Absorption and release of carbon monoxide by C3 and C4 turfgrasses in light and dark

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Absorption and release of carbon monoxide by C₃ and C₄ turfgrasses in light and dark by

David J. Brahm

A Thesis Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of
MASTER OF SCIENCE
Major: Horticulture

Iowa State University
Ames, Iowa
1986

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INTRODUCTION

Carbon monoxide (CO) is a colorless, odorless gas that can be harmful to humans at a relatively low level (Stewart, 1975). The ambient concentration of CO in the atmosphere is estimated to be 0.04 to 0.90 \( \mu l/\text{liter} \) (Robbins et al. 1968). In addition to this ambient CO concentration, the estimated world-wide emissions of CO from anthropogenic sources is 200 \( \times 10^6 \) metric tons/year (Robbins et al. 1968). Several natural sources contribute additional CO to the atmosphere, and, of these, the oceans of the world are the largest producers. They generate approximately \( 10^7 \) metric tons of CO annually (Swinnerton et al. 1970; Seiler and Junge, 1970).

From these estimates, it has been calculated that the ambient level of CO could be expected to double in four to five years (Levy, 1970). Surprisingly, there has been no significant increase in the ambient CO level over the last twenty years (Pressman and Warneck, 1970). This suggests that a natural sink (or sinks) removes CO from the atmosphere soon after it is liberated. The three main methods of removal of CO from the environment are believed to be: 1) photochemical oxidation to carbon dioxide (Pressman and Warneck, 1970); 2) oxidation or reduction by soil microflora (Inman et al. 1971; Inman and Ingersoll, 1971; Heichel, 1973); and 3) removal by vegetation (Krall and Tolbert, 1957; Chappelle and Krall,
1961; Chappelle, 1962a,b; Delwiche, 1970; Bidwell and Fraser, 1972; Kortschak and Nickell, 1973; and Bidwell and Bebee, 1974).

When observing the CO levels in the atmosphere, it must be taken into consideration that there are two different CO regimes. One regime is that of ambient or "normal" concentration as viewed on a regional or global basis. This regime is characterized by low, relatively constant, CO concentrations. The other regime is that of large urban centers and is characterized by high, local concentrations which may vary widely (Inman and Ingersoll, 1971).

The NAAQS (National Ambient Air Quality Standard) for CO is 10 mg/m$^3$ and 40 mg/m$^3$ for 8-hour and 1-hour averaging times, respectively. Measurement of CO in U.S. urban areas shows that these levels frequently are violated. In 1973, 72 percent of the CO monitoring stations in the U.S. reported violations of the 8-hour NAAQS with 11 percent exceeding the 1-hour NAAQS (OAQPS, 1973). Since 1973, violations of the 8-hour NAAQS continue to occur. In 1974, 56 percent of the stations reported violations, 54 percent in 1975 and 46 percent in 1977 (OAQPS, 1974; OAQPS, 1975; OAQPS, 1977).

Because of the many violations of the NAAQS, the amount of CO emissions in the urban environment has caused serious concern among public health officials and representatives of
the automotive and petroleum industries (Ingersoll, 1971). Therefore, because it has been shown that vegetation may be a possible sink for CO, and since turfgrass makes a large portion of the plant life in the urban environment, it is important to determine the potential of turfgrasses to remove this serious pollutant from the environment.

The objective of this study was to observe four different turfgrass species, two warm-season and two cool-season, under light and dark conditions to determine which specie or species had the greatest potential to remove carbon monoxide from the atmosphere.
LITERATURE REVIEW

Carbon monoxide was first associated with plants when Langdon and Gailey reported its occurrence in the pneumatocyst (float bladder) of the kelp *Nereocystis leuкеana* as a product of respiration (Langdon and Gailey, 1920). This observation has been confirmed by other investigators (Rigg and Henry, 1935; Rigg and Swain, 1941). The uptake of CO by plants was first noted by Krall and Tolbert (1957). They found that CO was absorbed by intact barley leaves in the light. This led to other reports that observed the ability of certain plants to absorb CO (Chappelle and Krall, 1961; Chappelle, 1962a,b; Delwiche, 1970; Bidwell and Fraser, 1972; Kortschak and Nickell, 1973; Bidwell and Bebee, 1974), and alternatively, to produce CO in the presence of light and oxygen (Wilks, 1959; Loewus and Delwiche, 1963; Seiler and Giehl, 1977; Fischer and Luttge, 1978, 1979, 1980).

Absorption of Carbon Monoxide by Plants

Krall and Tolbert (1957) found that light was required to attain $^{14}$CO uptake in excised barley leaves, and that fixation in the dark was negligible. The $^{14}$C distribution in the light showed that serine contained about half the isotope fixed while considerable label was present in phosphate esters and small amounts in sucrose, glycine and glycolic
acid. In contrast, $^{14}\text{CO}_2$ mainly labels sucrose and phosphate esters of the photosynthetic carbon cycle. Infiltration of 2,4-dinitrophenol (DNP) into barley leaves before exposure to $^{14}\text{CO}$ caused the pattern of $^{14}\text{C}$ distribution to simulate that given by fixation of $^{14}\text{CO}_2$ in the light. Krall and Tolbert suggest that the oxidation of CO to CO$_2$ takes place on the cytochrome oxidase at the time of photodissociation of its complex with the enzyme. This reduced CO might then be the "active" molecule that appears in serine (1957).

In an extension of the work done by Krall and Tolbert, Chappelle and Krall (1961) demonstrated that, in order for $^{14}\text{CO}$ to be absorbed by barley leaves, O$_2$ must be present. They also found that $^{14}\text{CO}$ was absorbed by intact barley leaves in the dark, although at a much lower rate than in the light, and converted it to CO$_2$. CO$_2$ also was indicated as the primary product of the light reaction. Using cell-free extracts of spinach leaves, Chappelle and Krall (1961) found that, with no light stimulation of the extract, there was an O$_2$ dependency with CO$_2$ shown to be the only reaction product.

The finding of Chappelle and Krall (1961) showing that CO$_2$ was the first detectable product of CO conversion in plants creates a discrepancy with Krall and Tolbert (1957) who, using paper chromatography, observed that the major product of CO fixation by illuminated barley leaves was
serine. The question then arises as to whether CO$_2$ is the primary product or if it formed via an intermediate. It has been established that formate is a precursor of serine (Sakami, 1948). A hydration of CO would result in formic acid, which could go to CO$_2$ through the formate dehydrogenase system (Matthews and Vennesland, 1950) or to serine via the formate-activating system (Sakami, 1948).

Chappelle's work with algae gave evidence that CO$_2$ was the primary product of CO incorporation and eliminated formate as an intermediate in the reaction. This was done by using $[^{14}\text{C}]$ formate and paper chromatography (1962a). He also found that there was low fixation of $[^{14}\text{C}]$ formate into serine, while the fixation patterns and products of $^{14}\text{CO}_2$ and $^{14}\text{CO}$ were almost identical.

