POLYETHYLENE MULCHES: THEIR INFLUENCE ON SOIL ATMOSPHERIC CO_2 CONCENTRATION AND SUBSEQUENT ASSIMILATION BY EGGPLANT (*Solanum melongena* L.) ROOTS

A Thesis

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by

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INTRODUCTION

Mulching, the application of various materials to the soil surface to influence the microclimate of the soil, induces a beneficial effect on crop productivity. One type of material that is used for mulching is a thin layer of inert polyethylene film. The polyethylene film, usually 1.0 to 4.0 mil in thickness and 1 to 1.5 m wide, is laid over the soil and seeds or transplants are placed in slits made at desired intervals in the polyethylene film. The practice of growing a crop in a soil that is covered with a polyethylene film is termed "plastic mulching."

Plastic mulching has several advantages over conventional non-mulched growing practices. The polyethylene film protects the soil against wind and water erosion. It acts as a protective barrier to high winds and destructive raindrop action on the soil. In addition to the reduction of erosion, certain colors of polyethylene film have the ability to suppress weed growth. Weed growth is inhibited because little photosynthetically active radiation can penetrate certain opaque colors of polyethylene film. The most important advantage of plastic mulching is an increase in early and total yield for certain crops. Numerous hypotheses have been presented in an attempt to explain this increase. Factors often proposed for improved growth of polyethylene mulched plants have been increased soil temperature, conservation of soil moisture, weed control, elimination of root pruning by cultivation, maintenance of good soil physical condition, reduced leaching of nitrogen, and reduction of certain soil-borne diseases.

This study will examine an additional factor that may promote increased yields, soil atmospheric carbon dioxide. The objectives within this study are to:

1. Monitor the soil carbon dioxide concentration at several depths under black polyethylene film and in non-mulched soils.
2. Assay for growth of eggplant (*Solanum melongena* var. *esculentum*) in a soil environment of enriched carbon dioxide.
3. Assay for radioactive labeled $^{14}CO_2$ absorption, fixation and translocation of fixation products from intact eggplant roots.

The primary aim of this study is to obtain a better understanding of soil carbon dioxide effect on eggplant when the crop is grown on a polyethylene film.

LITERATURE REVIEW

Polyethylene mulch's beneficial influence in vegetable crop yield has been convincingly proven. In one of the first articles concerning polyethylene mulch's effect on vegetables, Emert (1957) reported that tomatoes grown on three foot wide black polyethylene film had an increase of 1.4 pounds of fruit per plant, compared to tomatoes grown on unmulched soil.

Studies were conducted in Michigan comparing yields of tomatoes, muskmelons, summer squash, and cucumbers grown on black polyethylene mulch to unmulched soil. Early planted tomatoes on the polyethylene mulch had a 47% early and 132% total yield increase. Average yields of several varieties of muskmelon exhibited 271% early and 100% total yield increases when the plants were grown on polyethylene mulch. Similar yield increases were reported with summer squash (182% early, 58% total) and cucumbers (126% early, 28% total) (Carolus, 1961).

Courter and Oebker (1964) observed substantial increases in cucumber yields when they were grown on polyethylene mulch. In 1958, the early yield of U.S. #1 cucumbers was 53% higher when the plants were on a polyethylene mulch. In 1962, there was a 45% early and 22% total increase with polyethylene mulching.

Clarkson and Frazier (1957) evaluated cantaloupe growth on paper and polyethylene mulches. Cantaloupe yields were two to three times as great on polyethylene as on the unmulched plots. There was greater foliage and flower production on the mulched areas.

Hopen and Oebker (1975) reported increases in yields of several cool season vegetables when they were grown on black polyethylene mulch. They noted a 94% increase in the total number of heads and 89% increase in the total weight of broccoli. Significant increases in marketable and total weight of lettuce was also reported.

A recent study in California on the effect of polyethylene mulches on desert area cantaloupes reported a 155% and 168% total yield increase with black and clear polyethylene mulch, respectively (Johnson and Mayberry, 1981).

Eggplant, like many warm season vegetables, respond favorably to polyethylene mulch. Gerakis and Tasangarakis (1969) observed that the mean yield of eggplant mulched with polyethylene was 1.99 kilograms (kg) per plant, as compared with .018 kg per plant from the unmulched control. They concluded that the yield increase was due to a greater number of fruit per plant.

Paterson and Smith (1973), in a study on nitrogen, mulches, and trickle irrigation effects on eggplant production, reported that mulching with clear polyethylene combined with irrigation increased yields of eggplant 43% over unmulched treatments. Total yields and individual fruit weight of eggplant grown under irrigation and polyethylene mulched conditions increased as the nitrogen increased. They concluded that clear polyethylene significantly encouraged early growth and large early yields of eggplant.

Courter, _et al_. (1968) used eggplant and other warm season vegetables in a comparison between polyethylene coated paper and polyethylene film. In most cases, the difference in yield between the two treatments was not significant. 'Black Magic' hybrid eggplant had a 79% early and 61% total yield increase when the plants were grown on polyethylene, compared to unmulched soil. When the crop was grown on black paper coated with clear polyethylene, there was a 98% early and 62% total yield increase over unmulched soil.

Pollack, et al. (1969), in a summary of crop responses to various polyethylene mulches, observed that summer squash, muskmelon, and eggplant benefited in early and total season yields when grown on polyethylene film mulch. They observed that eggplant yields were increased almost 300% with the use of a clear polyethylene mulch. Average yield of early harvested eggplant between 1966 and 1968 with clear polyethylene was 266 bushels per acre (bu/A), and 155 bu/A on unmulched soil. Total harvest data for these years are just as striking. Average total bushels per acre was 574 for the unmulched soil, and 1,183 for the clear polyethylene. In all years, both the early and total harvests showed significant differences between treatments.

Salman (1981), in a Master's Thesis concerned with the effect of polyethylene mulch on the productivity of eggplant in Ohio, reported a 71.4% yield increase with clear polyethylene and a 13.5% increase with black polyethylene compared to the unmulched treatments. He felt that crop response to polyethylene mulch was due to several factors such as increased soil temperature and moisture, weed control without cultivation, and less soil compaction resulting in a more friable and well-aerated soil.

Alderfer and Merke (1944) indicated that the primary effect of mulching is a physical modification of the soil environment. Many of the modifying influences of a polyethylene mulch on the soil environment have been recognized for several years. Clarkson (1960) found that the average minimum and maximum temperature under the polyethylene mulch was generally higher than the corresponding temperature in the unmulched soil. The maximum differences between the polyethylene and the unmulched soil were recorded at the one and three inch depths. Soil temperature at the six inch depth tended to be consistent between treatments. Clarkson concluded that the somewhat higher temperatures found under the polyethylene were not harmful to plant growth.

A study that compared soil temperatures under black polyethylene and in unmulched soil reported that soil temperatures were 3^{o} to 7^{o} F warmer under the black polyethylene at the 3 inch depth during clear days (Black and Greb, 1962). Homna, et al. (1959) noted that daytime unmulched soil temperatures at the soil surface were higher than the black polyethylene's. At night, this trend was reversed; night temperatures were higher in the soil beneath the black polyethylene mulch than in unmulched soil.

Shadbolt, et al. (1962) compared soil temperatures under clear and black polyethylene and unmulched soil. They noted that temperatures in the unmulched soil were sometimes higher than the temperatures under the polyethylene. This would occur only for a short period during the midday hours. They concluded that bare soil warmed faster and reached a higher maximum temperature, but it cooled faster than the soil that was covered with the polyethylene.

Schales (1963) observed that clear polyethylene mulch had the greatest temperature increase over unmulched soil. In early season readings at the 1 inch depth, there was an average increase of 10° F. The black polyethylene treatments increased the soil temperature an average of $3-5^{\circ}$ F. White opaque polyethylene did not bring about any difference in soil temperature. Increased soil temperature was greatest early in the season before plant growth was able to shade the mulch.

