min}^{-1}.$$

The flow rate most frequently used was between 5.5 to 6.0 liters min^{-1} .

¹ $1 \text{ m}^3 \text{ sec}^{-1} = 60,000 \text{ liters min}^{-1}$.

Environmental Parameters

Irradiance was measured during the study with a Belfort[®] pyranograph located at the research center. Recovery rates of applied CO₂ by plants are known to be positively correlated to radiation intensity.

Takami (57) and Takami and VanBavel (58) found the efficiency of CO₂ release to be insignificant below an irradiance of 0.3 cal cm⁻² min⁻¹. Maximum efficiency was predicted at an irradiance of about 1.4 cal cm⁻² min⁻¹ depending on canopy type. Harper (26) also stated that below 0.2 cal cm⁻² min⁻¹ efficiency was negligible.

Efficiency of CO₂ release is negatively correlated with wind speed. Normally between a windspeed of 1 and 3 m sec⁻¹ at canopy surface, resulting efficiencies are acceptable (57, 58). However, at velocities above 3 m sec⁻¹ the released CO₂ is rapidly swept away, dropping efficiencies to very low levels. Consequently, the higher the windspeed the greater the release rate must be to compensate if the same expected CO₂ concentrations are to be maintained.

From these findings, CO₂ releases were not made if irradiances were below 0.3 cal cm⁻² min⁻¹ at the beginning of the release period or windspeed above 3.5 m sec⁻¹ (7.8 mi hr⁻¹). This usually resulted in a continuous CO₂ release on clear, calm days between approximately 1000 and 1700 MDT.

Carbon dioxide applications began on 10 July 1982, 30 days after planting. The plants were approximately 6 cm tall by this time. Establishment and growth had been delayed due to rainy conditions during the latter half of June.

Harvesting Procedures

A randomized complete block design was used for obtaining dry and/or fresh weights for the treatments. Each bed was an experimental unit and each treatment was replicated 4 times.

On 7 August 1982, 58 days from planting, five plants from each treatment bed from each of the 4 reps were severed at the soil surface and fresh weight for each plant was measured. Immediately after a plant had been harvested it was weighed on a Fisher/Ainsworth Model SC-2000, battery-operated electronic balance with a 2000 g capacity and accurate to ± 1 g. This balance was designed for portability for use in the work area. An average weight for each treatment replicate was reported. This procedure was repeated on 15 August with the same sample size. In addition to fresh weight determination, dry weights for each plant were also measured.

After recording fresh weights, plants were placed in a 70° C forced draft drying oven in labeled paper bags for 72 hours. Whole plant dry weights were obtained with a Mettler P163 balance.

Air Sampling Procedure

Air samples for CO₂ determination were collected at 1 cm above the soil surface and just above canopy height (25 cm). Samples were collected before and after harvest during light and dark hours. However, post harvest samples were reported due to an improved sampling technique.

Four one-liter tedlar sample bags (SKC Inc., Mod. 231-01), one for each treatment, were used to obtain the surface air samples. Each

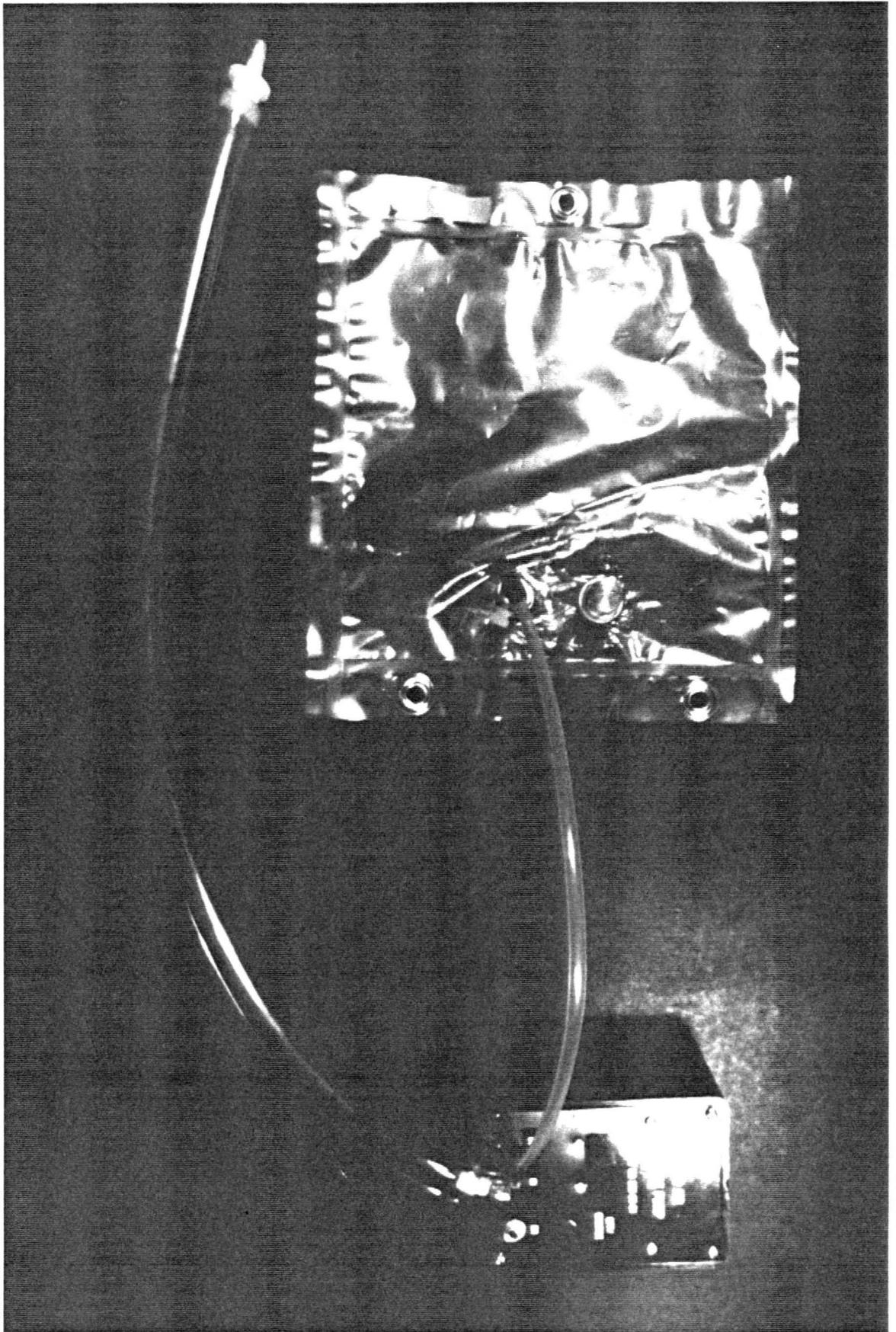
sample bag had a dual fitting consisting of a replaceable septum and a hose and valve. A battery-operated portable pump (SKC. Inc., Mod. 222-23-115) equipped with vacuum and pressure connections and designed for grab-bag sampling was used to inflate the sample bag. A 3 mm I.D. teflon tube extending from the pressure connection on the pump was connected to the valve fitting on the sample bag for collecting samples. One end of a 1 cm I.D. tygon tube was positioned at the appropriate height and the other end attached to the vacuum connection on the pump. A photograph of the pump and sample bag is presented in Figure 4. Air was drawn through the tygon tube into the pump and out through the pressure connection and teflon tube to the sample bag. A 0.5 liter air sample was drawn in approximately 30 seconds. After sampling, the valve fitting on the bag was closed and the teflon tube removed. Samples were collected from each treatment at every sampling time. Samples were analyzed within 1 hour.

Soil-air samples were collected from a 5 cm depth in the soil using the same procedure as in the previous studies. There were some changes in the apparatus involved, however. A 2.5 ml glass, gas-tight syringe was used in sample collection and the horizontally buried glass tube length was increased to 6 cm. Fifteen samples were taken between July 20 and August 3.

Carbon Dioxide Analysis

Samples for the determination of CO₂ concentrations at all levels were analyzed with a Hewlett-Packard 5840A gas chromatograph with the column packed with Porapak QS (Applied Science Laboratory). The sample was passed through the column and then into a nickel catalyst

Fig. 4. Experiment 3. Battery powered pump and sample bag used in collecting surface air samples.



methanator that converted CO_2 quantitatively to methane for detection by a Flame Ionization Detector. Areas under the peak for known CO_2 concentrations of 295, 413, 691, and 1000 ppm were used for determining the standard curve.

A 2.5 ml glass, gas-tight syringe was used for sampling from the collection bags, for the above surface samples. The needle was inserted into the septum fitting on the bag and a sample drawn for immediate injection into the GC. Collection bags were not used for the soil-air samples which were drawn directly from the sampling tubes by means of the syringes. Since the GC could not accurately analyze CO_2 concentrations above 0.1% CO_2 (1000 ppm) due to incomplete conversion to methane, samples thought to be above this concentration were diluted with N_2 before injection into the gas chromatograph.

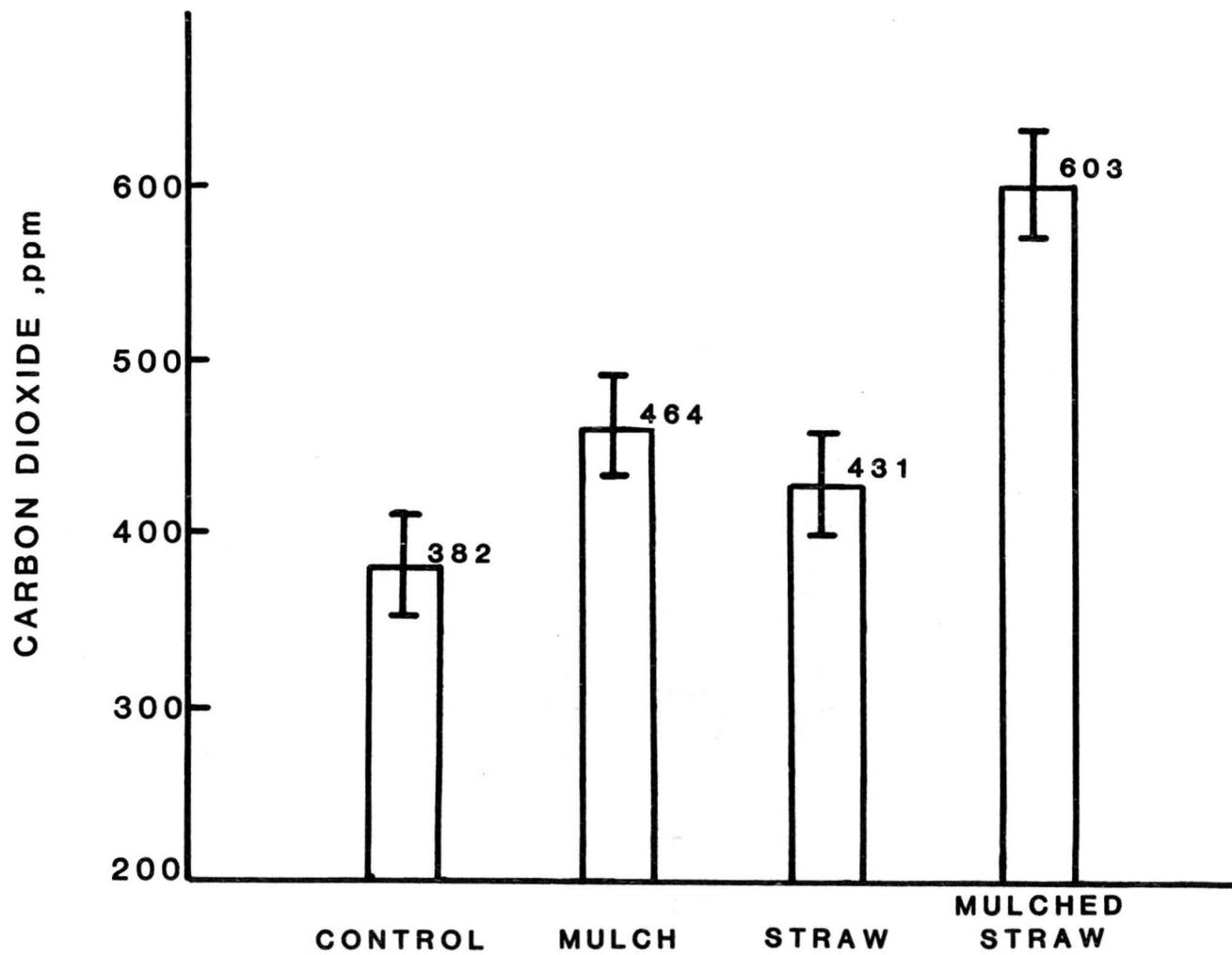
RESULTS AND DISCUSSION

Experiment 1 - Low Irradiance Growth Chamber Study

Mean CO₂ concentrations did not differ significantly between sampling dates and were combined. Measurements were taken at 1 cm above the soil surface and at a depth of 5 cm in the soil. Mean separation between treatments at both positions was determined by Tukey's Honestly Significant Difference (hsd) procedure.