Chappelle (1962b) observed that, in the dark, the only product of CO incorporation was CO$_2$. He believed that oxidation of CO to CO$_2$ by molecular O$_2$ was an oxidase reaction. His work indicated the involvement of an active metallic center in the CO incorporation reaction. This lends support to the possibility of the enzyme being an oxidase, as practically all of the enzymes studied up to 1962 that were involved in the transfer of molecular oxygen were metal-containing proteins. In this respect, the oxidation of CO by cytochrome oxidase, as reported by Breckenridge (1953), is of great interest.
Cytochrome oxidase functions normally as a terminal electron acceptor in respiration, and it contains a heme protein whose iron moiety is capable of undergoing reversible oxidation and reduction. In a reduced state, it will bind with CO and become inactive until the CO is removed by illumination. It is in the reduced form that the cytochrome oxidase functions as a catalyst for the oxidation of CO, by activating the CO and making it susceptible to oxidation by molecular oxygen. In the light, the oxidized CO, now CO$_2$, would be released either to the atmosphere or be fixed by RuDP carboxylase/oxygenase.

Chappelle (1962a) also found that incorporation of CO by algae was affected by changes in temperature, thus ruling out the possibility of the incorporation being a nonenzymatic photochemical reaction because photochemical reactions are virtually temperature-independent. This also adds support to his oxidase theory.

In 1971, Inman and Ingersoll reported no absorption of CO by seedlings of a number of species exposed to an atmosphere containing 100 to 120 µl CO/liter in air. By using a gas chromatographic technique, Inman and Ingersoll concluded that most CO absorption occurs in the soil (Inman et al. 1971). Because of this, Bidwell and Fraser (1972) reinvestigated the problem of CO absorption by leaves using
$^{14}\text{CO}$ in a circulating gas apparatus that used an infrared gas analyzer to measure CO$_2$ concentration and an array of Geiger-Mueller tubes to measure radioactivity. This apparatus has been described in detail (Steward et al. 1971).

Bidwell and Fraser (1972) exposed bean leaves to 200 to 360 µl $^{14}$CO/liter in air and found that they metabolized CO in light and dark. In light, CO was converted mainly to sucrose and proteins. The distribution of $^{14}$C among the products suggested that most of the absorbed CO was reduced and incorporated into serine, in agreement with Krall and Tolbert (1957), which then was converted to sucrose. Not observed in previous studies, Bidwell and Fraser (1972) reported that some CO was oxidized to CO$_2$. In darkness, CO was absorbed nearly as fast as in light but was almost completely converted to CO$_2$ and subsequently released.

The distribution of $^{14}$C in the insoluble fraction was very different from $^{14}$CO or $^{14}$CO$_2$. The CO labeled primarily protein in light while CO$_2$ labeled carbohydrates. This shows that CO metabolism follows a completely different pathway than CO$_2$. This is further supported by the soluble fraction which states that although both CO and CO$_2$ were converted to sucrose, only CO$_2$ labeled those intermediates of the photosynthetic carbon reduction cycle. The CO, on the other hand, labeled serine and glycolate.
The data presented by Bidwell and Fraser (1972) suggests that, in light, CO is fixed mainly in serine, much of which goes through the serine pathway to sucrose (Hess and Tolbert, 1966). The entry of CO into serine may be mediated by the formation of CO-derivative (e.g., tetrahydrofollic acid, Sakami, 1948), which becomes reduced. However, not all CO carbon goes into this pathway. The proportion of $^{14}$C from $^{14}$CO entering starch was increased greatly when the CO$_2$ concentration was lowered, suggesting that some of the $^{14}$CO was converted to CO$_2$ and metabolized as such. However, the data from Bidwell and Fraser (1972) indicated that at physiological levels of CO$_2$, CO is absorbed and metabolized as CO.

The low rate of CO reduction in the dark suggested that photosynthetic reducing power is used in light (Bidwell and Fraser, 1972). The majority of CO absorbed in the dark is oxidized to CO$_2$, which then follows the normal dark fixation pathway.

Bidwell and Fraser (1972) also found that the absorption of CO by leaves varied widely with species, which would be expected because often there is found to be variation between leaf samples in photosynthetic rate measurements (Kortschak and Forbes, 1969). The rate of CO uptake by plants was approximately proportional to the CO concentration;
therefore, it may be possible that plants act as CO absorbers under conditions of unnaturally high CO levels, converting CO to organic material by day and CO$_2$ by night (Bidwell and Fraser, 1972).

Until 1973, all of the data on CO absorption by plants was acquired from C$_3$ plants. Kortschak and Nickell worked on the CO metabolism by sugarcane leaves, a C$_4$ plant (1973). Their results showed that the leaves of sugarcane can metabolize low concentrations (2 μl/liter) of CO photosynthetically, but the rate of uptake, 10$^{-4}$ mg/dm$^2$/hr, was too low to be significant in removing CO from the atmosphere.

Kortchak and Nickell suggested that the pathway of CO metabolism was similar to that of CO$_2$ photosynthesis (1973). In contrast to the work done by Bidwell and Fraser (1972) with bean leaves, a C$_3$ plant, no activity was found in serine, suggesting that a C$_4$ plant has a different pathway of CO metabolism. Kortschak and Nickell (1973) did find that darkness reduced uptake of CO to 2% of that found in light, suggesting that CO metabolism is photosynthetic. Because the very low uptake rate of CO made it impossible to identify early products in tests of a few seconds, a conclusion concerning the mechanism was not drawn.
In 1974, Bidwell and Bebee observed the ability of 35 species of plants to absorb CO from a gas stream containing 6 \( \mu l \) CO/liter in air. The plants were tested in a closed, flowing gas system at 22-24\(^0\)C as described previously (Steward et al., 1971). Each plant was allowed to absorb CO for 15 to 45 minutes. They observed that the average rate of CO absorption for the 35 species was 0.19 \( \mu l/hr/g \) fresh weight of leaf tissue (Bidwell and Bebee, 1974). In agreement with Bidwell and Fraser (1972), Bidwell and Bebee found a wide variation in CO-absorbing capacity among plant species (1974).

In Bidwell and Bebee's (1974) attempt to determine the metabolism of absorbed \( ^{14}CO \), leaves of bean (a C\(_3\) plant) and corn (a C\(_4\) plant) were allowed to absorb \( ^{14}CO \) (135 \( \mu l/liter \) in air) for various times in light. Most of the fixed \( ^{14}C \) was converted into sucrose in both bean and corn leaves. Bean leaves showed evidence of fixation via \( CO_2 \) and via serine. Corn leaves showed no evidence for fixation via serine but seemed to fix \( ^{14}C \) by beta-carboxylation, which indicates the conversion of \( ^{14}CO \) to \( ^{14}CO_2 \). The serine formed by bean leaves was heavily labeled in C atoms 2 and 3, which is consistent with its formation by the reduction of CO and its transfer to glycine (e.g., by hydroxymethyl transferase) to form serine.
Data presented by Bidwell and Bebee (1974) on CO absorption by corn leaves were similar to that of Kortschak and Nickell (1973) on CO uptake by sugarcane leaves. They both suggested that C₄ plants do not metabolize CO rapidly.

Bidwell and Bebee concluded that plants can absorb a little over 1/10 of the total CO produced in the world (1974). Furthermore, they produce over twice as much as they absorb, indicating that plants are of little importance in the global balance of CO at ambient CO concentrations. However, it must be emphasized that CO absorption by leaves increases with higher CO concentrations. With average values of 5 to 9 ul/liter CO reported in atmospheres of urban areas, with maximum values reaching well over 100 ul/liter, the rate of CO absorption by plants may be greater by a factor of 10 to 100 (Altman and Dittmer, 1966; Jaffe, 1968; Hexter and Goldsmith, 1971).