Courter and Oebker (1964) believed that the increase in yields were due to factors other than high temperatures in the mulched soils. They based this conclusion on observations that soil temperature was consistently lower under paper mulch, intermediate in unmulched soil, and highest under black polyethylene. The similar yield increases that occur when crops are on paper or polyethylene mulch (1962 total yield of summer squash: black paper 199.8 cwt/A; black polyethylene 197.7 cwt/A), suggest that increased soil temperature is not the most important factor that promotes increased production of crops.

Hopen (1965) concluded that growth and yield differences on black polyethylene mulch cannot be explained solely by soil temperature differences. A slight soil moisture advantage is found under the polyethylene which may be partially responsible for the increased growth and yields of plants.

Generally, soils under polyethylene mulch have a slightly higher concentration of soil moisture than do soils without the mulch. The polyethylene film acts as a physical vapor barrier to soil water evaporation (Schales, 1963). Giddens (1965) reported that, when soil was not irrigated for two weeks, the unmulched soil contained 7% moisture and the black and clear polyethylene mulched soils contained 10.8 and 11.6%, respectively. After a three-inch rainfall, the percent moisture increased to 13% in the unmulched soil, 12.9% under the black, and 11.9% under the clear polyethylene.

Salman (1981) noted that soil moisture was significantly higher under the mulched treatments when compared to the unmulched soil. Soil moisture contents were: black polyethylene, 22.09%; clear polyethylene, 18.96%; and unmulched soil, 17.77%. He noted that soil moisture fluctuated less under the polyethylene films than in the unmulched soil, and fluctuations were closely associated with the amount of rainfall which occurred during the experiment.

Schales and Sheldrake (1964) also observed that soil moisture fluctuated less under the polyethylene mulch than in the unmulched treatments. Unmulched soil varied 7.3 units from before and after a 1.5 inch rainfall, while clear polyethylene varied 3.6 units and black varied 1.1 units. Buclon (1971) concluded that polyethylene mulch plays the part of a regulator as regards to rainfall and irrigation; initially there is less water penetrating the ground, but the water is not lost in soil evaporation.

Soil moisture readings under the polyethylene mulches are not always the highest levels. Schales and Sheldrake (1966) noted that soil moisture levels were generally lower in the upper 6 inches of the soil under the polyethylene film than in the unmulched control. They also noted that plant growth was most rapid, muskmelon fruit set was earliest, and yields were generally highest under the polyethylene mulched treatments. These results indicate that yield increases with polyethylene mulch are not totally due to higher moisture levels that are typically found in mulched soils.

Nutrients in the soil and the reduction of leaching of nitrogen have been linked as a factor that influences increased yield of polyethylene mulched plants. Waggoner, Miller and DeRoo (1960) found that polyethylene mulch did not change the quantity of available ammonia, phosphorus, potassium, calcium, magnesium, aluminum, or manganese in the soil. Nitrate concentration was consistently higher beneath the film than in the unmulched soil. Nitrate nitrogen concentration in parts per million (ppm) of soil were: unmulched, 4; black polyethylene, 33; clear polyethylene, 18. They concluded that the difference in nitrates was caused by differences in mineralization and removal of the nitrogen.

Clarkson (1960) reported that black polyethylene effectively reduced the loss of nitrogen from the soil profile. He suggested that supplemental nitrogen fertilizer that is normally applied during the growing season need not be applied if the crop is mulched with polyethylene.

Schales (1963) reported that soluble salts in the soil decreased less under the polyethylene than in the unmulched soil. The primary reason for this observation is a decrease in water percolation resulting in less leaching under the polyethylene film.

Another proposed factor that influences productivity of plants that are grown on polyethylene mulch is increased concentration of carbon dioxide in the microclimate of the plant. Sheldrake (1963) reported a four-fold increase in CO_2 concentration at the planting holes in the polyethylene. He questioned if physical changes to the microclimate under the polyethylene, such as temperature increase, early season moisture conservation, and decreased soil compaction, could explain the dramatic yield increases. Sheldrake proposed the chimney effect hypothesis to explain the yield increases. The chimney effect, defined, is the build-up of carbon dioxide under the polyethylene film and the emission of this gas through the planting holes in the polyethylene. The plant thus exists in an environment of enriched concentration of CO_2.

Hopen and Oebker (1975) examined CO_2 levels at the 4 to 9 cm height above the planting holes in the polyethylene. They found a slight increase of 50 ppm CO_2 over the holes in the polyethylene. The higher concentration of CO_2 dissipated rapidly into the ambient atmosphere. Only plant foliage a few centimeters from the holes could benefit from the small increase in CO_2. They concluded that the increased growth and yield of plants grown on polyethylene mulch cannot be attributed totally to the chimney effect.

Carbon dioxide may still play an important role in the increased productivity of plants that are grown on polyethylene mulch. Roots, like other non-green tissues, are capable of assimilating CO_2. Ruben and Kamen (1940), using short-lived radioactive carbon-11 dioxide ($^{11}CO_2$), showed that a preparation of ground barley roots could fix CO_2. Poel (1953), using similar techniques and long-lived carbon-14 dioxide ($^{14}CO_2$), identified several products of CO_2 fixation by radiochromatography. The compounds found to contain radioactivity were organic acids; malic, citric (or isocitric), aspartic, and amino acids; serine, asparagine, glutamine, and tyrosine. The chromatographic pattern of the products in barley were very similar to the pattern obtained by Benson and Calvin (1950) in the dark fixation of $^{14}CO_2$ by the leaves of barley.

Russian investigators have conducted extensive research programs on root assimilation of CO_2. Kursanov, _et al_. (1951) exposed 30-day-old bean plant roots to $^{14}CO_2$. Using a radioautograph, they found the radioactivity throughout the entire plant. They then quantified the radioactivity location and found twice as much assimilated carbon in the stem than in the roots. Little radioactivity was found in the leaves.

In a second report concerning CO_2 assimilation by plant roots, Kursanov, _et al_. (1952) reported that CO_2 uptake by the root system is not directly related to water uptake. They also reported that there was rapid movement of ^{14}C within the plant. Within five minutes of $^{14}CO_2$ exposure to the roots, ^{14}C was found in the upper leaflets of the bean plant. Distribution of the ^{14}C is apparently dependent on illumination of the stem. When the stem was shaded, the ^{14}C passed through the stem into the upper leaves. In a normally-illuminated plant, the ^{14}C was concentrated in the roots and in the middle part of the stem. Based on their experiments and calculations, 25% of the bean plant's carbon is fixed by the roots.

Jacobson (1955) examined the interaction of CO_2 fixation and ion absorption. He reported that when young excised barley roots were supplied with labeled CO_2 in solution, they fixed from 1.18 to 7.02% of the available CO_2 in three hours. The amount of $^{14}CO_2$ fixed was determined largely by the concurrent ion absorption treatment with the smallest percentage obtained during excess anion absorption and the largest percentage obtained during excess cation absorption. He also noted that in all treatments labeled, malate was the most abundant species produced.