Mean CO₂ concentrations at 1 cm above the soil surface are presented in Figure 5. Relative differences in CO₂ levels between treatments were found to be greater after plant harvest. Hence, a single container was selected from each treatment and its plant severed at the soil line for air sampling. The concentrations reported would then represent the maximum CO₂ levels available for plant uptake. Carbon dioxide levels over the mulch treatment were greater than those over the bare soil control, representing an increase of approximately 22 percent. The difference between means is significant at the 1% level indicating a real increase in CO₂ concentration. Previous field studies with this type of mulch indicated a similar increase in CO₂ levels near the soil surface (36, 40). Hopen and Oebker (28) measured CO₂ directly over the mulch hole and found slightly greater CO₂ levels (12%) there than over bare soil, but did not credit yield increase in mulched cucumber plants to this effect. They pointed out, however, that seedlings growing directly in the mulch hole would possibly benefit from these higher CO₂ concentrations.

Fig. 5. Experiment 1. Mean CO₂ concentrations collected at 1 cm above the soil surface.
Each mean represents 9 measurements made April 23-24, after plant removal.
5% hsd = 59.



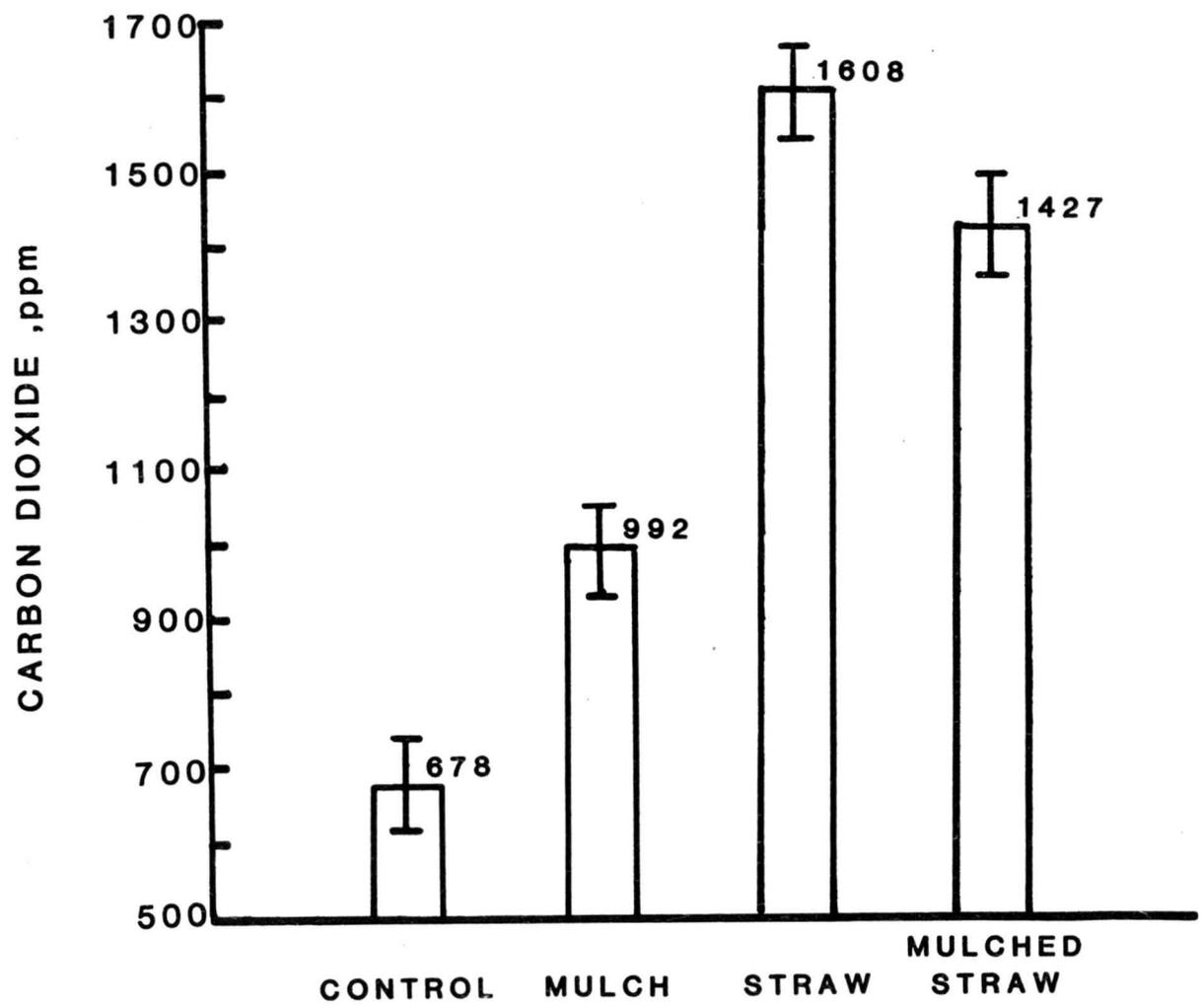
Mean CO₂ concentration over the straw treatment was not significantly greater than the control treatment level. The mulched straw treatment, however, raised CO₂ levels over 50% when compared to the bare soil control. This was nearly a doubling of the current ambient CO₂ concentration (335 ppm) and represented the maximum potential for plant response. These results indicated that although straw was providing a CO₂ source, any enrichment of surface concentrations was quickly dissipated without a mulch covering. The mulch created a barrier concentrating the increased soil evolved CO₂ under the mulch for subsequent funneling to the plant.

Measurements of soil-air CO₂ concentrations were taken at a depth of 5 cm. The mean of 6 measurements per treatment are presented in Figure 6. Carbon dioxide concentrations normally occurring in the soil atmosphere range from 1000 to 50,000 ppm (22, 46) depending on soil composition and depth of measurement. The levels reported in this study appear low because of the shallow sampling depth. However, the location would be that of the effective root-zone of a seedling.

Carbon dioxide concentration of the soil atmosphere was significantly increased by the addition of mulch but not to a phytotoxic level. The mulch reduced the rapid loss of CO₂ from the soil allowing it to concentrate in the upper soil profile. Non-mulched straw generated the most soil CO₂, more than twice as much as that of the control treatment. However, as previously noted, this enrichment source did not greatly affect surface concentration without a mulch covering.

No attempt was made in this study to evaluate the amount or effect on plant response of CO₂ uptake by the root. Recent research results, however, show that root absorbed CO₂ may contribute

Fig. 6. Experiment 1. Mean CO₂ concentrations collected at a 5 cm depth. Each mean represents 6 measurements made April 15-19. 5% hsd = 126.

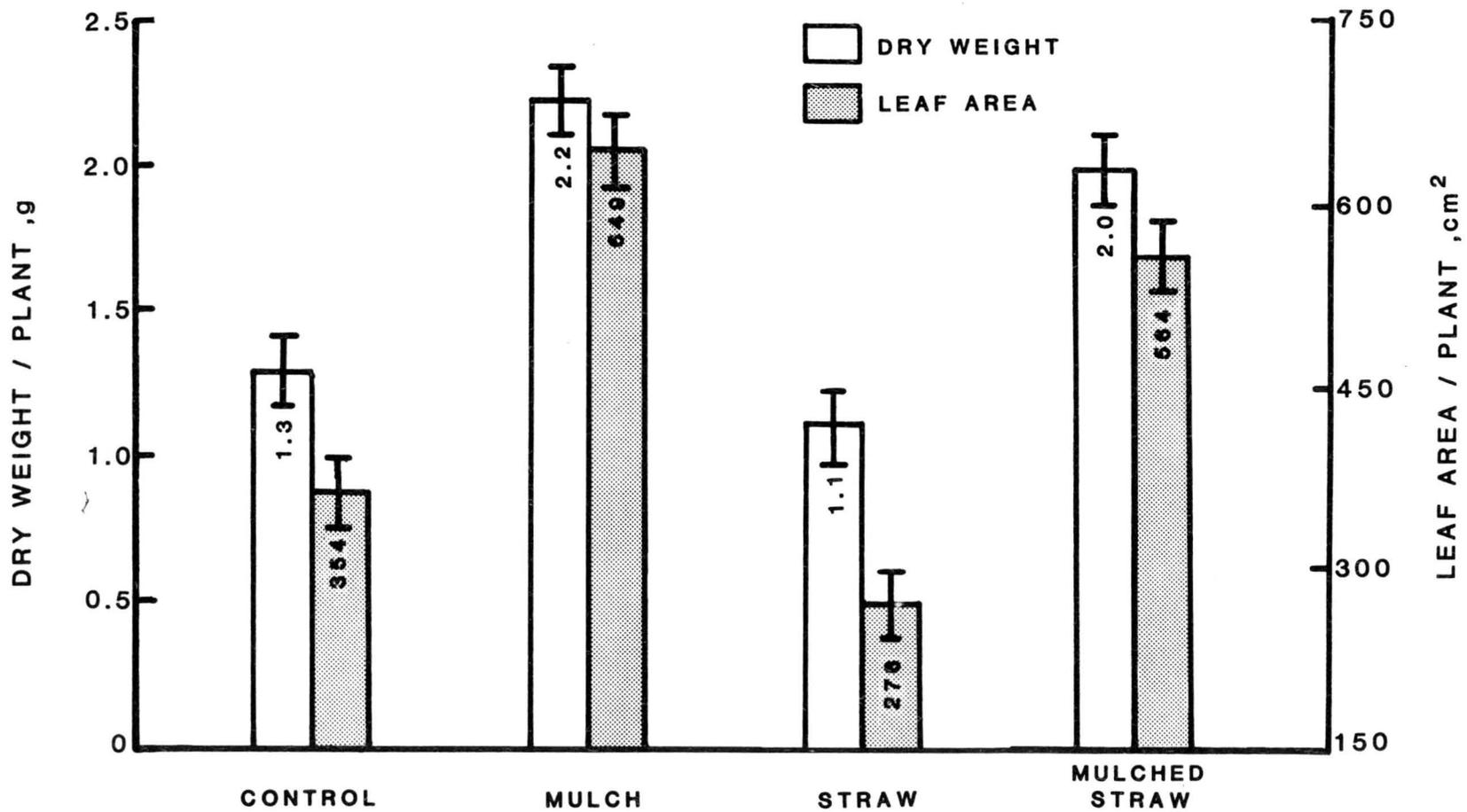


significantly to total plant CO_2 uptake (5). Therefore, treatments affecting subsurface CO_2 concentrations may play a more direct role in total plant growth than simply providing a greater soil CO_2 flux to the photosynthesizing tissues.

On 5 May the plants were harvested. Mean leaf area and dry weight for each treatment are presented in Figure 7. Plant response means did not differ significantly among the 3 growth chambers and, therefore, were combined. Mean leaf area of mulched plants indicated an 83% increase over the non-mulched plants. Mean dry weight of mulched plants was 78% greater than non-mulched plants. This early yield increase was a reflection of the enriched CO_2 levels available to the mulched plants. Lettuce grown in CO_2 enriched greenhouses commonly show yield increases comparable to those reported in this study (68). This suggests that differences in plant growth were mainly due to higher CO_2 concentrations found over the mulch since similar soil moisture status and nutrition levels were maintained for mulched and non-mulched plants.