Release of Carbon Monoxide by Plants

Langdon (1917) found that little CO was produced in algae, but little work after that was done concerning CO evolution in green plants. From reports observing unusually high CO content in a specimen of human muscle tissue into which a considerable quality of green vegetation had been impacted during an aircraft accident, Wilks (1959) took measurements of CO produced by various types of green plants.
His work seemed to point to the natural occurrence of a CO "generator" or precursor substance in green plants. The generation and liberation being brought about through some photodegradative activity involving the chlorophyll system, which requires both light and oxygen. Wilks' (1959) work looked only at the effects of O₂ concentration and the production of CO by plants. No work was done on the effects of CO₂ concentration.

In Fischer and Luttge's (1978) work with *Nerium oleander*, a C₃ plant, they agreed with Wilks (1959) that CO production in plants is increased considerably by light and that production of CO in the light was increased by increasing the concentration of oxygen. Fischer and Luttge (1978) went one step further than Wilks (1959) in that they studied the effects of CO₂ concentration on the rate of CO production. They reported that by increasing the O₂ concentration and decreasing the CO₂ concentration in the presence of light, the rate of CO production was increased. When the concentrations were reversed, the CO production was decreased. This was completely opposite of CO₂ fixation that is inhibited at higher O₂ concentrations. Their data also showed that small amounts of CO were released in the dark and increasing the temperature greatly increased the CO release, and decreased CO₂ uptake.
The above information suggested that CO evolution may occur via the photorespiration pathway. Zelitch (1979) states that photorespiration in C₃ plants occurred when conditions include high light intensities, high levels of O₂, and high temperatures, which are similar to conditions favoring CO production. Photorespiration in C₃ plants occurs when conditions include high light intensities, high levels of O₂, and high temperatures, which are similar to the conditions favoring CO production. Photorespiration occurs because, in the presence of O₂, the enzyme RuDP carboxylase/oxygenase of the Calvin cycle can function as RuDP oxygenase capacity, it adds oxygen to RuDP, converting it to one molecule of PGA, instead of producing two PGA molecules containing three carbons each. Thus, there is no net carbon fixation when photorespiration occurs (Fischer and Luttge, 1980). Phosphoglycolate later loses phosphate to become glycolate, which moves into another membrane bounded organelle, the peroxisome. In the peroxisome, glycolate reacts with oxygen to form glyoxylate and hydrogen peroxide; the latter immediately is broken down to water and oxygen. The glyoxylate is then converted into glycine which then is transferred to the mitochondria. In the mitochondria, two glycines combine to form the amino acid serine (which can be...
used directly in protein synthesis, or further transformed to glucose), simultaneously liberating CO₂ (Zelitch, 1971).

Fischer and Luttge (1978, 1979, 1980) suggested that light-dependent production of CO in a C₃ plant is a by-product of photorespiratory C₁ metabolism. CO could be derived from the glycolate pathway of photorespiration in two ways (Fischer and Luttge, 1978). First, direct oxidative decarboxylation of glycolate may produce formate (Tolbert and Ryan, 1976) and hence CO. Second, formate and CO may originate from the tetrahydrofolate activated C₁ unit formed during photorespiratory synthesis of one molecule of serine from two molecules of glycine (Chollet and Ogren, 1975).

Fischer and Luttge (1979, 1980) worked with the C₄ plant Amaranthus paniculatus and have results that show light-dependent net CO production. In their observations, they noted that, as the O₂ concentration increased, CO evolution was stimulated, but, as expected, it only slightly affected CO₂ uptake. The reason for the small effect of O₂ on the uptake of CO₂ in C₄ plants is the result of the Kranz anatomy of the C₄ leaf that gives the C₄ plant a very high affinity for CO₂. Phosphoenol pyruvate, PEP, a 3-carbon compound that occurs in the mesophyll of the leaf, is the initial acceptor of CO₂. CO₂ and PEP then is catalyzed by PEP carboxylase to form oxaloacetic acid, which is converted
to either malic acid or aspartic acid. They then diffuse from the mesophyll cells to the bundle sheath cells where they are decarboxylated to yield CO₂ and pyruvate, a 3-carbon compound. CO₂ then enters the Calvin-Benson cycle to form the intermediates formed in a C₃ plant. Meanwhile, pyruvate diffuses back to the mesophyll cells (Galston et al. 1980). Fischer and Luttge (1980) believe that photorespiration occurs in C₄ plants inside the bundle sheath. They base this assumption upon the fact that the CO₂-concentration at the site of RuBP carboxylase/oxygenase in the bundle sheaths is raised significantly as compared with the ambient atmosphere. Because the oxygenase function of this enzyme is responsible for glycolate synthesis, this result seems to support the conclusion that CO-evolution is a consequence of photorespiration.

By combining the work of Bidwell and Bebee (1974) with that of Fischer and Luttge (1980), it would seem that all the CO evolved in a C₄ plant would be fixed by the plant via beta-carboxylation, converted to CO₂ and then absorbed by PEP as soon as the CO₂ diffused into the mesophyll, and very little CO would be released to the atmosphere.

Measurement of Carbon Monoxide

Because CO can be harmful to humans at a relatively low level (Stewart, 1975), detection and measurement of the gas
has been very important. Over the years, 17 different methods for the quantitation of CO have been developed, with all but two having some sensitivity to ethylene as a source of interference: gas chromatography and infrared gas analysis (Gladon, 1977). The use of gas chromatography and infrared gas analysis to measure CO are not without problems. Rodkey, Collison and Engel report that CO is liberated from transparent acrylic and polycarbonate plastics after exposure to air (1969). The rate of CO produced by the plastics is increased after an exposure to CO. They also suggest a strong indication that rubber stoppers and tubing may absorb CO and release it at a later, possibly unexpected, time, giving erroneous results in the measurement of CO. Therefore, the CO measurement system must be constructed mainly of inert materials, such as glass and stainless steel, whenever possible.

**Gas chromatography**

Chromatography is a physical method of separation of components in a mixture (Willard et al. 1965). The process of chromatography occurs within the separation column, which normally is a small-diameter tubing packed with a material that selectively can pass substances through or absorb and desorb them.
Chromatography may be further defined as the method of removing the separated sample components from the column, this is sometimes referred to as development. The preferred method of development in gas chromatography is elution. In the elution method, a stream of an inert carrier gas, helium or nitrogen, flows through the column. The sample mixture is injected into the continuous stream of carrier gas that moves the individual components through the column at different rates. Each component moves at a rate depending on its partition coefficient $K$ where:

$$K = \frac{\text{weight of solute (sample per ml. of liquid phase)}}{\text{weight of solute per ml. of carrier gas}}$$

(Johns, 1959).

Under favorable conditions, the individual components will have different partition coefficients and will be separated completely upon emergence from the column. After the separated components leave the column, they immediately enter a detector attached to the column (in the case of CO measurement, CO is converted to Methane and a flame ionization detector is used). Here, the individual components register a series of signals that appear as a succession of peaks above a base line on a recorded curve. The area under the peak is a quantitative indication of the component; the time lapse between injection and emergence of the peak serves to identify it.
Carbon monoxide methanizer

The gas chromatograph used in this study is the Carle analytical gas chromatograph (AGC) model 211M. The system includes a methanizer that catalytically converts CO to methane via reduction so that it can be detected with a flame ionization detector (FID).