Jackson and Coleman (1959) indicated that plant roots could fix CO_2 via carboxylation of phosphoenolpyruvate (PEP). They performed several assays on snap bean root extracts to determine the mechanism of CO_2 fixation. They found that fixation was dependent upon a supply of PEP or substrate capable of being transformed to PEP. No fixation occurred when pyruvate or acetate were substituted for PEP, indicating that those compounds are not converted to PEP prior to fixation. In addition, they observed that when root extracts were pretreated with NH_4, 48% of the fixed $^{14}CO_2$ was recovered in aspartate and/or glutamate. In the absence of NH_4 pretreatment, the majority of the labeled carbon was recovered in malate. They suggested the reaction scheme of dark CO_2 fixation by succulent leaves was employed in CO_2 fixation by roots. Saltman, et al (1956) proposed the following scheme of reaction for the dark fixation by succulent leaves:

$$\text{1.) Carbohydrate} \xrightarrow{\text{Glycolysis}} \text{PEP}$$

$$2.)\quad PEP + CO_2 \xrightarrow[\text{Carboxylase}]{\text{PEP}} \text{Oxaloacetate}$$

$$3.)\quad \text{Oxaloacetate} \xrightarrow[\text{Dehydrogenase}]{\text{Malic}} \text{Malate}$$

$$\text{Oxaloacetate} \xrightarrow[\text{Transaminase}]{+\ NH_3} \text{Aspartate}$$

Several other authors have shown that CO_2 is absorbed and assimilated by roots. Graf and Aronoff (1955) reported that onion and soybean roots fixed CO_2. They found the pattern of fixation of excised and attached roots to be identical. Bendri, et al. (1960) found that bush beans, soybeans, avocado, trifoliate orange, and barley roots can fix $^{14}CO_2$. In several of these species, no PEP carboxylase activity had been noted. Mazelis and Vennesland (1957) found that turnip roots can fix $^{14}CO_2$. They noted that PEP carboxykinase can catalyze the fixation if ADP is added to the PEP.

In the past several years, there has been renewed interest in investigations of root fixation of CO_2. Coker and Schubert (1981), in a report dealing with CO_2 fixation by soybean roots and nodules, noted that the concentration of CO_2 to which plant tissues were exposed had a marked effect on the rates of CO_2 fixation. Higher rates of fixation occurred when the tissue was exposed to high concentrations (2-3.5%). Maximum CO_2 fixation occurred in younger secondary roots before nodules formed. They also found ^{14}C labeled organic and amino acids in the stem tissue. They concluded that soybean roots and nodules possess an active system for fixing CO_2. PEP carboxylase is the primary enzyme responsible for CO_2 fixation. Fixed CO_2 plays an important role in the carbon economy of soybean nodules by serving as a source of carbon skeletons, energy substrate, and counter ions.

Arteca, et al. (1979), working with potatoes in an enriched soil atmosphere of 45% CO_2, 21% O_2, and 34% N_2, found a significant increase in shoot dry weight as early as two to six days after the plants were exposed to elevated levels of CO_2. The CO_2 enrichment also caused a significant increase in tuber weight and a highly significant increase in the number of tubers per plant. They found that 18% of the increased dry matter in the tubers came from root fixation of CO_2.

In a different study, Arteca, et al. (1982a) observed that within two hours of $^{14}CO_2$ exposure to potato roots, the petioles became heavily labeled with ^{14}C. They suggested that increased growth of plants was caused by the CO_2 and/or malate lowering the pH of the cell sap, thereby increasing the CO_2/O_2 ratio in the leaves and decreasing photorespiration.

Carbon dioxide levels in the soil atmosphere vary considerably. Reported concentrations range from 0.03% to 25% (Stolzy, 1974). These wide differences have been attributed to variations in soil depth, soil moisture, climáte, and soil management (Stolzy, 1974). Sampling technique can result in some variation. A large gas sample may include gas from the soil surface or from very large pore spaces (Hack, 1956). Ritchie (1964) used a small volume gas sample and, with a gas chromatograph, measured CO_2 at various depths in the soil. He found that a Webster clay loam soil with normal tillage and chemical weed control had an average concentration of 0.73% at the 3-inch depth, 2.06% at 6 inches, 2.75% at 9 inches, and 2.99% at the 12-inch depth.

Polyethylene mulch may promote an increase of CO_2 in the soil. Salman (1981) observed that there was a significant build-up of CO_2 below the polyethylene mulch at the soil surface. The CO_2 concentration at noon was almost 0.50% under the clear and 0.25% under the black polyethylene. The unmulched soil had only 0.035% CO_2.

Tukey and Schoff (1963) measured CO_2 in the soil under decomposable and non-decomposable mulching materials. They found that, when a polyethylene film was placed over a glass-fiber mulch, the soil at a 6-inch depth contained 5.6% CO_2. Measurements at the same depth without the polyethylene film on the glass fiber mulch had 3.2% CO_2 and 1.2% CO_2 on the clean cultivated control. Baron and Gorske (1981) found considerably higher CO_2 concentrations under black polyethylene mulch at the 15 and 5 cm depths. Early season readings at the 15 cm depth measured an average of 113,280 ppm under the polyethylene mulch, and 20,123 ppm CO_2

in the unmulched check. Differences between treatments at the 5 cm depth were just as striking, 12,025 ppm under the polyethylene, and 1,186 ppm CO_2 in the unmulched soil.

Excessive CO_2 in the soil is often cited as a contributing factor to poor plant growth due to the toxic effect on various plant processes (Russell, 1952). Stolwijk and Thimann (1947) reported that root growth was reduced in four dicotyledonous plants by a CO_2 concentration as low as 1%.

Plants are normally tolerant to high CO_2 levels. Harris and VanBavel (1957) found no appreciable effect on growth of tobacco in aerated sand culture as long as the amount of O_2 is equal to or greater than the CO_2 concentration in the soil atmosphere. Geisler (1963), in a report on CO_2 influence on the morphology of pea roots, observed that CO_2 acts as a stimulating substance. After a three-week exposure period, the root length and number of lateral roots in the most favorable CO_2 concentration of 7.5% CO_2 exceeded the control by more than 50%. He attributed the increase in root growth to the uptake of CO_2 and the accumulation of metabolic products (organic and amino acids).

Grable and Danielson (1965), in a paper on the influence of CO_2 on corn and soybean seedlings, found that a soil atmosphere of 10 and 19% CO_2 with 21% O_2 gave a growth stimulation of corn plant height and dry weight. Carbon dioxide levels in the soil had little influence on soybean growth until the concentration was greater than 29%. The effect was then merely a slight decrease of foliar fresh weight.

Arteca (1982a,b) best summarized the effect of carbon dioxide fixation by roots and its influence on growth by saying, "generally, it is accepted that CO_2 in the root zone may be utilized by most plants, the degree of utilization appears to differ between species."

CHAPTER 1

SOIL CARBON DIOXIDE MONITORING STUDY

Introduction

Several authors (Hopen and Oebker, 1975; Carolus, 1961; Salman, 1981) have noted substantial increases in yields of vegetable crops when grown on soils covered with a polyethylene mulch. Increased soil temperatures and moisture, reduced crop root damage, and increased weed control are just a few of the factors which may be responsible for these yield increases. It has also been suggested (Baron and Gorske, 1981) that levels of CO_2 under the plastic mulch may be higher than on bare soil. This increased level of CO_2 may also be a contributing factor towards increased yields.

The objective of this study was to measure CO_2 levels at various soil depths, both under a black plastic mulch and on non-mulched soil.

Materials and Methods

Soil CO_2 levels were monitored at The Ohio State University Horticulture Teaching and Research Farm in Columbus, Ohio from June to August, 1981. The soil type was a Brookston silty clay loam with a 2% organic matter content. A preseason treatment of 12-12-12 fertilizer at 1120 kilograms per hectare (kg/ha) was broadcast and incorporated into the soil.