Mean dry weight of the non-mulched straw treatment was slightly lower than the bare soil control (Fig. 7). Leaf area was significantly lower than the control. The CO_2 concentrations over the straw and bare soil treatments were comparable; therefore, significant differences in plant response were not expected. Mulched straw generated the maximum CO_2 concentration and thus, the greatest potential for yield increase. Unexpectedly, the yield of this treatment was lower than the yield of the mulch treatment, disrupting the trend of greater yields related to significant increases in CO_2 concentrations. Positive yield response to CO_2 concentrations much higher than the maximum level in

Fig. 7. Experiment 1. Mean plant leaf area and dry weight data collected on May 5, 1981.
5% hsd (leaf area) = 59; 5% hsd (dry weight) = 0.2.

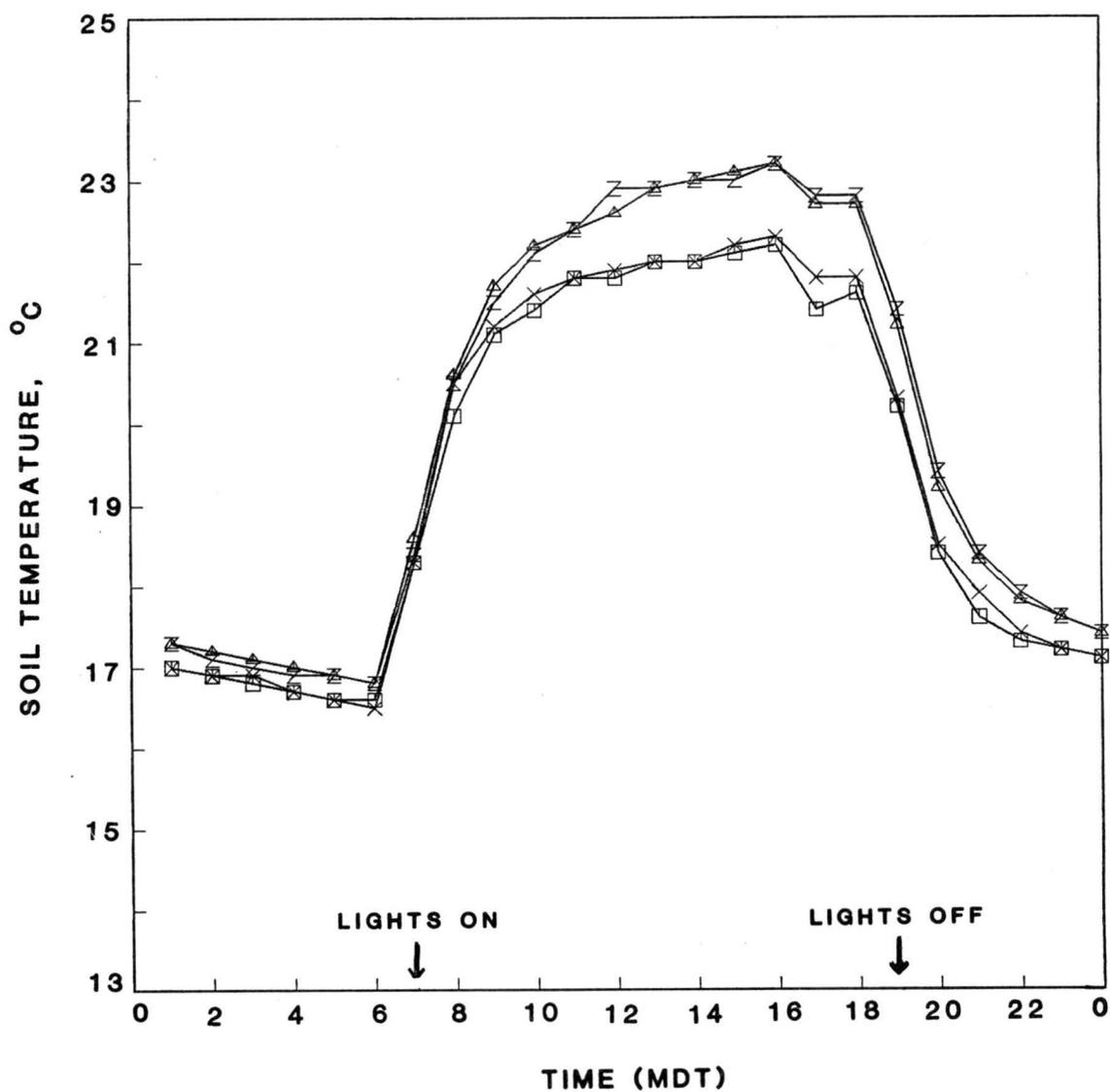


this study have been reported in greenhouse grown lettuce (68). Consequently, another factor in addition to CO₂ concentration was involved. An initial explanation might be the relatively low light intensities in the growth chambers compared to field conditions. The irradiance in the chambers was approximately 60 cal cm⁻² day⁻¹, which is only a fraction of the irradiance usually occurring in the field. At low irradiances, assimilation rate saturates at relatively low CO₂ concentrations (34, 41, 42, 55). Thus, the rate of assimilation would not be enhanced by CO₂ enrichment. The plants may have been unable to utilize the maximum CO₂ levels generated by the mulched straw treatment due to light limitation and not CO₂ limiting the photosynthetic process.

Soil temperature at a depth of 5 cm was monitored for all treatments after harvest (Fig. 8). Mulch appeared to be the only factor affecting soil temperature. The effect of straw on temperature was negligible. Both mulch treatments increased light period soil temperatures about 1° C over the two non-mulch treatments. Smaller differences were detected during the dark hours. Polyethylene coated black paper normally warms the soil similar to black plastic film (16). Although high temperatures may be measured at the mulch surface during the day, the soil temperatures even at shallow depths are not greatly increased and are frequently lower than bare soil (40, 53). Also, heat escaping from bare soil during the night is minimized with a mulch covering, often creating higher soil temperatures than bare soil. This is more apparent in field studies where temperature differences between mulched and non-mulched soils are usually greater than chamber studies. The slight increases in soil temperature under the mulch reported in this study could not have entirely accounted for the magnitude of plant response.

Fig. 8. Experiment 1. Mean hourly soil temperatures at a 5 cm depth, measured on May 6-8, 1981, with plants removed.

△ MULCHED STRAW
□ STRAW
Z MULCH
× CONTROL



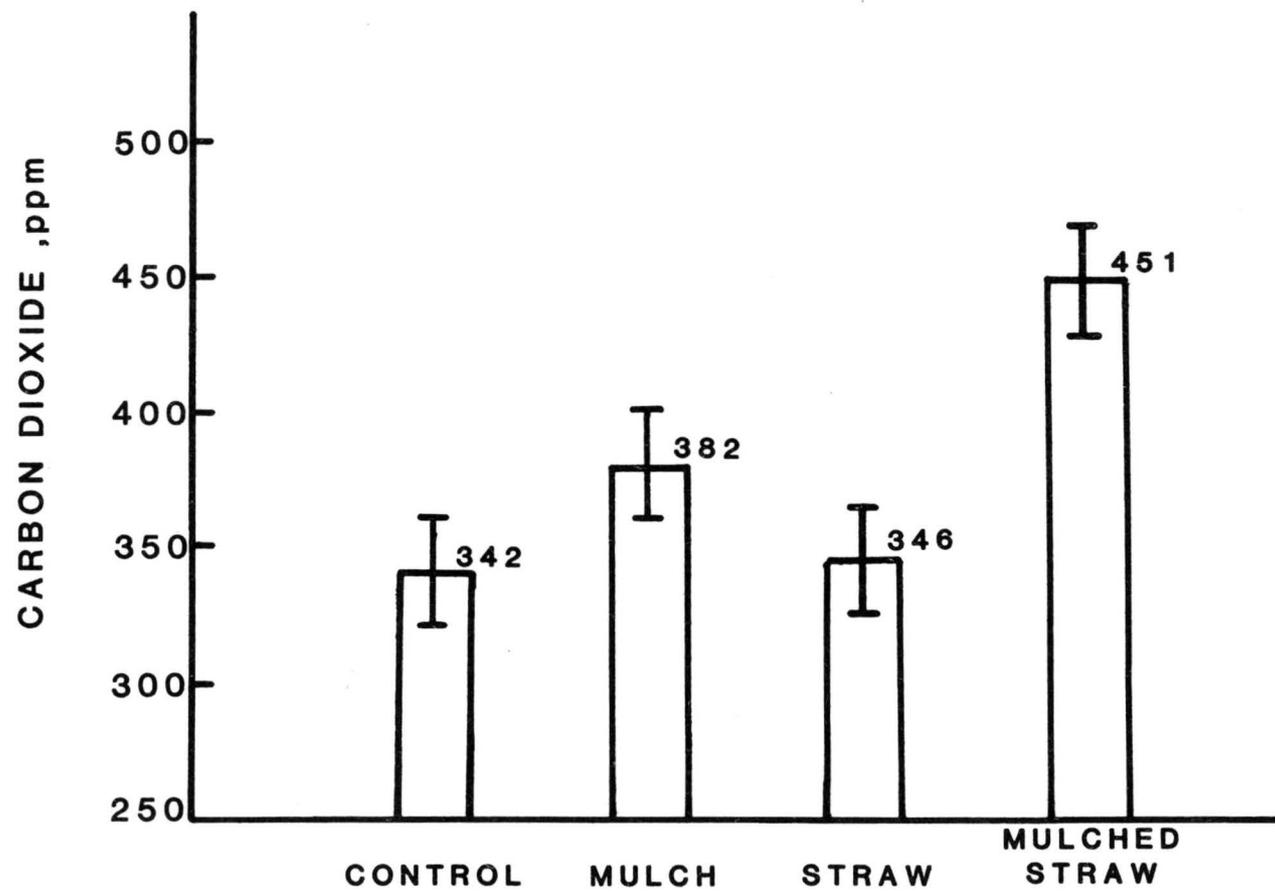
Even so, higher soil temperatures would raise CO₂ concentrations by stimulating soil respiration. Minor discrepancies in soil temperature would have been more important in determining plant growth if critical soil temperatures had been encountered.

Experiment 2 - High Irradiance Outdoor Study

Carbon dioxide measurements at 1 cm above the soil surface after final plant harvest were taken during the dark to take advantage of reduced windspeed and more thermally stable conditions. The means of 22 measurements at this height for each treatment are presented in Figure 9. The CO₂ concentration was enhanced by the mulch when compared to the bare soil control. This represented an increase of 12%, which was lower than the increase observed in Experiment 1. This was probably due to the less controlled conditions of an outdoor study when compared to growth chamber experiments. Carbon dioxide enrichment from a point source, such as the mulch hole, is inversely related to windspeed. Consequently, any wind movement during sampling may have affected the measured concentrations.

Carbon dioxide concentration over the non-mulched straw treatment was almost identical to concentrations measured over the bare soil control. The mulched straw, however, provided the greatest CO₂ enrichment with an increase of 32% over the control CO₂ concentration. This correlates well with Experiment 1, which determined that mulch was required to significantly increase CO₂ concentration near the soil surface. The mulched straw again produced the greatest potential for plant response on the basis of CO₂ enrichment.

Fig. 9. Experiment 2. Mean CO₂ concentrations at 1 cm above the soil surface. Each mean represents 22 measurements made September 27-29. 5% hsd = 40.



Higher CO₂ levels were found in the interface between the soil and mulch layer in both the mulch and mulched straw treatments than over the bare soil (342 ppm). The mean CO₂ concentration of 22 measurements for the mulched straw treatment (588 ppm) was significantly greater than the mean concentration of the mulch treatment (456 ppm). These measurements indicated that CO₂ was concentrating under the mulch for release through the mulch hole.