The first work done with a methanizer was conducted by Porter and Volman (1962), following the reaction reported by Sabatier and Senderens (1902), which demonstrated that CO will react with hydrogen in the presence of a reduced nickel catalyst in the following manner:

\[ \text{CO} + 3\text{H}_2 \rightarrow \text{CH}_4 + \text{H}_2\text{O}. \]

The methanizer developed by Porter and Volman (1962) consisted of 100 to 120 mesh silocel firebrick soaked in a saturated solution of nickel nitrate with surplus liquid removed by filtering under vacuum. The firebrick then was dried overnight at 100 to 110° C and then heated in air for five hours at a temperature between 400 and 500° C. The firebrick was then packed into a 12.5 cm length of stainless steel tubing (o.d. 4mm x i.d. 2mm) around which was wrapped a heater winding and insulation. This tube was placed immediately before the FID and just after the flame and carrier gases (hydrogen and helium). The catalyst was then heated to 400° C before the CO was passed through.
The Carle AGC-211M methanizer uses the basis of Porter and Volman's (1962) methanizer with improvement developed by O'Neil, Rodkey, and Collison (1969). The AGC-211M methanizer consists of a converter tube packed with an active hydrogenation catalyst plus a heater and thermocouple enclosed within an insulated stainless steel box. It has a separate, adjustable temperature control for regulating the temperature of the catalytic bed with a temperature readout from ambient to $1000^\circ C$ on a pyrometer. To obtain efficient conversion of CO to $CH_4$, the temperature of the catalyst should be between 375 and $400^\circ C$.

In the reduction process, the gas sample, following chromatographic separation from the other components of the sample, were brought to the methanizer via the carrier gas, nitrogen, and, at the same time, hydrogen was flowing through the catalyst. The CO of the sample was reduced by the catalyst to methane and was detected by the FID.

The catalyst is kept in a reduced state by passing the entire flow of hydrogen through the methanizer and having a bypass system for oxygen that may be present in the sample. The oxygen is bypassed to the FID.

If oxygen were to pass through the catalyst, the efficiency of the conversion process for CO would decrease. The methanizer can be returned to its efficient state by
passing hydrogen through it with the temperature of the converter at 500 to 525°C for 30 minutes.

The efficiency of CO conversion by the methanizer was found to be 99.7% by Rodkey (1970). He also found that when analyzing samples containing low CO levels, it was essential that the carrier gas be free of CO₂.
MATERIALS AND METHODS

Plant Material

Clones of Kentucky bluegrass *Poa pratensis* L. 'Baron'; tall fescue *Festuca arundinacea* L. 'Kentucky 31'; zoysiagrass *Zoysia matrella* (L.) Merr.; and St. Augustine grass *Stenotaphrum secundatum* (Walt.) Kuntze were vegetatively propagated from individual shoots and grown hydroponically under greenhouse conditions for at least three months before testing.

All species of turf in the study were treated with Dinocap 25% WP (2,4-Dinitro-6-octyl-phenyl-crotonate, 2,6-dinitro-4-octyl-phenyl crotonate, and nitro-octyl-phenols, a mixture of 1-methyl-heptyl, 1-ethylhexyl, and 1-propylpentyl isomers of the octyl 8-carbon chain) at the rate of 15 g/liter as needed to control powdery mildew and spider mites.

Hydroponic System

The hydroponic system used was similar to that described by Roberts and Lage (1965) with a modification of the culture lid that aided in the removal of individual shoots for testing purposes (Figure 1). The nutrient solution used was a slightly modified version of that used by Pellett and Roberts (1963) (Table 1).
Figure 1. Nutrient culture unit with modified lid used to grow turfgrass plants
The system was aerated by compressed air at 2.5 kg/cm², with air introduced to each culture unit through 1.5 mm i.d. (inside diameter) trickle tube via 7.9 mm i.d. tubing which was attached to the air line.

Table 1. List of nutrients, their sources and concentrations used in nutrient solution

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Source</th>
<th>Concentration (ppm)</th>
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</thead>
<tbody>
<tr>
<td>N</td>
<td>Ca(NO₃)₂ • 4H₂O</td>
<td>156¹</td>
</tr>
<tr>
<td></td>
<td>(NH₄)₂SO₄</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>H₃PO₄</td>
<td>25</td>
</tr>
<tr>
<td>K</td>
<td>KOH</td>
<td>. 50 *</td>
</tr>
<tr>
<td>Mg</td>
<td>MgSO₄ • 7H₂O</td>
<td>19</td>
</tr>
<tr>
<td>Fe</td>
<td>Fe₂(SO₄)₃</td>
<td>1.2</td>
</tr>
<tr>
<td>Mn</td>
<td>MnSO₄ • H₂O</td>
<td>0.25</td>
</tr>
<tr>
<td>B</td>
<td>H₃BO₃</td>
<td>0.1</td>
</tr>
<tr>
<td>Zn</td>
<td>ZnSO₄ • 7H₂O</td>
<td>0.1</td>
</tr>
<tr>
<td>Cu</td>
<td>CuSO₄ • 5H₂O</td>
<td>0.01</td>
</tr>
<tr>
<td>Mo</td>
<td>MoO₃ a</td>
<td>0.01</td>
</tr>
</tbody>
</table>

¹Nutrient solution contains 80 ppm Ca and 90 ppm S. Different from Pellet and Roberts (1963).

Nutrient solutions were changed at weekly intervals to maintain a near neutral pH, and to keep the culture units
clean. Fresh nutrient solution had a pH of 6.5, but increased to 7.2 after one week in the culture units. Distilled water was added daily to the culture units to keep them full.

Lighting System

For the irradiance study, the exposure system was placed 1 meter below two 1000-watt metal halide lamps enclosed in Sylvania bat-wing fixtures. A 10 cm deep water bath was installed under the lamps to absorb heat. Physan 20 was used to control the growth of algae, fungi, and bacteria in the water bath. Light measurements taken with a Li-Cor quantum radiometer inside the exposure chamber gave a reading of 910 \text{umol/m}^2/\text{sec}.

Exposure System

A recirculating system was designed for the exposure of the plant specimens to carbon monoxide in air balance (Figure 2). The exposure system consisted of six subunits that were connected by either 6.25 mm inside diameter stainless steel or glass tubing.

The exposure chamber was a 250 mm diameter, approximately 9 liter glass vacuum desiccator with a modified lid for the induction and removal of gases. Circulation of the gases through the system was performed by an air pump with a stainless steel diaphragm. A rheostat connected to
Figure 2. Schematic diagram of the CO recirculating exposure system
hydraulic vacuum pump

vacuum flask

3-way valve

quick coupler

humidity sensor

sample port

rheostat

humidifiers

exposure chamber
the air pump controlled the air flow through the system. The gases in the system were humidified by passing through a series of two humidifiers, which were modified gas traps, filled three-quarters full of distilled water (Figure A1). The relative humidity inside the system was monitored by a Dew-All humidity analyzer. Evacuation of the exposure system was performed by the use of a three-way stainless steel valve, a 4000-ml vacuum flask, a vacuum gauge and a hydraulic vacuum pump.

Connection of the subunit to the stainless steel tubing was accomplished by stainless steel swagelok fittings. Stainless steel swagelok fittings also were used in the connection of stainless steel tubing to the glass tubing. Standard 18/9 ground glass ball and socket fittings with silicon vacuum grease and ball and socket clamps were used on all glass to glass connections.