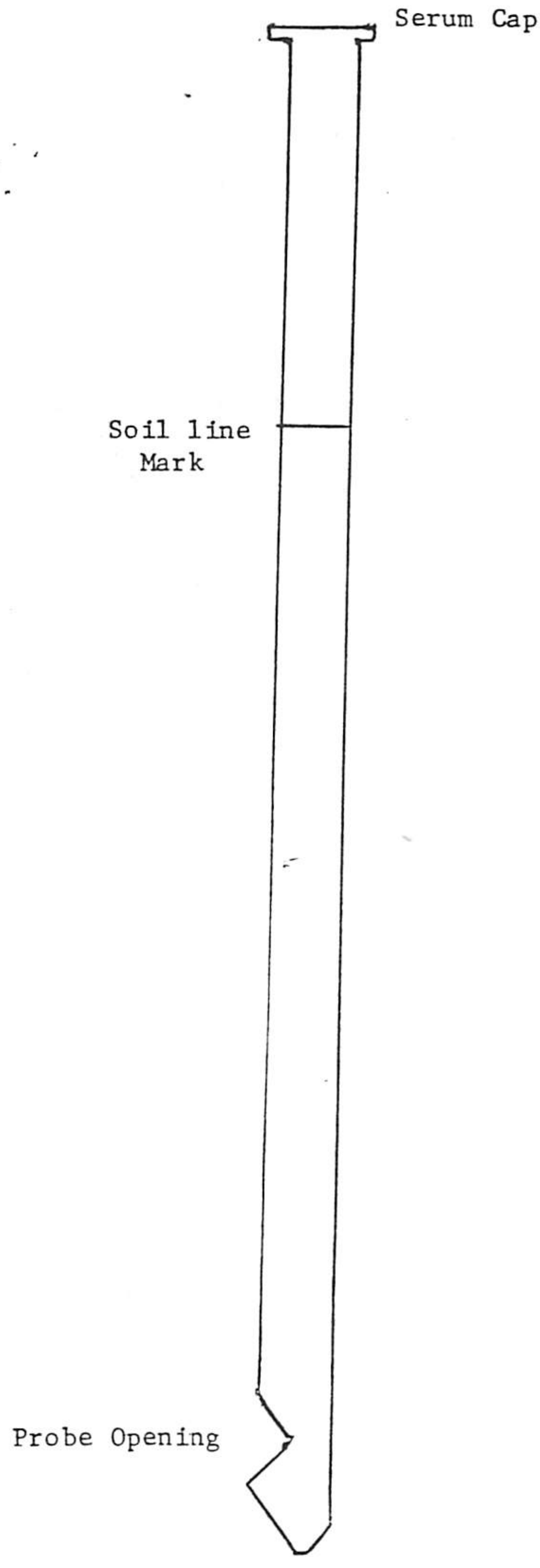
'Harris Classic 465' eggplant (Solanum melongena var. Esculentum L.) seeds were germinated and seedlings were transplanted into 60 cm^3 plastic cells. Plants were grown in the greenhouse for approximately 5 weeks (third leaf was fully expanded).

On May 27, trifluralin (α, α, α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) was broadcast at 1.12 kg/ha and incorporated 5 cm deep in the soil for weed control. Raised planting beds 8 cm high were shaped, and the polyethylene film was laid. Black polyethylene mulch 1.5 meters wide x 1.5 mil thick with a CO_2 permeability rate of 4350 ml per 24 hours per 645.3 cm^2 was used (Edison Plastic, East Rutherford, NJ). Eggplant transplants were planted on 76 cm centers. Planting holes were approximately 10 cm in diameter. A randomized complete block design was used with 4 replicates.

Soil atmospheric samples were obtained by using probes that were placed at 5 and 15 cm depths in the soil. The probes were constructed from 9 mm outside diameter copper tubing. The copper tubing was cut in 10 and 20 cm lengths. Probes were fabricated by crimping one end of the tube. Approximately 1 cm above the crimp a small piece was cut out of the side wall of the tubing. The cut in the tube permitted diffusion and equilibration of gases between the probes and the soil. The above ground end of the probe was fitted with a rubber serum cap that formed an airtight seal (Figure 1).

Soil atmospheric samples, consisting of 3 ml of air, were taken weekly for 10 weeks beginning June 26, 1981. Soil air was drawn into a syringe from each of the soil probes placed 5 and 15 cm below the soil

Figure 1. Gas sampling probe.



surface. Air samples were also collected at the soil surface (under the polyethylene mulch, when present), and at 5 cm above the soil surface (in the plant canopy). Six replications of air samples were taken from all locations.

Quantitative analysis of carbon dioxide was performed by using gas-solid absorption chromatography. A Packard Model 417 Becker Gas Chromatograph was used to analyze gas samples. This chromatograph was equipped with a thermal conductivity detector and a copper separation column, filled with 60-80 mesh silica gel. Helium was used as the carrier gas, with a flow rate of 60 ml per minute. The temperatures of operation were: detector, 190° C; injector port, 120° C, oven, 90° C. The detector current was set at 200 milliamperes.

Gas samples were injected into the gas chromatograph through the injection port. An electrical current surge that corresponds to the concentration of CO_2 was recorded as the length of a peak on a Houston Instruments OmniScribe strip-chart recorder. The recorder was operated at a chart speed of 0.5 cm per minute.

Four replications of a standard reference gas that contained 4.85% CO_2 were injected into the gas chromatograph. The average of the resulting peak heights was multiplied by the attenuation factor (a resistance factor that is used to keep peak heights on the strip-chart recorder) and then introduced into the following equation that was used to calculate the composition of CO_2 in the unknown sample:

$$\frac{\text{Sample (\%)}}{\text{Attenuation Factor} \times \text{Peak Height (Sample)}} = \frac{\text{48500 (\%)}}{\text{Attenuation Factor} \times \text{Peak Height (Standard)}}$$

Data were statistically analyzed using a standard F test at the 0.10 level of significance.

Results and Discussion

Results indicate that when a polyethylene mulch is used, there is an increase in the CO_2 concentration of the soil atmosphere at the 5 and 15 cm depths (Tables 1 and 2). In some instances, these levels are not statistically different; however, a clear trend exists in that higher levels of CO_2 are found under the mulched treatments. One exception to this is the August 28 measurements where the CO_2 levels in unmulched soil were greater than the mulched soil. Carbon dioxide levels at the soil surface under the polyethylene mulch were consistently higher than the unmulched treatment throughout the entire experimental period (Table 3). Due to the extreme amount of variability in the concentration of CO_2 measured in the soil, the differences between the polyethylene mulched treatment and the unmulched treatment at the 15 cm, 5 cm, and at the soil surface locations were not always statistically significant.

Gas exchange between the soil and the atmosphere involves both mass flow and diffusion mechanisms, with diffusion being the main factor involved in gas exchange (Romell, 1922). Polyethylene mulch, acting as a gas barrier, limits the movement of CO_2 from the soil to the atmosphere. The CO_2 diffusion rate through polyethylene mulch is quite slow (4,350 ml/24 hrs/645.3 cm^2). Therefore, any significant movement of CO_2 from the soil to the atmosphere would be through planting holes or tears in the polyethylene.

Increased soil moisture, temperature, and nitrate levels under the polyethylene mulch are at least partially responsible for the increased concentration of soil CO_2. Carbon dioxide levels are undoubtedly increased due to an acceleration of biological activity in the polyethylene mulched soil. Plant roots and soil microorganisms become more active, which results in an increase in respiration and evolution of CO_2.

The CO_2 levels under the polyethylene mulch at the 5 and 15 cm depths decreased as the season progressed until a point where there was a higher concentration in the unmulched soil than in the mulched soil (Figures 2 and 3). In the beginning of the season, there was a substantial difference between the two treatments. On June 26, there was a 463% higher concentration of CO_2 under the polyethylene mulch at the 15 cm depth, and a 914% increase at the 5 cm depth. By week 9, the concentration of CO_2 under the polyethylene mulch diminished and at the 15 cm depth there was only a 3% difference between the treatments. At week 10, the CO_2 level at 15 cm, although not statistically different, was 62% higher in the unmulched soil.

The decreasing CO_2 concentrations as the season progressed is probably due to the soil environment becoming non-conducive to soil microorganism growth. Schales and Sheldrake (1964) reported that soil moisture decreases in the later part of the season under black polyethylene mulch. Moisture reduction, combined with depleted nutrient supplies and the build-up of microbial waste products, may cause a reduction in the population of soil microorganisms to a significantly lower level. The

Figure 2. Soil CO_2 levels as recorded at the 15 cm depth under a polyethylene mulch or bare soil. Readings were taken weekly beginning June 26, 1981.