Carbon dioxide measurements made at a depth of 5 cm under the mulch were slightly greater than measurements made under the bare soil (Fig. 10). The addition of straw significantly increased the CO₂ concentration in the soil with mulched straw creating the maximum concentration (1453 ppm). These results differ from Experiment 1 in which CO₂ levels under non-mulched straw were greater than concentrations under mulched straw. However, subsurface CO₂ concentrations would not be expected to have an adverse affect on root or plant growth as previously discussed.

Leaf area and dry weight data for plants harvested on 19 and 26 September are presented in Figures 11 and 12, respectively. A reversal of treatment means is observed in comparing these two harvests. The mulch yield for the first harvest was slightly less than the control yield. In contrast, the final yield of mulched plants was approximately 13% greater than the control yield. This increase reflected the 12% rise in surface CO₂ concentration available to the mulched plants during the same growing period.

Final yield of the straw treatment was similar to the control yield. However, it should be noted that mean leaf area of straw treated plants was lower than control plants. This same response was observed

Fig. 10. Mean CO₂ concentrations at a 5 cm depth in the soil. Each mean represents 30 measurements made September 10-25. 5% hsd = 228.

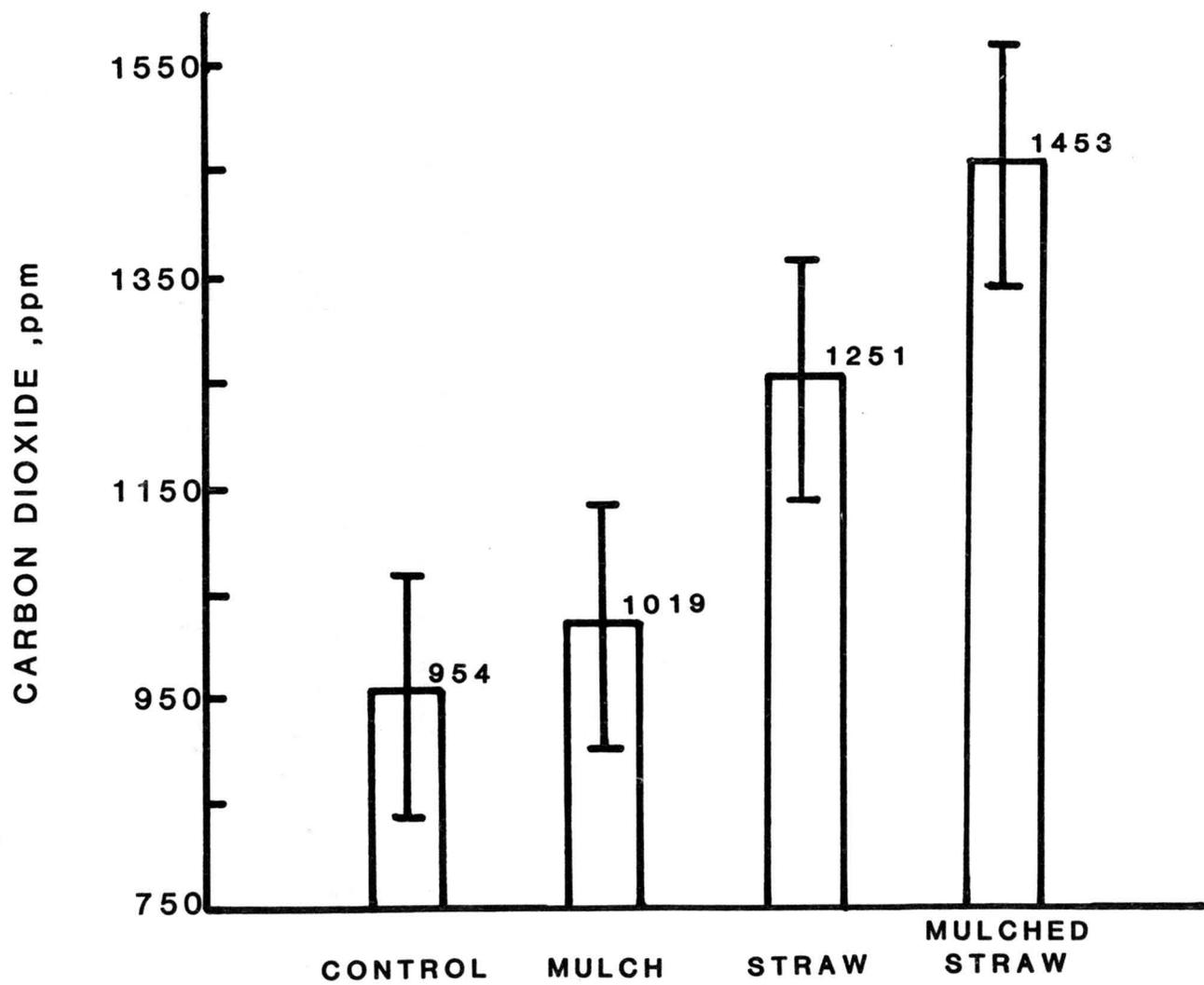


Fig. 11. Experiment 2. Mean plant leaf area and dry weight. Data were collected on September 19, 1981. Treatment means did not differ significantly at the 5% level.

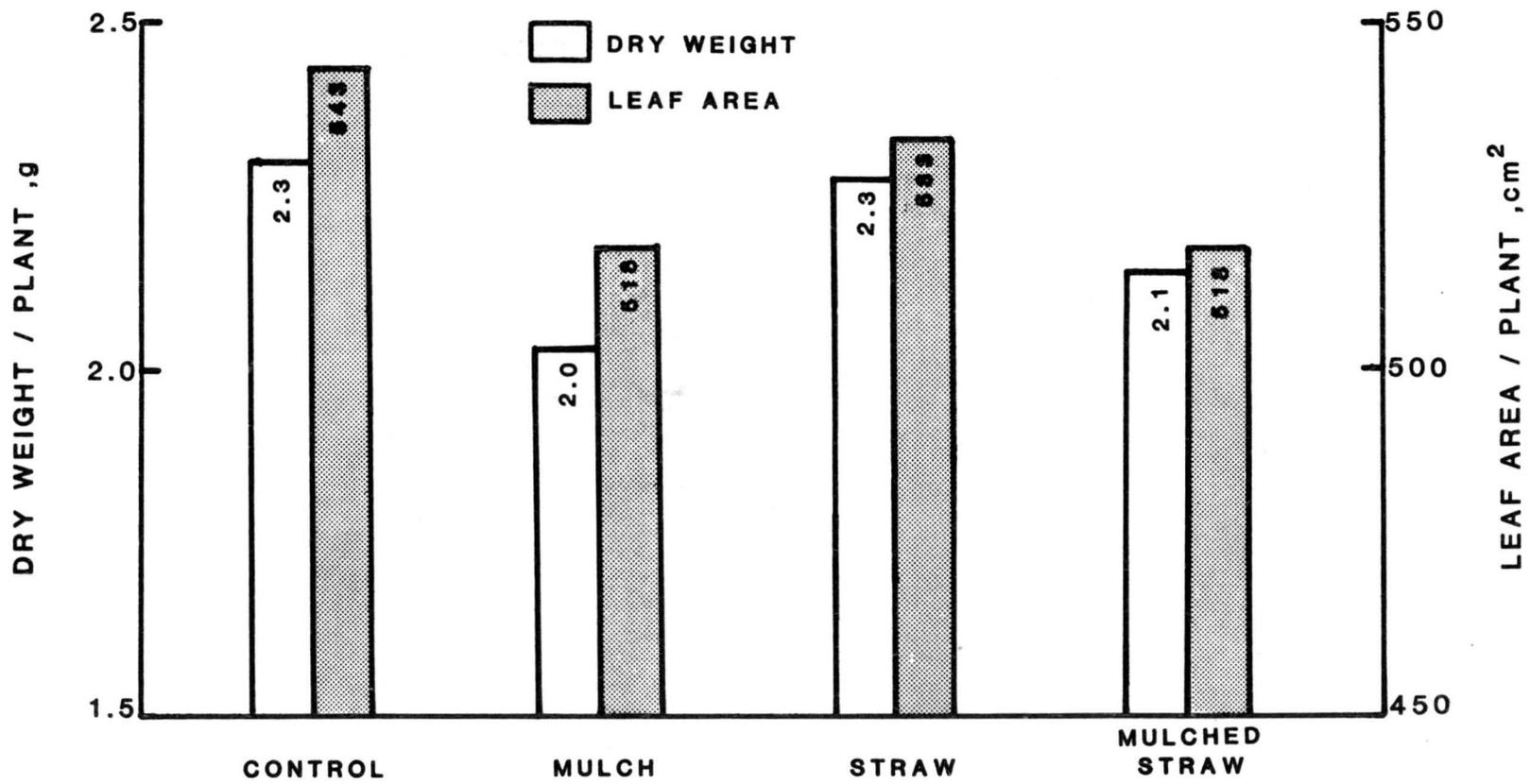
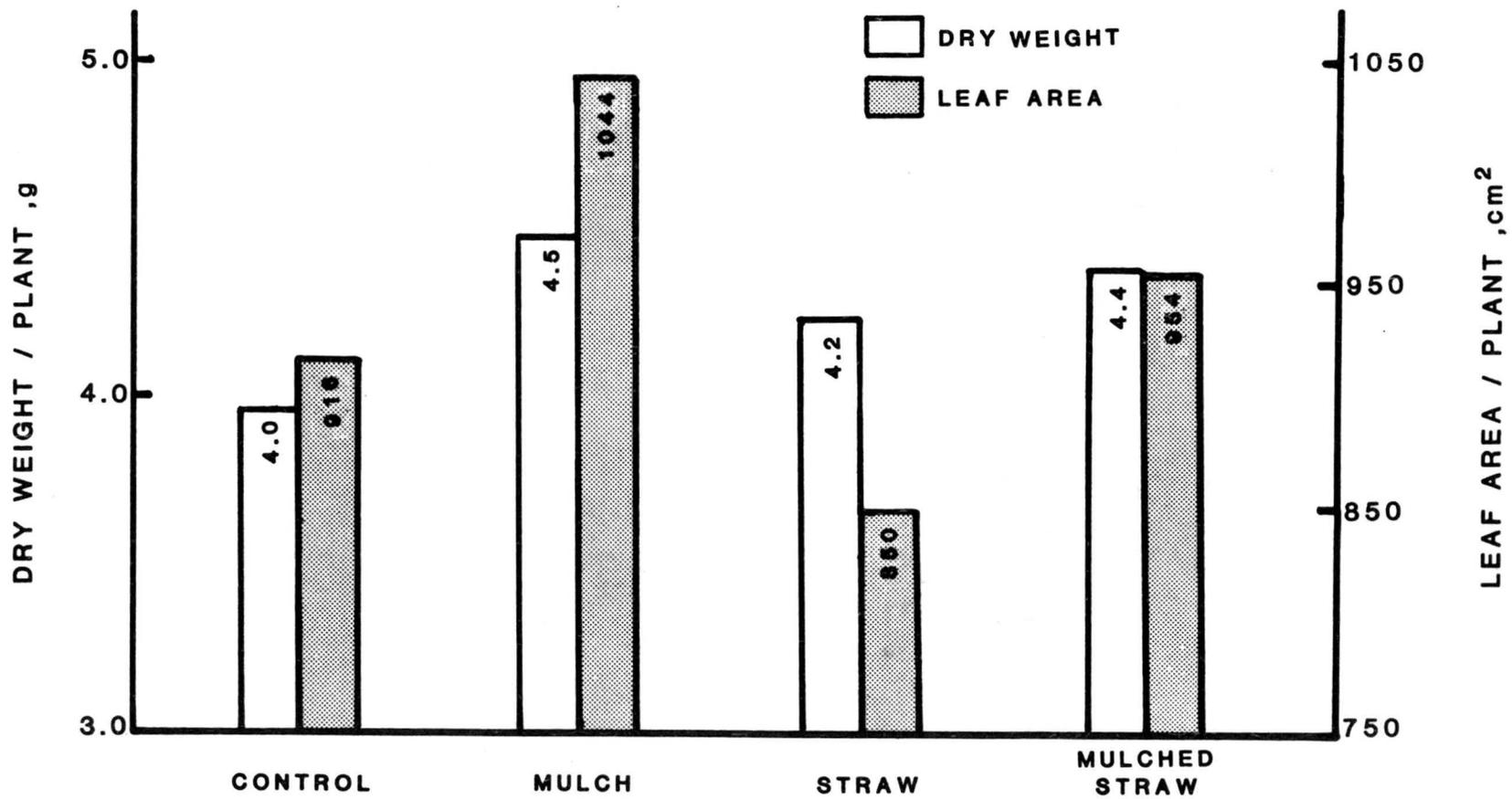


Fig. 12. Experiment 2. Mean plant leaf area and dry weight data collected on September 26, 1981. Treatment means did not differ significantly at the 5% level.