Preparation of the Exposure System

One thousand ml of distilled water were placed in the bottom of the exposure chamber prior to insertion of the plant samples. After placement of the specimens, the chamber was sealed and evacuated to 7.5 cm Hg by the hydraulic vacuum pump. A gas-tight quick-coupler in the system was detached and a teflon bag, filled with 101 μl/liter carbon monoxide in air, fitted with a quick-coupler was connected to the
exposure system. The contents of the bag were allowed to fill the system and return it to atmospheric pressure, at which time the bag was removed and the system was connected together and the recirculating pump was started.

Light Testing Procedure

Fresh plant material was placed in the exposure chamber with roots immersed in water. The chamber then was covered with .15 mm black polyethylene and placed under the lighting system. Once the system was filled with 101 μl/liter carbon monoxide in air, the lighting system was turned on and the plastic cover was removed. The recirculation pump was allowed to run for five minutes before the initial gas sample was taken. Subsequent samples were taken hourly for six hours. At the end of the run, the plant material was removed from the chamber and divided into leaf, sheath, stem, and root parts and oven-dried, at which time plant dry weight was recorded. The chamber temperature was measured by a glass thermometer enclosed within the exposure chamber. The chamber temperature during each run was $33 \pm 0.2^\circ C$, the relative humidity was 100%, and the light measurement reading was 910 μmol/sec/m².

Dark Testing Procedure

The exposure system was set up in the laboratory for the dark study. Fresh plant material was placed in the exposure
chamber with roots immersed in water, and the chamber then was covered with .15 mm black polyethylene. The system was filled with 101 ul/liter carbon monoxide in air. The recirculation pump was allowed to run for five minutes before the initial gas sample was taken, then subsequent samples were taken hourly for six hours. At the end of the run, the plant material was removed from the chamber and divided into leaf, sheath, stem, and root parts and oven-dried, at which time plant dry weight was recorded. The chamber temperature during each run was 23°C. The relative humidity was 100%.

Gas Analysis Apparatus

A Carle 211M analytical gas chromatograph (AGC-211M) equipped with a flame ionization detector and methanizer was used for carbon monoxide analysis. A 1.5m by 3mm OD stainless steel column containing 60/80 mesh silica gel and a 1m by 3mm stainless steel column containing 60/80 mesh molecular sieve type 13X preceded the methanizer. The AGC-211M was connected to a Spectra Physics integrator, which in turn was connected to a Houston Instruments recorder. A Hamilton (5000 ul) gas-tight syringe was used for all gas samples.

Five ml samples of the test atmospheres were analyzed for carbon monoxide content by means of gas chromatography. The samples were withdrawn from the exposure system with a
calibrated glass syringe by puncturing a septum in the sample port. The syringe was flushed three times with the sample gas before the sample for analysis was withdrawn.

The analytical technique was based on the catalytic reduction of carbon monoxide to methane followed by flame ionization detection of methane. The method used has been described by Porter and Volman (1962). Before reduction of carbon monoxide to methane, carbon monoxide was separated from other components of the sample gas by using a 1.5m by 3mm OD stainless steel column containing 60/80 mesh silica gel and a 1m by 3mm OD stainless steel column containing 60/80 mesh molecular sieve type 13X. Column temperature was 72°C, with nitrogen as the carrier gas (30 ml/min.). Reduction of carbon monoxide took place at 400°C in the catalytic bed.

To ensure that changing conditions within the system would not affect the accuracy of the method, a 101 µl/liter standard gas sample was analyzed before each experimental sample. Interpolation of the two calibration gas readings then became the standard basis for comparison. An empty, sealed exposure system was filled and tested with 101 µl/liter carbon monoxide in air periodically between experiments to ensure that no leaks had developed.
Statistical Analysis

The experimental design for both the light and dark study was a completely randomized design of five treatments (four turfgrasses and control) replicated five times. The treatments were exposed to 101 ul/liter CO for a six hour period.

Data collected after exposure included dry leaf weight, hourly changes in CO concentration (ul/liter/hr) and cumulative changes in CO concentration (ul/liter). From this information, concentrations were calculated on the basis of both ug CO/g dry leaf weight and ug CO/g dry leaf weight/hour (see Appendix).

Analysis of variance was performed and differences between the treatment means were compared using Fisher's least significant difference (Steel and Torrie, 1980).
RESULTS

Light Study

Results of the analysis of variance for cumulative absorption or release of carbon monoxide (CO) by the four grasses over a six-hour period showed that there was no difference in the response between C$_3$ and C$_4$ grasses when exposed to 100 ul/liter CO (Table 2).

Table 2. Analysis of variance for cumulative absorption or release of CO by four grasses over a six hour period in light

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>m.s.</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass</td>
<td>3</td>
<td>146.07</td>
<td>0.0001**</td>
</tr>
<tr>
<td>C$_3$ vs C$_4$</td>
<td>(1)</td>
<td>30.41</td>
<td>0.0730</td>
</tr>
<tr>
<td>KB vs TF</td>
<td>(1)</td>
<td>141.76</td>
<td>0.0002*</td>
</tr>
<tr>
<td>SA vs ZY</td>
<td>(1)</td>
<td>266.05</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Hour</td>
<td>5</td>
<td>95.36</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Grass by Hour</td>
<td>15</td>
<td>9.15</td>
<td>0.4709</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>90.43</td>
<td></td>
</tr>
</tbody>
</table>

*Denotes significant LSD at the 0.05 level.
**Denotes significant LSD at the 0.01 level.

There was a difference in the response exhibited in the comparison of the two C$_3$ grasses against one another, and the two C$_4$ grasses against one another. The amount of time the
grasses were exposed to CO also caused a difference in the amount of CO absorbed or released by the grasses.

Figure 3 presents the comparison of the four grasses to each other after they were exposed to CO for six hours. All four grasses were statistically similar in their ability to absorb CO from the atmosphere in the exposure chamber. Tall fescue and St. Augustinegrass removed 3.96 and 3.53 μg CO/g dry leaf weight, respectively, while Kentucky bluegrass removed 0.89 μg CO/g dry leaf weight. Zoysiagrass was the only grass that did not show a cumulative absorption of CO after six hours. Instead, it resulted in a net increase of 0.68 μg CO/g dry leaf weight within the exposed chamber.

The combination of the mean absorption or release of CO by the four grasses gave a variable response on an hourly basis (Figure 4). In the first hour, the grasses released 0.90 μg CO/g dry leaf weight into the exposure chamber. The evolution of CO by the grasses reoccurred after the fourth hour. Removal of CO from the exposure system took place during hours two, three, five, and six, with hours two and six having the greatest amount of CO removed (2.70 and 3.98 μg CO/g dry leaf wt/hr, respectively).