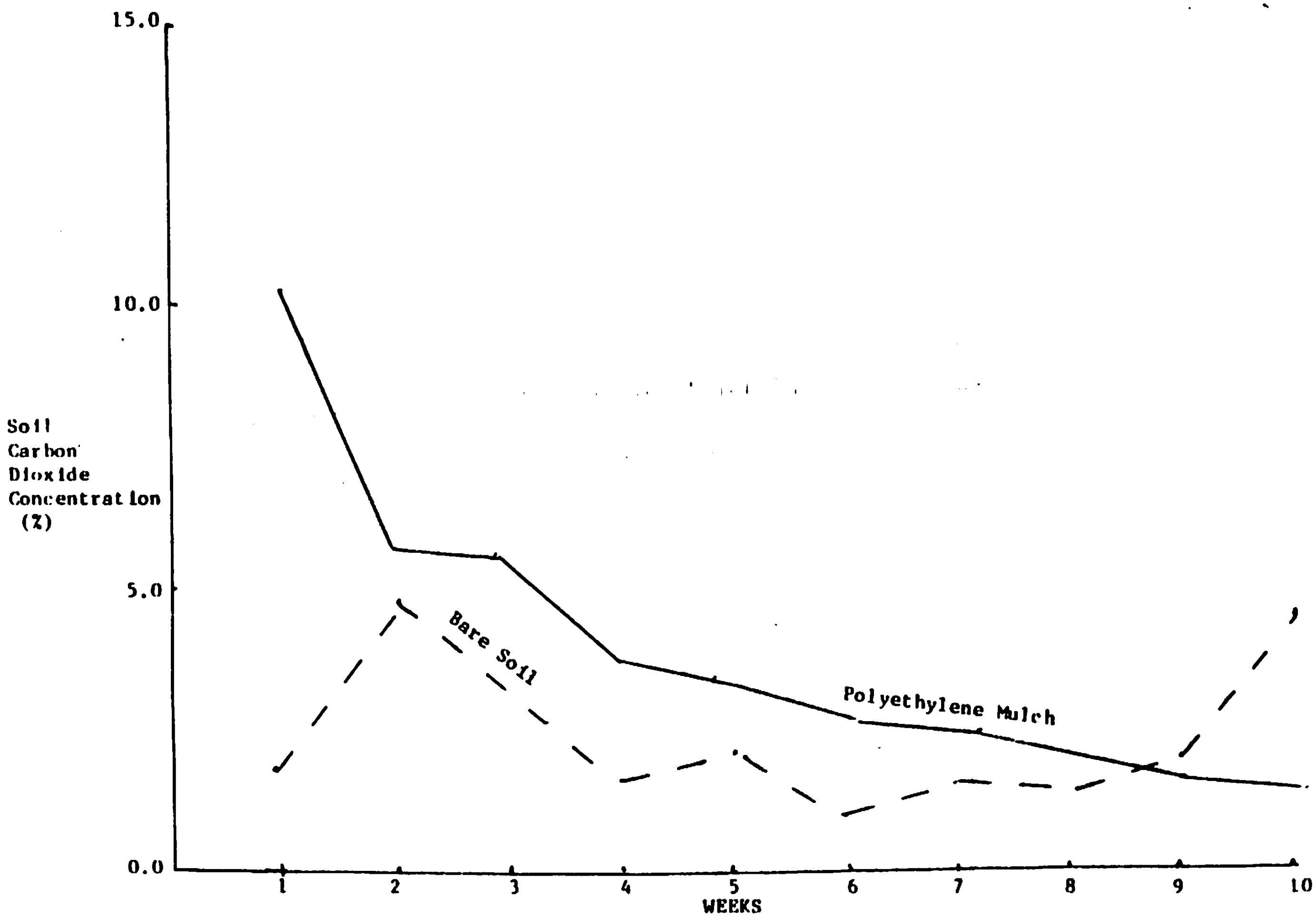
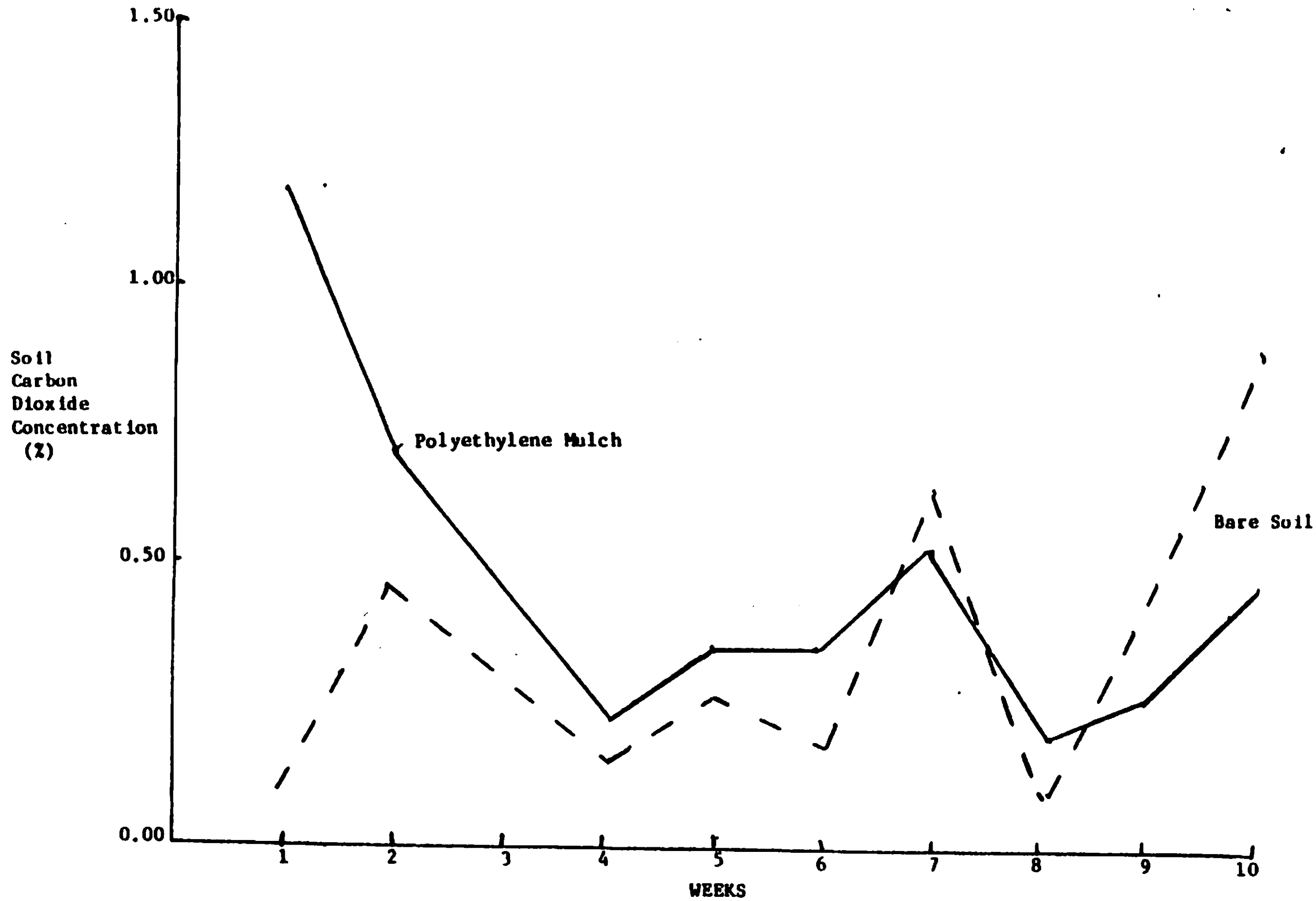


Figure 3. Soil CO_2 levels as recorded at the 5 cm depth under a polyethylene mulch or bare soil. Readings were taken weekly beginning June 26, 1981.



lower population of soil microorganisms would decrease the quantity of CO_2 evolved.