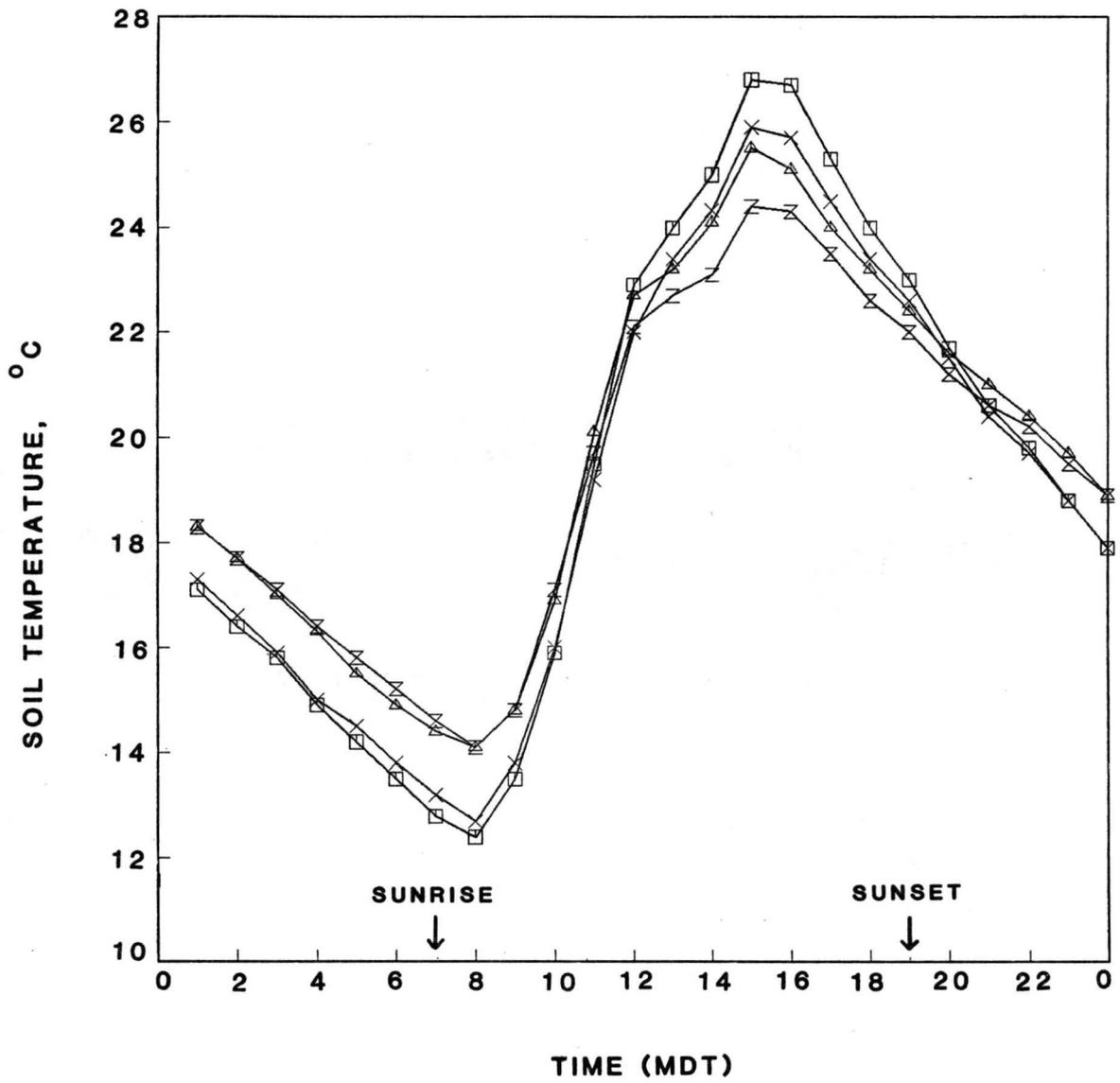


in Experiment 1, where straw reduced leaf area approximately 22 percent. The final yield of mulched straw plants was not significantly greater than the yield of mulched plants. In fact, the mean yield of mulched straw plants was lower than the mean yield of mulched plants. A similar response occurred in Experiment 1 and was attributed to low light intensities during the study. The irradiance level in this experiment averaged $356 \text{ cal cm}^{-2} \text{ day}^{-1}$, over 5 times the level of Experiment 1. Therefore, light intensity should not have limited photosynthesis at the maximum aboveground CO_2 level (451 ppm) encountered in this experiment (7, 8). The greater enrichment level of the mulched straw treatment should have initiated a beneficial plant response under this higher light intensity. Consequently, from these results, straw would not be recommended as a CO_2 source. This conclusion is supported by plant response in this experiment to the straw treatment (no mulch) in which leaf area was depressed under essentially the same surface CO_2 concentration as the control.

Soil temperatures measured after harvest at a depth of 5 cm are plotted in Figure 13. Temperature was lower in the mulch soil than either the mulched straw or control during much of the light period. The addition of straw to the soil slightly raised light period temperatures with the non-mulched straw increasing soil temperature to a maximum of 26.8° C at 1400 MDT. Differences in soil temperature during the dark hours were attributed to the mulch. Both the mulched straw and mulch treatments maintained similar night temperatures and were slightly higher than those of the bare soil or straw treatments. The mulch acted as a barrier preventing soil heat loss to the atmosphere during the night. These effects of PE-coated black paper mulch and/or black

Fig. 13. Experiment 2. Mean hourly soil temperatures at 5 cm in the soil, measured on September 27-29 with plants removed.

△ MULCHED STRAW
□ STRAW
Z MULCH
X CONTROL



plastic on soil temperature have been observed in the field (40, 52, 53). The maximum difference between day and night soil temperature of the bare soil was approximately 13.0° C, whereas under the mulch this difference was only about 10.3° C. Thus, mulch tends to moderate soil temperature fluctuations. These slight changes in soil temperature make it difficult to explain any yield response on the basis of temperature differences alone.

Experiment 3 - Field Study

Table 1 gives the meteorological conditions and flow rates for those days when CO₂ was metered into the release lines. Optimum air temperature for photosynthesis is between 15-30° C for most C₃ plants (51). Favorable conditions of low windspeed and high irradiance were the criteria used to determine if CO₂ releases would be made on any particular day. On favorable days, approximately 23.1 x 10² liters of CO₂ were released per day. Samples from the CO₂ lines taken at the point where CO₂ was metered into the drip lines and also at the end of the lines were similar, indicating a uniform flow throughout the release line.

Carbon dioxide concentrations were determined for each treatment at 1 cm and 25 cm above the soil surface and at 5 cm below the soil surface. Treatment mean separations were determined separately at each measured level by the hsd procedure.

Figure 14 presents the results of CO₂ concentrations at 1 cm above the soil surface during the day. Mulching caused a significant 19% increase in CO₂ concentration over that of the bare soil control during the light period. Similar increases in CO₂ levels were observed in

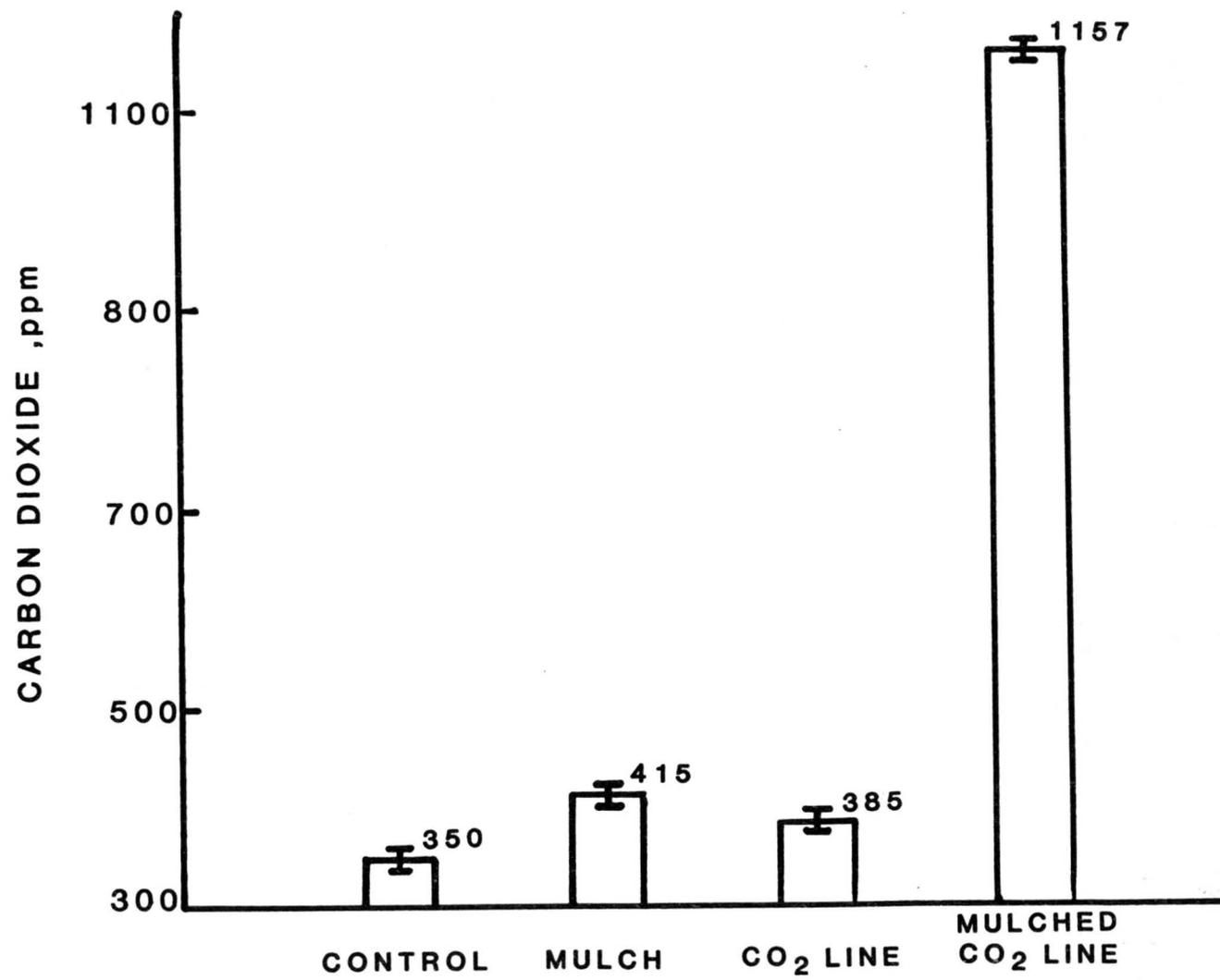
Table 1. CO₂ release duration, flow rate, irradiance, and air temperature data during the CO₂ release periods for Experiment 3. Total growing period was from June 10 to August 15, 1982; however, leaves were present for only 59 days. Releases were not made if irradiance was below 0.3 cal cm⁻² min⁻¹ or windspeed above 3.5 m s⁻¹ at the scheduled start of the release period. Average flow rate, irradiance, and beginning and ending temperature during the 23 release days were 5.57 liters min⁻¹, 539 cal cm⁻² d⁻¹ and 24.7° C and 30.5° C, respectively.