Figure 5 presents the cumulative absorption or release of CO over the six hour period by the four grasses combined. Again, as in Figure 4, hours one and four gave the lowest
Figure 3. Cumulative absorption or release of CO in light by four grasses over a six-hour period. The 0 line represents the control. Bars extending above the 0 line signify absorption of CO, whereas bars extending below the 0 line signify release of CO. Each bar is the mean of 30 values, with six hours of data from each of five replications.
Figure 4. Hourly absorption or release of CO in light for four grasses over a six-hour period. The 0 line represents the control. Bars extending above the 0 line signify absorption of CO, whereas bars extending below the 0 line signify release of CO. Each bar is the mean of 20 values from the four grasses at each hour from each of five replications.
\[ \mu_{8} \text{ CO/g dry leaf wt/hr} \]

LSD 0.05-2.11

TIME (HR)

<table>
<thead>
<tr>
<th>TIME (HR)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.90</td>
</tr>
<tr>
<td>2</td>
<td>2.70</td>
</tr>
<tr>
<td>3</td>
<td>0.68</td>
</tr>
<tr>
<td>4</td>
<td>-1.63</td>
</tr>
<tr>
<td>5</td>
<td>0.86</td>
</tr>
<tr>
<td>6</td>
<td>3.98</td>
</tr>
</tbody>
</table>
Figure 5. Cumulative absorption or release of CO in light over a six-hour period. The 0 line represents the control. Bars extending above the 0 line signify absorption of CO, whereas bars extending below the 0 line signify release of CO. Each bar is the mean of 20 values from the four grasses at each hour from each of five replications.
\[ \text{LSD } 0.05 = 1.91 \]
values, with the grasses releasing 0.92 µg CO/g dry leaf weight into the exposure chamber during the first hour. The amount of CO removed from the exposure chamber during hours two, three, four and five were similar statistically. After six hours, the grasses were collectively removed 5.69 µg CO/g dry leaf weight from the exposure chamber.

Individually, each of the four grasses exhibited a release of CO into the exposure chamber after the fourth hour, followed by the removal of CO during the fifth hour (Figure 6). Three grasses had their greatest absorption of CO during the sixth hour of exposure. Kentucky bluegrass had its largest absorption of CO during the second hour.

Comparing the grasses with each other at hourly intervals shows that all of them, statistically, have the same response to CO during the third, fourth, and fifth hour of exposure (Table 3 and Figure 6). During the first hour, Kentucky bluegrass absorbed less CO than both tall fescue and St. Augustinegrass, while zoysiagrass differs only from St. Augustinegrass. In the second hour, Kentucky bluegrass absorbed more CO from the exposure chamber than any of the other grasses. Tall fescue, St. Augustinegrass and zoysiagrass all removed the same amount of CO during the second hour. Zoysiagrass and tall fescue absorbed the most CO from the exposure chamber during the sixth hour.
Figure 6. Hourly absorption or release of CO in light by each grass over a six-hour period. The 0 line represents the control. Bars extending above the 0 line signify absorption of CO during that hour, whereas bars extending below the 0 line signify release of CO during that hour. Each bar is the mean of five replications.
Table 3. Analysis of variance for hourly absorption or release of CO by four grasses over a six-hour period in light
<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>m.s.</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass</td>
<td>3</td>
<td>3.74</td>
<td>0.8158</td>
</tr>
<tr>
<td>Hour</td>
<td>5</td>
<td>90.42</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Treatment (Grass by Hour)</td>
<td>23</td>
<td>36.14</td>
<td>0.0001**</td>
</tr>
<tr>
<td>KB HR1 vs TF HR1</td>
<td>(1)</td>
<td>66.50</td>
<td>0.0174</td>
</tr>
<tr>
<td>KB HR1 vs SA HR1</td>
<td>(1)</td>
<td>87.98</td>
<td>0.0065*</td>
</tr>
<tr>
<td>TF HR1 vs ZY HR1</td>
<td>(1)</td>
<td>25.06</td>
<td>0.1407</td>
</tr>
<tr>
<td>SA HR1 vs ZY HR1</td>
<td>(1)</td>
<td>38.83</td>
<td>0.0675</td>
</tr>
<tr>
<td>KB HR2 vs ZY HR2</td>
<td>(1)</td>
<td>106.32</td>
<td>0.0029*</td>
</tr>
<tr>
<td>KB HR2 vs TF SAHR2</td>
<td>(1)</td>
<td>92.24</td>
<td>0.0054*</td>
</tr>
<tr>
<td>TF SA HR2 vs ZYHR2</td>
<td>(1)</td>
<td>5.29</td>
<td>0.4962</td>
</tr>
<tr>
<td>KB HR3 vs TF HR3</td>
<td>(1)</td>
<td>27.37</td>
<td>0.1239</td>
</tr>
<tr>
<td>KB HR3 vs C4 HR3</td>
<td>(1)</td>
<td>7.50</td>
<td>0.4183</td>
</tr>
<tr>
<td>TF HR3 vs C4 HR3</td>
<td>(1)</td>
<td>10.90</td>
<td>0.3297</td>
</tr>
<tr>
<td>KB HR4 vs ZY HR4</td>
<td>(1)</td>
<td>6.19</td>
<td>0.4618</td>
</tr>
<tr>
<td>KB HR5 vs TF HR5</td>
<td>(1)</td>
<td>12.47</td>
<td>0.2973</td>
</tr>
<tr>
<td>KB HR5 vs ZY HR5</td>
<td>(1)</td>
<td>12.47</td>
<td>0.2973</td>
</tr>
<tr>
<td>KB HR5 vs SA HR5</td>
<td>(1)</td>
<td>10.41</td>
<td>0.3408</td>
</tr>
<tr>
<td>TF HR6 vs KB HR6</td>
<td>(1)</td>
<td>28.10</td>
<td>0.1190</td>
</tr>
<tr>
<td>TF HR6 vs SA HR6</td>
<td>(1)</td>
<td>14.71</td>
<td>0.2579</td>
</tr>
<tr>
<td>ZY HR6 vs SA HR6</td>
<td>(1)</td>
<td>48.68</td>
<td>0.0411*</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>8.80</td>
<td></td>
</tr>
</tbody>
</table>

*Denotes significant LSD at the 0.05 level.
**Denotes significant LSD at the 0.01 level.
Dark Study

Results of the analysis of variance for cumulative absorption or release of CO by the four grasses over a period of six hours showed that there was a difference between grasses (Table 4). It also showed that C₃ grasses respond differently than C₄ grasses and that the two grasses within each of the groups respond differently.

Table 4. Analysis of variance for cumulative absorption or release of CO by four grasses over a six hour period in the dark

<table>
<thead>
<tr>
<th>source</th>
<th>d.f.</th>
<th>m.s.</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass</td>
<td>3</td>
<td>4188.35</td>
<td>0.0001**</td>
</tr>
<tr>
<td>C₃ vs C₄</td>
<td>(1)</td>
<td>5113.46</td>
<td>0.0001**</td>
</tr>
<tr>
<td>KB vs TF</td>
<td>(1)</td>
<td>2943.11</td>
<td>0.0001**</td>
</tr>
<tr>
<td>SA vs ZY</td>
<td>(1)</td>
<td>4508.48</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Hour</td>
<td>5</td>
<td>349.60</td>
<td>0.1051</td>
</tr>
<tr>
<td>Grass by Hour</td>
<td>15</td>
<td>188.94</td>
<td>0.4450</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>2252.94</td>
<td></td>
</tr>
</tbody>
</table>

**Denotes significant LSD at the 0.01 level.

Data presented in Figure 7 visually describe the variation among the grasses in their ability to either absorb or release CO following exposure to it for six hours. Tall fescue absorbed the largest amount of CO while Kentucky
Figure 7. Cumulative absorption or release of CO in dark by four grasses over six hours. The 0 line represents the control. Bars extending above the 0 line signify uptake of CO, whereas bars extending below the 0 line signify release of CO. Each bar is the mean of 30 values, with six hours of data from each of five replications.
Kentucky Bluegrass

Tall Fescue

St. Augustinegrass

Zoysiagrass

LSD 0.05-11.18

μg CO/g DRY LEAF WT

12.95

4.38

1.76

15.77

4.38

St. Augustinegrass

Tall Fescue

Kentucky Bluegrass
bluegrass and zoysiagrass absorbed amounts similar to one another but different from that of either tall fescue or St. Augustinegrass. St. Augustinegrass was the only one to release CO during the six hour exposure period.