Carbon dioxide concentrations 5 cm above the planting holes in the polyethylene mulch coincided with the concentrations found at the same location in the unmulched treatment (Table 4). Findings in this study concurred with a study by Hopen and Oebker (1975) in which they stated that CO_2 levels a short distance above the plant holes in the polyethylene mulch did not significantly differ from the levels over the unmulched soil.

The concentration of CO_2 in the soil is dependent on the sampling depth. There is approximately a ten-fold increase in soil CO_2 concentration at the 15 cm depth, compared to the 5 cm depth. There is another ten-fold difference in comparing CO_2 levels between the 5 cm depth and the levels at the soil surface. The presence or absence of the polyethylene mulch did not appreciably alter this relationship. However, the polyethylene mulch was responsible for increased levels of CO_2 at the respective depths.

Table 4. CO_2 levels measured at 5 cm above a black polyethylene mulched and unmulched soil.

Date Measured	CO_2 Concentrations (%)		% Difference[a]
	Polyethylene Mulch	Unmulched	
6/26/81	0.0379	0.0370	2
6/29/81	0.0390	0.0392	1
7/6/81	0.0424	0.0426	1
7/13/81	0.0389	0.0376	3
7/24/81	0.0438	0.0447	2
7/31/81	0.0407	0.0387	5
8/7/81	0.0410	0.0400	3
8/14/81	0.0328	0.0328	0
8/21/81	0.0340	0.0351	3
8/28/81	0.0397	0.0363	9

[a]Differences between treatments for a particular sampling date are not statistically significant at the 0.10 level of significance.

CHAPTER II

GROWTH RESPONSE OF EGGPLANT TO A SOIL ENVIRONMENT OF ENRICHED CO_2

Introduction

Several studies have shown that plant roots are capable of absorbing and fixing CO_2 (Poel, 1953; Coker and Schubert, 1981; Arteca, 1982a). As much as 25% of the bean plant's carbon may be fixed by the roots (Kursanov, et al., 1952). An increase in the concentration of CO_2 in the soil atmosphere could have a beneficial influence on plant growth.

The present study was undertaken to determine the effect of enrichment of the soil atmosphere with CO_2 on the growth of eggplant.

Materials and Methods

This experiment was conducted at The Ohio State University Horticultural Weed Control Greenhouse in Columbus, Ohio. Eggplant seeds were germinated and transplanted into 60 cm^3 plastic cell paks. Plants were watered daily or as needed and fertilized every 10 days with a 10-10-10 analysis soluble fertilizer at the rate of 12 grams per 3.75 liters (l) of water.

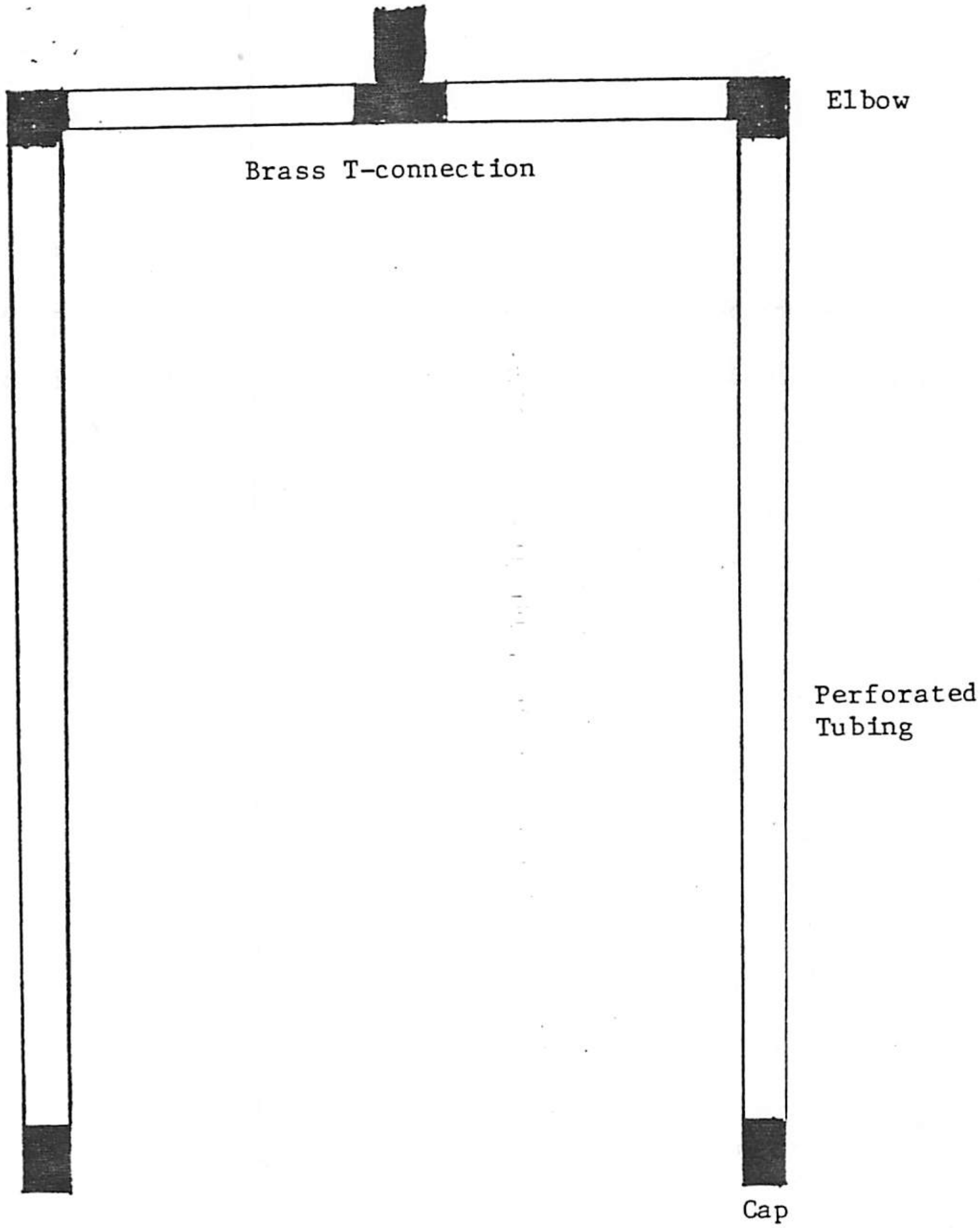
When the sixth true leaf was exposed but less than 2 cm long, the plants were retransplanted into a 16 cm tall x 15 cm diameter polyethylene container. The container was painted black to eliminate sunlight from penetrating to the roots. Drainage holes were cut in the bottom of the container and a 2 cm layer of coarse sand was added. The container was then filled with a 50:50 mixture of sphagnum moss and vermiculite. Pore space of oven dry media was calculated to be 71%.

A gas distribution system was placed in all treatment containers prior to their being filled with media. The gas distribution system consisted of an air line that was connected to a T-joint. Two pieces of 6 mm outside diameter perforated tubing, 14 cm long, were set vertically in the container. The bottom end of the perforated tubes were capped, while the upper ends were attached to a 90° elbow. The perforated tubing was linked together by connecting a short piece of 6 mm tygon tubing to the elbows and to a T-joint. The open end of the T-joint was then fastened to a 6 mm tygon tube which served as the gas supply line (Figure 4).

In addition to the gas distribution system, other utilities were placed in the container. A soil probe was installed to allow gas sampling at the 5 cm depth. An irrigation line, consisting of 2 mm inside diameter spaghetti tube, was added to the container. The spaghetti tube was anchored above the soil level with a 12 cm plastic spike.

Diffusion of gases between the soil atmosphere and the ambient atmosphere was reduced by placing a 1.5 mil black polyethylene sheet on the soil surface. The polyethylene was sealed to the sides of the container with a 4 mil polyethylene tape. All openings in the polyethylene sheet were sealed with this tape.