Day	Release period MDT	CO ₂ flow rate liters min ⁻¹	Irradiance cal cm ⁻² d ⁻¹	Temperature °C			
				S ¹	E ²	Max	
July	10	1000-1600	5.50	660	20.0	27.2	27.2
	11	1000-1600	5.50	607	22.2	24.4	28.9
	12	1000-1600	5.50	566	21.1	28.9	28.9
	13	1000-1600	6.00	412	21.1	24.4	27.8
	14	1000-1330	5.50	450	24.4	27.8	27.8
	16	1000-1600	6.00	613	24.4	32.8	34.4
	17	1000-1600	5.50	561	17.8	26.1	26.1
	18	1000-1600	5.00	551	23.9	30.0	31.1
	20	1000-1600	5.00	541	29.4	33.3	36.1
	21	1000-1630	5.50	488	28.3	34.4	34.4
	22	1000-1630	6.00	557	27.8	36.7	36.7
	23	1000-1600	5.50	584	28.9	35.6	35.6
	24	1000-1600	5.50	597	28.3	32.8	35.6
	25	1000-1600	6.00	531	30.0	31.1	35.6
26	1000-1600	6.00	421	25.0	27.2	29.4	
31	1000-1700	5.50	590	22.8	28.9	29.4	
Aug.	1	1000-1700	5.50	614	25.0	31.1	31.1
	3	1000-1700	5.50	501	--	--	--
	4	1000-1700	5.50	453	26.7	28.9	28.9
	5	0900-1600	5.50	549	22.2	31.1	31.1
	6	0900-1700	5.50	531	22.2	31.1	31.1
	9	0900-1700	5.50	496	26.7	35.6	35.6
	14	1000-1630	5.50	516	24.4	31.7	32.2

¹ Measured at start of release.

² Measured at end of release.

Fig. 14. Experiment 3. Mean CO₂ concentrations at 1 cm above the soil surface during the day. Each mean represents 20 measurements made August 22 through September 1 at 1400-1600 MDT. 5% hsd = 15.



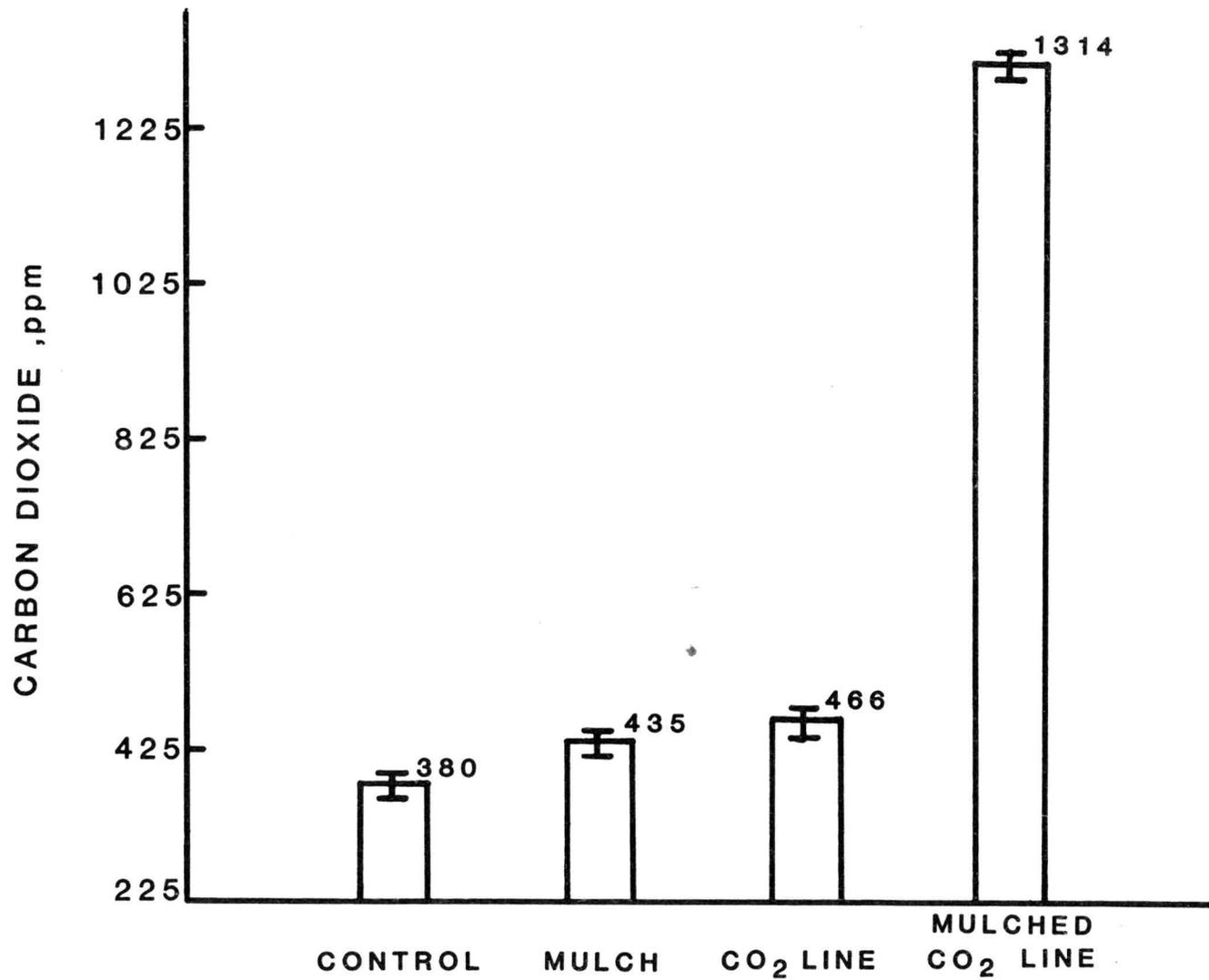
Experiment 1 and by others in field mulch studies (40). The CO₂ line did significantly increase surface concentrations, but to a lesser extent than the mulch. In contrast, when a mulch barrier was placed over the CO₂ line, CO₂ concentration during the day more than tripled the concentration measured over the bare soil.

The level of CO₂ enrichment from a release line under field conditions is quite variable as has been observed by previous researchers (3, 26, 57). Under normal situations, the fluctuations of windspeed and the thermally unstable air near a crop make ground releases of CO₂ inefficient.

This experiment shows that mulch creates a means of CO₂ enrichment, and combined with a surface release line, increases the efficiency and potential for enrichment by providing a physical barrier to rapid gas exchange. Without a mulch barrier, CO₂ release from a line source is quickly dissipated, nor is it possible to pinpoint releases directly at the plant base.

Figure 15 presents CO₂ concentrations at 1 cm above the soil surface during the dark period (2200 MDT). Carbon dioxide levels were greater in the dark because of soil and tissue respiration and the lack of a CO₂ sink. When compared to bare soil, the percentage increase in CO₂ from the mulched plots (no CO₂ line) was less at night than during the day. This was due to cooler soil temperatures at night which would decrease the amount of CO₂ evolved from the soil. A greater percentage of the CO₂ from the night releases was retained than during the day. This reflects the favorable conditions of low and steady windspeed along with near neutral thermal stratification prevalent at night which lessen horizontal mixing of released CO₂ with ambient air.

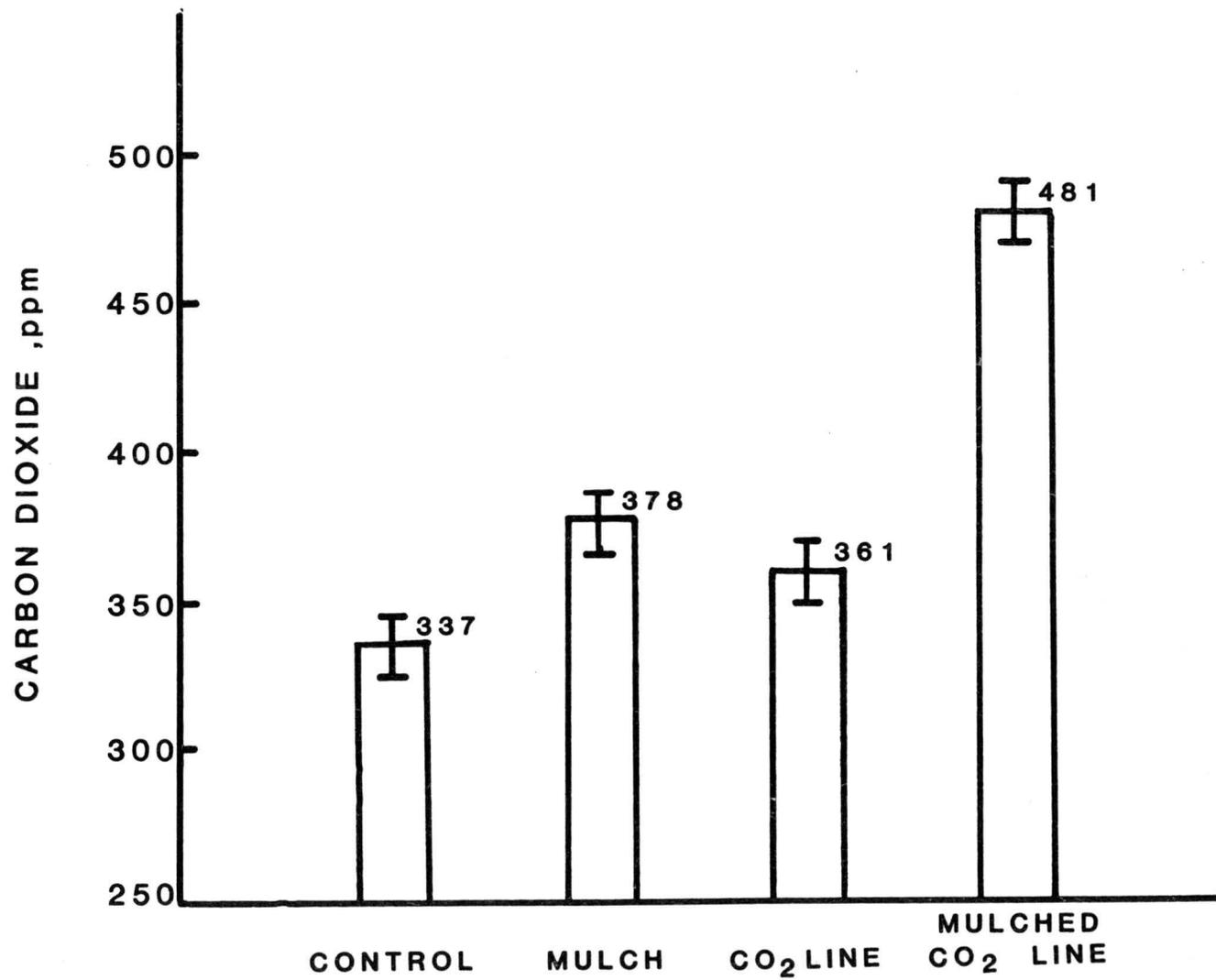
Fig. 15. Experiment 3. Mean CO₂ concentrations at 1 cm above the soil surface at night (2200 MDT). Each mean represents 15 measurements made on September 1-3.
5% hsd = 27.



For these reasons, efficient field enrichment of economically important Crassulacean acid metabolism (CAM) plants has been proposed (26). Percent capture of applied CO_2 may be greater because release could be made under nighttime conditions. Enrichment under these conditions requires further study. The mulched CO_2 line produced the highest CO_2 level at night with a concentration of 1314 ppm. This level was generated by the combination of mulch and the aforementioned greater enrichment capable from a CO_2 line at night.