The analysis in table five shows that the hourly effect on the absorption or release of CO by the four grasses is a significant factor, with the fourth and fifth hour different than the other hours.

Table 5. Analysis of variance for the hourly absorption or release of CO by four grasses over a six-hour period in the dark

<table>
<thead>
<tr>
<th>source</th>
<th>d.f.</th>
<th>m.s.</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass</td>
<td>3</td>
<td>117.79</td>
<td>0.7807</td>
</tr>
<tr>
<td>Hour</td>
<td>5</td>
<td>1123.41</td>
<td>0.0068*</td>
</tr>
<tr>
<td>Hour 4 vs others</td>
<td>(1)</td>
<td>1929.15</td>
<td>0.0107*</td>
</tr>
<tr>
<td>Hour 5 vs others</td>
<td>(1)</td>
<td>2741.60</td>
<td>0.0025*</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>90.11</td>
<td></td>
</tr>
</tbody>
</table>

* Denotes significant LSD at the 0.05 level.

Combining the mean absorption or release of CO by the four grasses on an hourly basis shows that the response during the first hour was similar to every hour except the fourth hour, which showed a release of 8.80 μg CO/g dry leaf
weight/hr (Figure 8). The release of CO during the fourth hour was followed by the absorption of 12.43 μg CO/g dry leaf weight/hr during the fifth hour.

Observing the cumulative absorption or release of CO by the four grasses over the six hour exposure period shows that the grasses exhibit little variation (Figure 9). Hours one, four and six are statistically similar as are hours one, two, three, five and six. The largest change occurs between the fourth and fifth hour, with the grasses during the fourth hour releasing 5.28 μg CO/g dry leaf weight and fifth hour absorbing 7.14 μg CO/g dry leaf weight.
Figure 8. Hourly absorption or release of CO in the dark over a six hour period. The 0 line represents the control. Bars extending above the 0 line signify absorption of CO, whereas bars extending below the 0 line signify release of CO. Each bar is the mean of 20 values from the four grasses at each hour from each of five replications.
LSD 0.05 = 8.57
Figure 9. Cumulative absorption or release of CO in dark over a six-hour period. The 0 line represents the control. Bars extending above the 0 line signify absorption of CO, whereas bars extending below the 0 line signify release of CO. Each bar is the mean of 20 values from the four grasses at each hour from each of five replications.
DISCUSSION

Light Study

This study showed that there were variations between the grasses in their rate of absorption and release of CO. This variation comes as no surprise, because previous studies, most notably Bidwell and Bebee (1974), that looked at thirty-five species of temperate and tropical plants, found a wide variation in the efficiency of CO absorption and/or release by green plants (Wilks, 1959; Chappelle and Krall, 1961; Inman and Ingersoll, 1971; Bidwell and Fraser, 1972).

In the study by Bidwell and Bebee (1974), they tested thirty-five species of plants and their ability to absorb CO from a gas stream containing 6 µl/liter CO in light. The average uptake for the thirty-five species was 0.19 µl CO/hr/g fresh leaf weight. They also found that CO uptake was linear with CO concentration to at least 100 µl/liter CO. From this information, the average uptake of CO by the thirty-five species exposed to 101 µl/liter CO would be 3.32 µl CO/hr/g fresh leaf weight.

Data presented in our study observed that tall fescue exposed to 101 µl/liter CO in the light would absorb 0.51 µl CO/hr/g dry leaf weight, while the average absorption rate for the four grasses was 0.75 µl CO/hr/g dry leaf weight. These values are less than what would be expected according
to the data presented by Bidwell and Bebee (1974), but were equal to or greater than seven of the thirty-five species tested.

Some variability in the results comparing our study and that of Bidwell and Bebee (1974) can be expected since our study calculated uptake of CO using dry leaf weight and Bidwell and Bebee calculated uptake of CO using fresh leaf weight. Additional differences could be caused by the different exposure systems used. Our system was constructed entirely of glass or stainless steel, while that of Bidwell and Bebee (1974) used plexiglass chambers, a rubber diaphragm pump, and heavy-walled butyl-rubber tubing.

Figure 3 presents the comparison of the four grasses with each other after they were exposed to CO for six hours and their cumulative absorption or release of CO. The grasses with the widest leaf blades, tall fescue and St. Augustinegrass, absorbed the most CO. Hence, the area of the leaf blade may have an effect on the amount of CO absorbed. It has been shown that the external surface of the leaf is populated by a variety of microorganisms (Ruinen, 1961) including those that have been found to oxidize CO into CO$_2$ (Ingersoll et al. 1974). It may be possible that with a larger leaf area to support a larger population of microorganisms, more CO would be absorbed.
This theory also may be supported by the results found by Bidwell and Fraser (1972) showing an incorporation of CO into the plant material during the day time and formation of CO$_2$ during the dark period. This would seem to indicate microbial processes on the leaf surface, whereby CO is oxidized to CO$_2$, that is afterwards utilized by the plant in the photosynthetic process during the light period (Seiler et al. 1978).

Bidwell and Bebee (1974) stated in their results that a large variability sometimes occurred in measurements made on individual species at different times. Variability of results also were acknowledged by Kortschak and Nickell (1973) in their study of sugarcane leaves.

Both of these studies indicated variations in results over a period of time but did not go into specifics. In this present study, all four grasses had a drop in CO absorption, which resulted in a release of CO during the fourth hour after exposure to CO. This fourth hour release of CO was then followed by CO absorption in the next two hours.

Again, it must be noted that the experimental design of this study was a completely randomized design with five replications of each treatment. Each treatment was assigned a number and a random number table was used to determine the order in which the treatments were exposed to carbon.
monoxide. The function of randomization is to ensure that there is a valid or unbiased estimate of experimental error and of treatment means and differences among them; it also tends to destroy the correlation among errors and make valid the usual tests of significance (Steel and Torrie, 1980). Therefore, randomization of this study eliminated any artifacts that may have given biased results.

One possible reason for the fourth hour release of CO could be caused by a regulatory mechanism of enzymes, feedback inhibition. In feedback inhibition, the enzyme that catalyzes the first step in a biosynthetic pathway is inhibited by the ultimate product (Stryer, 1981). Feedback inhibition affects many reaction sequences, resulting in the synthesis of small molecules such as amino acids. Bidwell and Fraser (1972) suggested that most of the absorbed CO in plants was reduced and incorporated into serine which was converted to sucrose. It is possible that an enzyme, during the synthesis of serine, is inhibited by serine itself when the concentration of serine reaches a sufficiently high level. Therefore, when enough CO has been taken up by a plant, and enough serine is produced, in this case four hours, the enzyme is inhibited and any excess CO is then released into the exposure chamber.
Another possible reason for the fourth hour release of CO could be the saturation of the active sites on the enzyme, at which time excess CO would be released into the chamber only to be absorbed once active sites on the enzyme again became available.