Figure 4. Design of system used to deliver treatment gases to pots during experiment examining the effect of CO_2 enrichment of the soil atmosphere on eggplant growth.



Treatment gases were injected into a four-chamber manifold. Gas flow was regulated in the manifold by thumbscrew valves. The flow rate entering the gas supply lines was kept at a constant rate of 40 ml gas per minute.

The growth response of eggplant to the different concentrations of soil CO_2 was repeated twice. The first testing period was conducted from March 2 to March 30, 1982. The experiment was then repeated from April 29 to May 21, 1982. Treatments injected consisted of CO_2 concentrations ranging from 0.04 to 14.75% (Table 5). Ambient air was obtained from a Second Nature Whisper Air Pump. Aeration treatment gases were in high pressure cylinders that were purchased from Liquid Carbonic (Chicago, Illinois) or mixed according to procedure by Saltuiet and Dilley (1977).

There were two control treatments in both testing periods. Control 1 had no aeration to the soil with polyethylene cover on the soil surface. Control 2 again received no aeration to the soil and did not have a polyethylene cover.

During the experiment, plants were irrigated every third day, or as needed. Fertilizing was accomplished by injecting a concentrated fertilizer solution into the water line. The concentrated fertilizer solution was prepared by mixing 11.25 kg of 20-20-20 fertilizer, dissolved in 113.6 liters of water. The injector delivered one part concentrated fertilizer into every 100 parts of water. This rate was equivalent to 200 ppm nitrogen.

Table 5. Composition of gases injected and CO_2 levels measured in polyethylene containers during CO_2 enrichment of soil atmosphere.

Aeration Treatment[a]	Input Gases			Measured CO_2 Concentration in pots (%)[b]
	CO_2 (%)	O_2 (%)	N_2 (%)	
15% CO_2	14.75	19.88	61.17	12.99
10% CO_2	10.00	20.43	64.71	8.22
5% CO_2	4.87	20.67	74.40	3.88
1% CO_2	1.03	21.81	77.14	1.07
Ambient Air	.04	21.47	76.19	0.33
Control 1	---	---	---	0.39
Control 2	---	---	---	0.11

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

[b]Average CO_2 levels as monitored in pots during the experimetal period.

At the termination of the experiment, several growth parameters were measured. Stem diameter was measured at the soil surface with a hand micrometer. Plants were cut at the soil surface and the fresh shoot weight recorded. Leaf area was measured using a Li-Cor portable leaf area meter. The root system was washed and excess water was blotted away and fresh root weight recorded. Roots and shoots were oven dried at 60° C for 48 hours and dry weights recorded.

Data were statistically analyzed using a Fisher's LSD test at the 0.05 level of significance.

Results and Discussion

In general, eggplant grown in soil-less media that was aerated with high levels of CO_2 had larger stem diameters (Tables 6 and 7). During the March 2 to March 30 experiment, the 15% CO_2 treatment had a stem diameter that was 30% larger than the polyethylene covered non-aerated control. The next largest stem diameter was obtained with the 5% CO_2 treatment, followed by the 10% CO_2, and then the 1% CO_2 treatment. Stem diameters for the CO_2 enrichment treatments were statistically similar except for the 1% vs. 15% treatments. The 1% CO_2 treatment had a significantly smaller stem diameter than the 15% treatment. All the enrichment treatments were significantly larger than the controls.

Injections of CO_2 into the soil atmosphere resulted in significant stem diameter increases, ranging from 14% to 18% over the non-aerated polyethylene covered control during the April 29 to May 21 experiment. There was no significant difference in stem diameter between the CO_2 soil enrichment treatments (Table 7).

Table 6. Effect of CO_2 enrichment of the soil atmosphere on eggplant growth. Evaluation of stem diameter measured at the soil surface of March 2 to March 30 experiment.

Aeration Treatment[a]	Stem Diameter (mm)
15% CO_2	9.07
10% CO_2	8.21
5% CO_2	8.91
1% CO_2	8.13
Ambient Air	6.76
Control 1	7.00
Control 2	6.00
LSD 5%	0.84

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Table 7. Effect of CO_2 enrichment of the soil atmosphere on eggplant growth. Evaluation of stem diameter measured at the soil surface of April 29 to May 21 experiment.

Aeration Treatment[a]	Stem Diameter (mm)
15% CO_2	10.65
10% CO_2	10.28
5% CO_2	10.72
Ambient Air	9.81
Control 1	9.05
Control 2	7.57
LSD 5%	0.80

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

The significant increase in stem diameter with treatments that contained CO_2 enriched soil suggest that eggplant roots have some mechanism that allows the root system to fix CO_2. It also seems probable that the "fixed CO_2" is translocated out of the roots. The stem seems to be one area where assimilated CO_2 is deposited. This observation agrees with the findings that were reported by Kursanov, et al. (1952), where they noted that bean plants localized assimilated $^{14}CO_2$ in the stem.

It would be advantageous to plant roots to fix CO_2 and deposit the assimilated CO_2 in the stem. The stem, like other tissues that contain chlorophyll, is capable of photosynthesis. Stems are covered with cutin, a wax covering that limits the exchange of gas between the stem cells and the atmosphere (Salisbury and Ross, 1978). It might be possible for roots to fix and translocate the assimilated CO_2 to the stems where light energy that was trapped by the stem chlorophyll drives the further reduction of carbon. Though the stem is known to be a major photosynthate sink, a plant that has roots that fix and translocate CO_2 to the stem can reduce the amount of photosynthate the leaves must translocate to the stem.

Total leaf area was significantly increased with CO_2 enrichment of the soil atmosphere. During the March 2 to March 30 experimental period (Table 8), the leaf area of the 10% CO_2 treatment was significantly larger than the non-aerated polyethylene covered control. There was no significant difference between the other CO_2 enriched soil atmosphere treatments and the non-aerated polyethylene covered control.

Table 8. Effect of CO_2 enrichment of the soil atmosphere on eggplant growth. Evaluation of leaf area measured during March 2 to March 30 experiment.

Aeration Treatment[a]	Leaf Area (cm^2)
15% CO_2	863.2
10% CO_2	1016.0
5% CO_2	967.8
1% CO_2	871.3
Ambient Air	846.3
Control 1	912.5
Control 2	582.5
LSD 5%	69.95

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

The leaf area response due to the concentration of CO_2 in the soil was similar during the April 29 to May 21 experiment (Table 9). All CO_2 soil enrichment treatments had larger, although often not significant, leaf area than the controls. The highest level of CO_2 injection (15% CO_2) influences a significant increase of almost 48% more total leaf area than the non-aerated polyethylene covered control.

During the March 2 to March 30 experiment, a maximum increase in leaf area was found with a 10% CO_2 injection into the soil atmosphere. Carbon dioxide concentrations above or below 10% reduced the total amount of leaf area. In the April 29 to May 21 experiment, the highest CO_2 level did not reduce the leaf area. The differences in growth trends between the two experimental periods can possibly be explained by the differences in the growing conditions. During the first experimental period, the days were short and the sky was often cloudy. The second experimental period had longer days with clear skies. This would infer that better growing conditions (period 2) would influence increased use of soil atmospheric CO_2. During unfavorable environmental conditions, high levels of CO_2 might become toxic.

After drying, shoot and root portions were weighed and total dry weight determined. Results of the March 2 to March 30 experiment show that eggplant total plant dry weight increased with the 10% (significant) and 5% (non-significant) CO_2 treatments when compared to the non-aerated polyethylene covered control (Table 10). There was a slight decrease (non-significant) in total plant dry weight with the 15% CO_2 and the 1% treatments.

Table 9. Effect of CO_2 enrichment of the soil atmosphere on eggplant growth. Evaluation of leaf area during April 29 to May 21 experiment.

Aeration Treatment[a]	Leaf Area (cm^2)
15% CO_2	1108.0
10% CO_2	879.3
5% CO_2	871.0
Ambient Air	1043.0
Control 1	750.5
Control 2	607.8
LSD 5%	195.01

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Table 10. Effect of CO_2 enrichment of the soil atmosphere on eggplant growth. Evaluation of total dry weight measured during March 2 to March 30 experiment.