The mean CO_2 concentrations of 15 samples taken just above crop height (25 cm) are given in Figure 16. The concentration over the bare soil indicated little change from the daytime concentration at 1 cm. Concentrations over the mulched plots and CO_2 line plots were not significantly different; however, the mulch did maintain a slightly higher enrichment level. This reflected the initially lower concentration from the non-mulched line source at 1 cm above the soil surface during the day. The mulched CO_2 line maintained the highest CO_2 level with increasing height. Under non-enriched conditions, CO_2 concentration at this height was only 336 ppm; however, with the mulched CO_2 line this concentration was elevated to 481 ppm. This was due to the tremendous initial concentration and also to vertical transport out of the canopy. Harper (26) in his field release experiments also found quite high CO_2 concentrations (measured at 4 m above the ground) over cotton and Coastal bermudagrass which he attributed to vertical transfer. He found that loss of CO_2 was primarily in the vertical direction. In any case, the concentration reported here remained significantly elevated at the top of the canopy. These enrichment levels are especially important for dense, low-growing crop canopies that favor a CO_2 concentration buildup.

Fig. 16. Experiment 3. Mean CO₂ concentrations at 25 cm above the soil surface during the day (1400-1600 MDT). Each mean represents 15 measurements made on September 1 and 2, 1982. 5% hsd = 20.



Soil carbon dioxide measurements taken at a 5 cm depth in the soil are presented in Figure 17. Mulch was the only factor affecting soil CO₂ concentrations. Both mulch treatments produced CO₂ levels 30% greater than the two non-mulched treatments. This mulch affect on subsurface CO₂ concentration was noted in the two previous experiments. The CO₂ line did not influence soil CO₂ concentration. Apparently, CO₂ released from the mulched line immediately mixed with ambient air and did not concentrate in the upper soil profile. In other words, there is a greater resistance to CO₂ diffusion into the soil from a surface application than to dispersion in ambient air where CO₂ concentrations are lower.

Fresh weights of plants harvested on 7 August are presented in Figure 18. The mean weight of mulched plants was over 30% greater than the mean weight of control plants. Irradiance and soil moisture levels were similar for all plots; therefore, increases in weight were attributed to the greater CO₂ concentration available to the mulched plants. Fresh weight of plants subjected to the non-mulched CO₂ line was slightly greater than control plants, but less than the weight of mulched plants. The CO₂ line increased CO₂ levels by only 10%, resulting in minimal plant response. The most interesting result was plant response to the mulched CO₂ line which had the highest CO₂ concentration and hence, the greatest potential for yield increase. However, plant fresh weight was lower with this treatment than that of mulched plants which had less CO₂ available for uptake. Figure 19 presents plant fresh and dry weight data from the 15 August harvest. The same trend in plant response is depicted in this final harvest as in the first harvest. Treatment means in both harvests did not

Fig. 17. Experiment 3. Mean CO₂ concentrations at 5 cm depths in the soil. Each mean represents 15 measurements made July 20 through August 3, 1982. 5% hsd = 22.

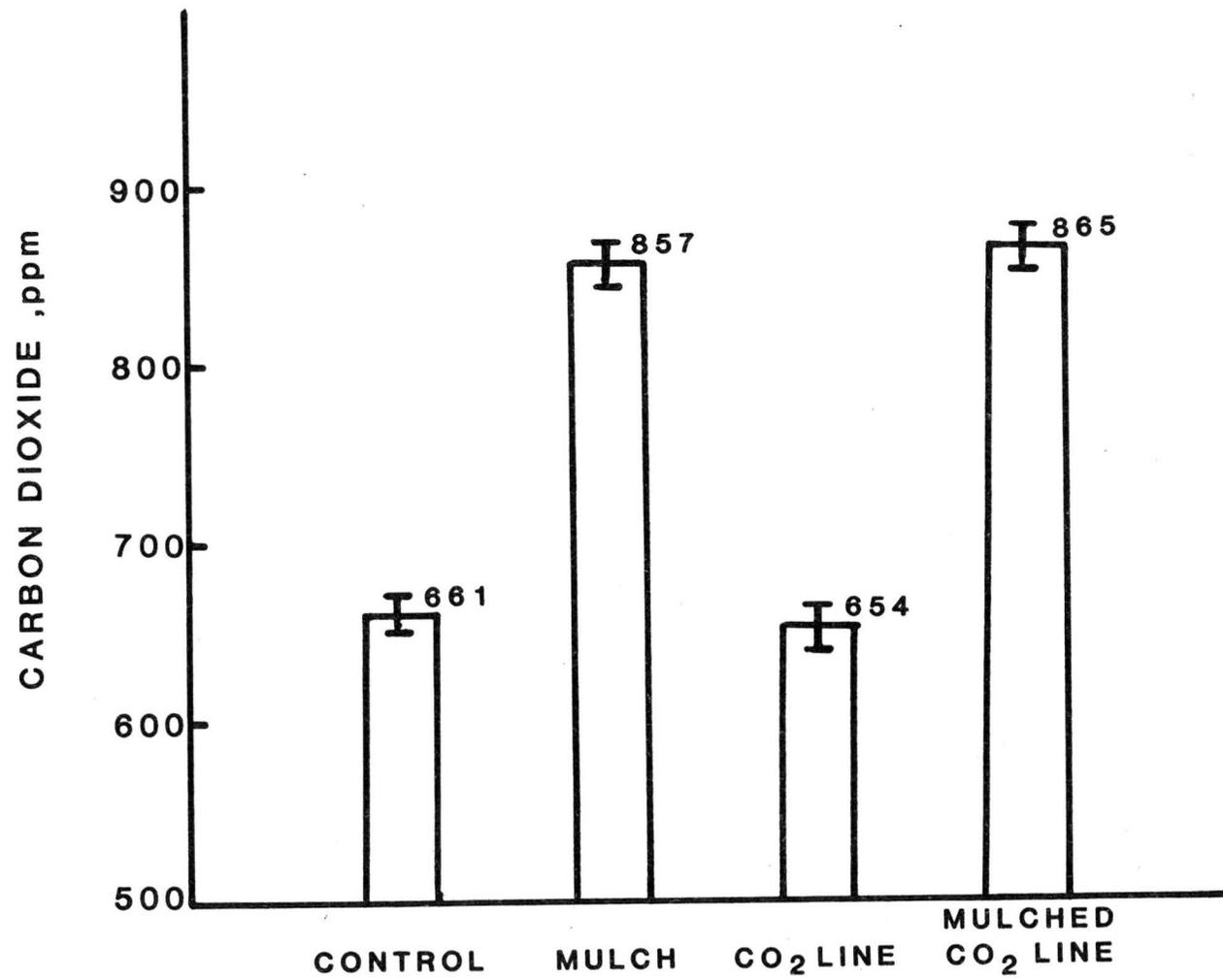


Fig. 18. Experiment 3. Mean plant fresh weight data collected on August 7, 1982.
Treatment means did not differ significantly at the 5% level.

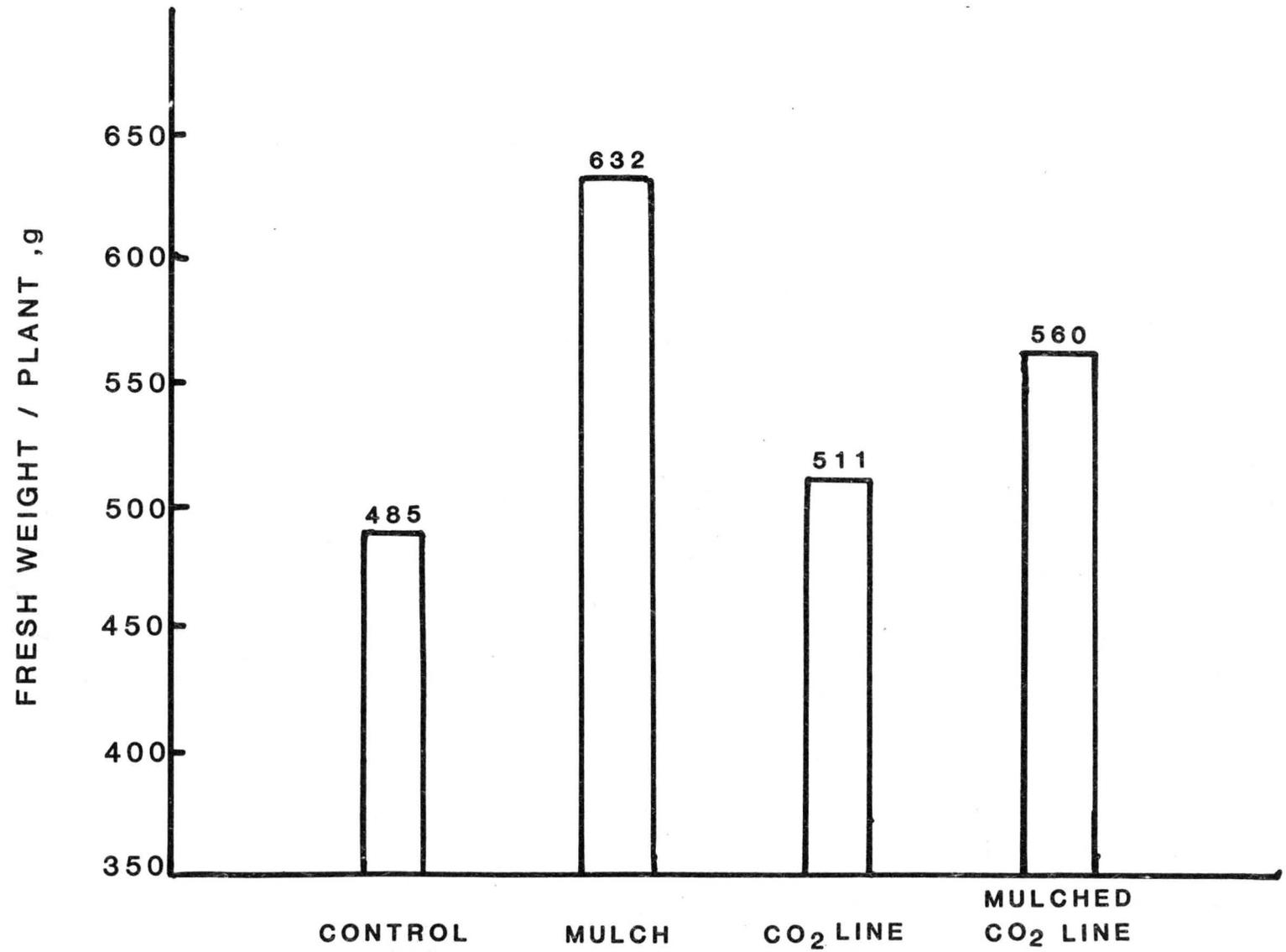
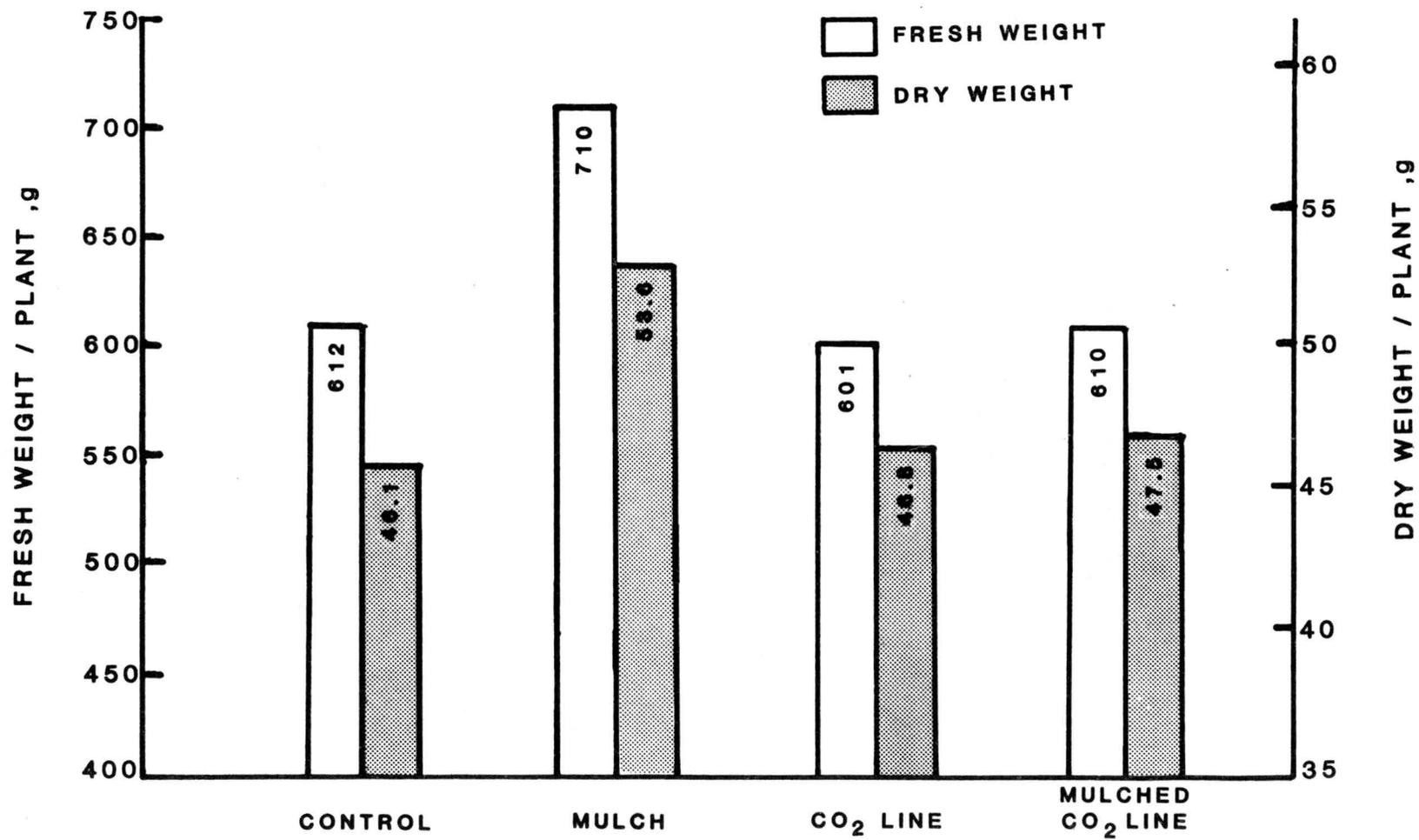