A third possibility could be de novo synthesis of an enzyme, that catalyzes the reaction is present in very small amounts until the substrate, CO, becomes available. Once the substrate is present, the enzyme then begins to be synthesized (Stryer, 1981). This could explain why there is an initial absorption of CO for the first three hours (saturation of the present enzyme), a release of CO the fourth hour (excess CO), and an absorption of CO the next two hours (de novo synthesis of enzyme).

Data presented in table 2 and figure 3 show that there is no significant difference in the absorption of CO by C₃ and C₄ grasses. If CO was absorbed via the photosynthetic pathway, there should have been a difference between the C₃ and C₄ grasses, since they both have different photosynthetic pathways. Our findings are then in accordance with those of Bidwell and Fraser (1972) who stated that CO metabolism followed a completely different pathway than CO₂.

Future studies concerning the absorption of CO by green plants should look at the effect of CO concentrations in the
fourth hour release, to see if there is a shift in the timing of the release. Lowering the CO concentration should cause the release to occur later. It will also be important to look at the effect changes in temperature have on the rate of CO absorption to determine if it is an enzymatic or non-enzymatic reaction.

Dark Study

This study showed that there was much variation between the four grasses in their ability to either absorb or release CO. Not only did the C_3 grasses react differently than the C_4 grasses, but the two grasses within each group reacted differently from one another.

Tall fescue, Kentucky bluegrass, and zoysiagrass all had a net absorption of CO, with tall fescue absorbing the largest amount during the six-hour exposure period. St. Augustine grass was the only one to release CO during this period.

The fact that this study indicates absorption of CO by three grasses and release of CO by one grass causes some discrepancy with previous studies concerning the absorption or release of CO.

Studies published by Wilks (1959), Seiler, Giehl and Bunse (1978), and Fischer and Luttge (1980), all indicated that the release of CO was light dependent, while Loewus and
Delwiche (1963) indicated minimal CO production in the dark. These previous findings conflict with our data of St. Augustine grass, which released a large amount, 12.95 ug CO/g dry leaf wt, of CO in the dark.

Other studies showed that absorption of CO was light-stimulated, while a small amount is absorbed in the dark (Krall and Tolbert, 1957; Chappelle and Krall, 1961; Kortschak and Nickell, 1973), which contradicts our findings with Kentucky bluegrass, tall fescue, and zoysiagrass.

The study with which our results, with the exception of St. Augustine grass, are nearest in agreement is by Bidwell and Fraser (1972), who stated that the absorption of CO by green plants continues at nearly the same rate in darkness as in light, which suggests that CO absorption is not photosynthetic.

As in the light study, this study also witnessed the fourth hour release of CO by the grasses, which was then followed by a large absorption of CO the fifth hour. Unlike the light study, this study had another release of CO during the sixth hour. The possible reasons for the release of CO by the grasses during the fourth and sixth hour could be the same as those stated in the discussion of the light study.

Future studies concerning the absorption or release of CO by green plants should concentrate on tall fescue and St.
Augustinegrass, which were complete opposites as to their ability to absorb or release CO. Another aspect of this study that should be observed more closely is the effect CO concentration has on absorption or release of CO, which may involve lengthening the exposure period. It will also be important to observe the effect changes in temperature have on the rate of CO absorption to determine if it is an enzymatic or non-enzymatic reaction.
SUMMARY

Light Study

All four grasses, though they exhibited variation in the amount of CO absorbed, were statistically similar. The similarity of the four grasses in their ability to absorb CO suggested that the pathway for CO absorption is not photosynthetic. The combination of the mean absorption or release of CO by the four grasses, showed that CO was absorbed every hour except the first and fourth, where CO was released. The cumulative absorption or release of CO by the four grasses over the six-hour period was 5.69 μg CO/g dry leaf weight, with an hourly rate of 0.75 μl CO/hr/g dry leaf rate. Data collected from this study indicated that all four turfgrasses have the potential for removal of carbon monoxide from the environment in urban areas.

Dark Study

All four grasses exhibited variation in the amount of CO absorbed or released during the six hour exposure period. Tall fescue absorbed the greatest amount, 15.77 μg CO/g dry leaf weight, with zoysiagrass and Kentucky bluegrass absorbing less, thus suggesting that CO absorption is not photosynthetic. St. Augustinegrass was the only grass to exhibit a cumulative release of CO, 12.95 μg CO/g dry leaf
weight. The cumulative absorption or release of CO by the four grasses over the six-hour period was 0.98 μg CO/g dry leaf weight, with the grasses having the highest absorption rate of CO during the fifth hour. Tall fescue, zoysiagrass, and Kentucky bluegrass showed the most potential for significant removal of carbon monoxide from the atmosphere in urban areas.
LITERATURE CITED


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APPENDIX
Figure A1. Humidifier in carbon monoxide recirculating exposure system
INLET AND OUTLET MUST BE ON SAME LEVEL

18/9 GROUND GLASS BALL

SOLID GLASS SUPPORT BAR 5 mm O.D.

18/9 GROUND GLASS SOCKET

5.0 cm

3.0 cm

1.5 cm

35/20 GROUND GLASS BALL

35/20 GROUND GLASS SOCKET ATTACHED TO RB FLASK

3.0 cm

3-4 mm (I.E., 14-16 mm I.D. so that there is 3-4 mm on each side of the tube)

SOMEPLACE IN HERE WILL HAVE TO SWITCH FROM 9 mm I.D. TO 8 mm O.D.

200 ml ROUND BOTTOM FLASKS

G 3.0 cm

8 mm STEM DIAM.
FISHER No. 17-138B
1979 CATALOG p. 449
6 FOR 45.95

2.0 cm FROM TOP OF FRITTED CYLINDER TO LINE DRAWN ACROSS FLASK

COARSE FRITTED CYLINDER 12 mm DIAM.

5 mm BETWEEN CYLINDER AND BOTTOM AND SIDES
Sample calculation the conversion of ul/liter CO/g dry leaf weight/hr to ug CO/g dry leaf weight/hr.

Molecular weight CO = 28.05g = 28050000 ug

Volume of 1 mole of gas at standard temperature and pressure = 22.4 liters = 22400000 ul

Volume of exposure system = 10.12 liters

1 ppm = 1 ul/liter

Dry leaf weight of grass sample = 3.28 g

Change in ppm CO from previous hour = 1.84 ppm = 1.84 ul/liter

\[
\frac{\text{ppm CO}}{\text{g dry leaf weight}} = \frac{1.84 \text{ ppm}}{3.28 \text{ g}} = 0.561 \text{ ppm CO/g dry leaf weight}
\]

0.561 ppm CO/g dry leaf weight = 5.61 ul CO/liter/g dry leaf weight

Volume of CO = (0.561 ul CO/l/g dry leaf wt) (10.12 liter) = 5.677 ul in exposure system

\[
\frac{\text{Mole wt. CO}}{\text{Volume 1 mole gas}} = \frac{\text{ug CO in exposure system}}{\text{Volume CO in exposure system}}
\]

\[
\frac{28050000 \text{ ug CO}}{22400000 \text{ ul}} = \frac{x \text{ ug CO}}{5.677 \text{ ul CO}} = 7.1 \text{ ug CO/g dry leaf wt/hr}
\]