Fig. 19. Experiment 3. Mean plant fresh and dry weight data collected on August 15, 1982.
Treatment means did not differ significantly at the 5% level.



significantly differ from one another. The only variation between mulched and mulched CO₂ line plots was the surface CO₂ concentrations. Both treatments were mulched and therefore exposed to the same sub-surface CO₂ concentrations. Since aboveground concentrations were greater in the mulched CO₂ line plots than the mulched plots and yet plant response was lower, it would seem that an optimal concentration had been reached and that levels greater than this tended to suppress lettuce growth. Elevated CO₂ concentrations have been shown to decrease photosynthesis in some cases. Thomas et al. (59) suggested that this decrease in photosynthesis following prolonged exposure to high CO₂ is caused by accumulation of starch in the leaves. Mauney et al. (39) also reported that accumulation of starch in cotton leaves reduced the rate of photosynthesis with time. If accumulations of this type continued with little translocation out to strong sinks, leaves would start with elevated carbohydrate levels every morning. Consequently, a self-inhibitory process in photosynthesis rates could occur (17). However, greenhouse lettuce has been grown in CO₂ concentrations greater than the maximum daytime concentration reported here (1157 ppm) with significant yield increases (68). On the basis of the inconclusive results in plant response for this experiment, further field studies are warranted.

SUMMARY AND CONCLUSIONS

Experiment 1

Carbon dioxide measured at 1 cm above the soil surface was 22% greater over the mulch than over bare soil. This enrichment was generated by soil evolved CO_2 accumulating between the soil surface and the mulch. Subsurface CO_2 concentrations were also increased from the use of mulch. Straw incorporated into the soil mix did provide a CO_2 source as evidenced by the subsurface concentrations, but did not increase surface CO_2 levels unless a mulch covering was present. Mulched straw provided the highest surface enrichment levels with a mean CO_2 concentration of 603 ppm.

Mulching did increase plant tissue dry weight and leaf area when compared to control plants. Leaf area was significantly decreased by the use of non-mulched straw. Plant response to mulched straw did not reflect the maximum CO_2 concentrations generated by that treatment, in that growth tended to be less than that of mulched plants.

Soil temperature measured at a 5 cm depth was slightly greater under the mulch than in the bare soil. However, this difference would not be great enough to affect plant growth. Since nutrition and soil moisture were also eliminated as factors causing differences in plant growth, it was concluded that accumulation of CO_2 under the mulch and its subsequent funneling out to the plants was responsible for the 80%

increase in average growth of mulched lettuce seedlings compared to non-mulched seedlings.

Low light intensity, removing CO_2 as the limiting factor, was suspect when the lack of significant plant response to mulched straw which provided the greatest enrichment level was observed. Thus, even significant increases in CO_2 levels would not be expected to enhance plant growth.

Experiment 2

The main purpose of this experiment was to determine if under higher irradiance, mulch over incorporated wheat straw could significantly increase plant growth as shown by greater plant tissue dry weight and leaf area.

Mulching, as in Experiment 1, did elevate above and below surface CO_2 concentrations with subsequent increases in dry weight and leaf area. Non-mulched straw, again as in Experiment 1, did not significantly increase surface CO_2 concentration, nor did plant growth benefit from this treatment. Mulched straw generated the highest CO_2 concentrations; however, even under the high irradiance level of this outdoor study, plant growth was less than that of mulched plants. Since nitrogen deficiency of plants subjected to the straw treatments was prevented in both experiments, it was concluded that straw, although it did provide a CO_2 source, should not be used as a natural supply of CO_2 for plant growth.

Soil temperature at a depth of 5 cm under the mulch was slightly lower than bare soil during the day and warmer during the night. It was concluded that mulch had a moderating effect on soil temperature

fluctuations. Maintenance of warmer night temperatures would not be expected to benefit plant growth since increased root respiration would be the result.

Experiment 3

Carbon dioxide was applied to a field of lettuce and was found to significantly increase ambient concentrations. Average daytime CO₂ concentration at 1 cm above the soil surface was 385 ppm over non-mulched CO₂ line plots, but was elevated to 1157 ppm over the mulched CO₂ line plots. It was concluded that application of a poly-coated paper mulch improved the effectiveness of field CO₂ releases by supplying a physical barrier allowing released CO₂ to concentrate under the mulch for direct release to individual plants via the mulch hole. Significant enrichment levels were maintained to 25 cm above the soil surface with the use of a mulched CO₂ line.

Carbon dioxide depressions over a field crop are most likely to occur on calm, sunny days. Thus, CO₂ release should coincide with these meteorological conditions. Moreover, the low windspeed reduces rapid mixing of released CO₂ with the bulk air and the high irradiance assures efficient use by the crop.

Subsurface CO₂ concentrations were significantly increased by the application of mulch as observed in the two previous experiments. The effect on soil CO₂ levels by the use of the CO₂ line was negligible.

Plant response, as shown by increased fresh or dry weight, was greatest in the mulched plots (no line source). However, no significant differences among treatments suggest further field study is needed before a cause and effect relationship can be established.

GENERAL SUMMARY AND CONCLUSIONS

The objective of these experiments was to determine if concentrations of CO₂ sufficient to promote plant growth could be achieved and maintained in a lettuce canopy. Experiment 1 was a controlled study using wheat straw to provide a subsurface CO₂ source upon its decomposition, and also a poly-coated paper mulch. The mulch was shown to significantly increase above and below surface CO₂ concentrations. Mulched straw generated the highest CO₂ levels, but plant response was similar to mulched plants. Experiment 2 was a continuation of Experiment 1, but in an outdoor, higher irradiance situation. The results of these two experiments indicated that mulch did increase CO₂ levels to an average of 17% over ambient concentrations (22% - Experiment 1 and 12% - Experiment 2) near the soil surface. Mulched straw provided even greater enrichment levels. However, plant growth was apparently suppressed by the use of straw. This was concluded since plant growth, particularly leaf area, was less than that of bare soil control plants.

Mulched plants (no straw) in both experiments had greater leaf areas and dry weights than non-mulched plants. This response was due to the increased CO₂ available to the mulched plants and not to any soil temperature or moisture effect. Soil temperature differences between mulch and non-mulch were not considered great enough to cause the observed plant responses. Temperature under the mulch in the outdoor study was found to be slightly lower during the day and

warmer during the night. Different irrigation schedules were used for mulch and non-mulch treatments, thereby maintaining equal soil moisture levels so that soil moisture was not limiting in the non-mulch treatments.

Experiment 3 was a field study employing drip irrigation lines as the CO₂ distribution system to a lettuce canopy. A mulched CO₂ line proved to be the most effective in elevating CO₂ concentrations and in maintaining significantly higher CO₂ levels to 25 cm above the source. Observed differences in plant response were not significant so conclusions on crop behavior under this type of enrichment could not be determined. It is suggested that CO₂ application begin immediately upon germination.

From these results, it was concluded that CO₂ enrichment of lettuce is possible in the field. The best efficiency of released CO₂ would be obtained with well-watered, low-growing C₃ species with extensive canopies under conditions of high irradiance and low windspeed. Many of the vegetable species suit these requirements with the added advantage of high unit dollar value.

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APPENDIX

Table 2. Characteristics of soil mix used in Experiment 1.

pH	7.5
salts (cond.)	1.6
lime (est.)	4
O.M. %	4.7
NO ₃ -N ppm	47
P ppm	60+
K ppm	243
Zn ppm	1.7
Fe ppm	8.3
Mn ppm	7.4
Cu ppm	1.7
Sand %	51
Silt %	22
Clay %	27
Texture	SCL
C:N	11

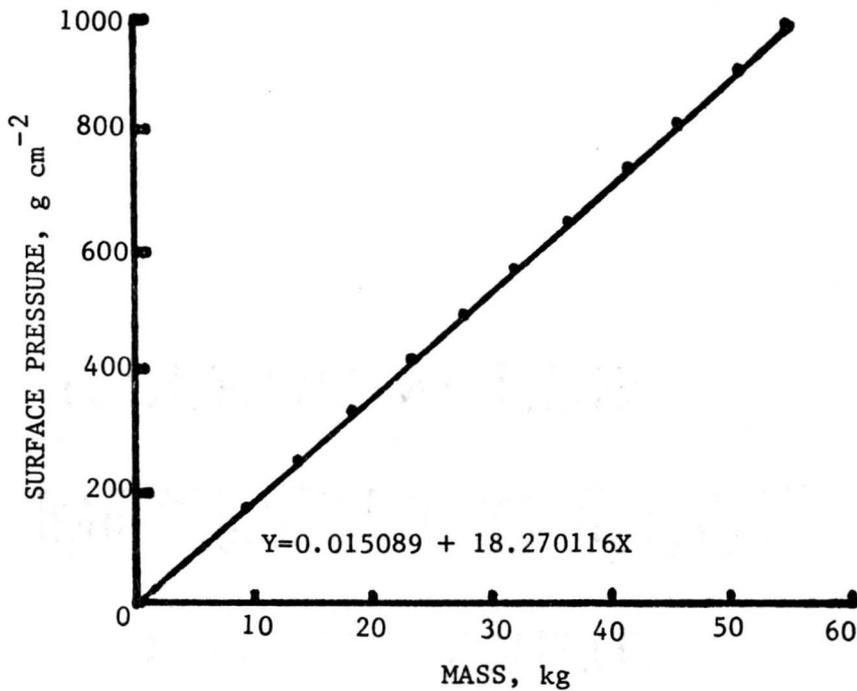


Fig. 20. Calibration curve for compaction device (see Fig. 1) used on containers in Experiment 1.

Table 3. Characteristics of field soil in Experiment 3.

pH	7.7
salts (cond.)	3.4
lime (est.)	High
O.M. %	1.7
NO ₃ -N ppm	43
P ppm	12
K ppm	425
Zn ppm	1.8
Fe ppm	6.3
Mn ppm	2.9
Cu ppm	3.1
Texture	CL