Aeration Treatment[a]	Total Dry Weight (g)
15% CO_2	7.7
10% CO_2	10.2
5% CO_2	9.1
1% CO_2	8.4
Ambient Air	9.0
Control 1	8.6
Control 2	5.5
LSD 5%	1.24

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Results for the April 29 to May 21 experiment (Table 11) show that the 5% and 15% CO_2 treatments had a significantly larger (16% and 32%, respectively) increase in total plant dry weight over the non-aerated polyethylene covered control. Differences between the 10% CO_2 soil enrichment treatment and the non-aerated polyethylene covered control were not significant. The 15% CO_2 treatment produced significantly heavier plants than the 5% and 10% treatments.

High concentrations of CO_2 in the soil atmosphere influenced an increase in total plant dry weight of eggplant. This increase is probably due to the roots fixing CO_2. Arteca (1979) showed that 45% CO_2, combined with 20% O_2 applied to the root zone of potato, increased dry matter content of the plants. Recently, he was able to increase dry matter accumulation with tomato and corn with 1% CO_2 soil enrichment (Arteca, 1982b). Arteca's results agree with the results that have been found in this study that the concentration of CO_2in the soil environment does influence the growth of plants.

Root dry weight was not as significantly affected as other variables. Only one significant increase in dry weight over the non-aerated polyethylene covered control existed (Tables 12 and 13). This occurred in the April 29 to May 21 experiment where the 15% CO_2 was 43% larger than the control. In all other cases, the dry root weight for the CO_2 enriched soil atmosphere treatments was not significantly larger than the non-aerated polyethylene covered control.

The lack of a significant increase in dry root weight would infer that root absorbed CO_2 does not remain in the root system. Significant

Table 11. Effect of CO_2 enrichment of the soil atmosphere on eggplant growth. Evaluation of total dry weight measured during April 29 to May 21 experiment.

Aeration Treatment[a]	Total Dry Weight (g)
15% CO_2	30.0
10% CO_2	24.3
5% CO_2	26.3
Ambient Air	27.5
Control 1	22.7
Control 2	20.7
LSD 5%	3.32

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Table 12. Effect of CO_2 enrichment of soil atmosphere on eggplant growth. Evaluation of dry root weight measured during March 2 to March 30 experiment.

Aeration Treatment[a]	Dry Root Weight (g)
15% CO_2	1.9
10% CO_2	3.1
5% CO_2	3.2
1% CO_2	2.8
Ambient Air	3.2
Control 1	2.7
Control 2	1.7
LSD 5%	0.50

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Table 13. Effect of CO_2 enrichment of soil atmosphere on eggplant growth. Evaluation of dry root weight measured during April 29 to May 21 experiment.

Aeration Treatment[a]	Dry Root Weight (g)
15% CO_2	10.3
10% CO_2	8.3
5% CO_2	8.9
Ambient Air	9.2
Control 1	7.2
Control 2	6.2
LSD 5%	1.92

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

increases in plant dry weight had to be in the shoot portion of the plant (the stem and leaves). One must question why more CO_2 was not deposited in the roots. Root washing to remove soil is a delicate task that, even with the best of care, will not facilitate complete recovery of all the root system.

Analysis of the growth parameters resulted in the ambient aeration treatment often having growth responses that were statistically equal to the optimum CO_2 treatment concentration (10-15%). This observation was noted during both experimental periods for total dry and root dry weight, and for total leaf area during the April 29 to May 21 experiment. This finding might suggest that the positive growth response noted when CO_2 is injected into the soil environment is not due to the root fixation of CO_2 but due to the increased aeration of the soil. It could also imply that the rate of root fixation of CO_2 is maximum when the soil atmosphere contains 0.33% CO_2 (level monitored with ambient aeration treatment).

A third possible explanation of why the growth with the ambient aeration treatment was similar to the optimum CO_2 soil enrichment treatments is that an inhibitor may be present in the CO_2 enrichment treatments. It could be possible that an inhibitor, such as ethylene, was present in the high pressure cylinders that were used as the source of gas for the CO_2 enrichment treatments. The inhibitor would not be present in an inhibiting concentration in the ambient atmosphere; therefore the air pump would not inject the inhibitor into the ambient atmosphere treatment.

Analysis of fresh root, fresh shoot, total fresh and dry shoot weights for both experimental periods are presented in Tables 18-25 of the Appendix. Measurements of these other growth parameters further substantiate the findings reported here. Carbon dioxide enriched soil atmosphere increases the growth of eggplant. The optimum level of CO_2 in the soil appears to be variable and dependent on the existing environmental conditions. The root absorbed CO_2 is apparently being transported to the stem and leaf areas of the eggplant.

CHAPTER III

$^{14}CO_2$ ASSIMILATION BY EGGPLANT ROOTS

Introduction

Plant roots are capable of assimilating CO_2, but the degree of utilization differs between species (Arteca, 1982b). Grable and Danielson (1965) found that a soil atmosphere of 19% CO_2 influenced an increase in dry weight of corn seedlings. A soil atmosphere of 45% CO_2 influenced a significant increase in potato tuber weight and tuber number (Arteca, 1979).

This study examines the amount of radioactive labeled $^{14}CO_2$ that is assimilated and translocated when intact eggplant roots are exposed to various concentrations of this gas.

Materials and Methods

Eggplant seeds were germinated and when the cotyledons of the seedlings were fully expanded, they were transplanted into a 250 ml Erlenmeyer flask. Plant roots were inserted through a hole in the #6 rubber stopper that was placed in the mouth of the flask. Another hole was placed in the stopper to facilitate the aeration line.

Plants were grown for approximately 5 weeks under hydroponic culture in a growth chamber. The Erlenmeyer flasks were filled with a solution consisting of Hydrosol (W.R. Grace) at 23 g/l. After the second week, calcium nitrate ($CaNO_3$) was added to the nutrient solution at a rate of 15 g/l. The nutrient solution was changed weekly, with water being added as needed between solution changes.

The growth chamber was set on a photoperiod of 16 hours light and 8 hours dark. The temperature during the dark period was 21° C and 25° C during the light period. The relative humidity varied between 60 and 80%. Lighting consisted of fluorescent tubes and incandescent bulbs with a measurement of 800 foot-candles.

When the fifth true leaf was exposed, but less than 2 cm long, the plant was removed from the growth chamber and taken to the laboratory where it was prepared for the root fixation assay. Plants were transferred from the Erlenmeyer flask into a side-arm Erlenmeyer flask. Care was taken to seal all possible areas to prevent leakage of the gas. The holes in the rubber stopper around the stem and the air line were sealed with Mortite caulking cord and the space between the mouth of the flask and the rubber stopper was sealed with DAP all-purpose caulk.

Different concentrations of the radioactive $^{14}CO_2$, with a specific activity of 1.01 micro curri per millimole, were injected from a gas cylinder into a glass manifold (Table 14). Connected to the manifold were 3 glass air lines that went through the hole in the rubber stopper into the flask. The flow rate exiting the air lines was calibrated and kept at a constant rate of 40 ml of gas per minute. Gas was exhausted out of the flask through the side arm. The side arm was connected to a CO_2 trap that contained 250 ml of 0.75 molar (M) sodium hydroxide (NaOH).

Table 14. Effect of concentration of $^{14}CO_2$ on assimilation of carbon by eggplant roots. Concentrations of input gases.

Treatment	Input Gases (PPM)		
	CO_2 (%)	O_2 (%)	N_2 (%)
13% CO_2	129,582	196,757	637,872
4% CO_2	42,966	198,367	653,921
2% CO_2	19,662	221,867	748,093
1% CO_2	11,580	224,417	776,593

Plant roots were exposed to the treatment gases for 30 minutes. At the end of this period, a vacuum was started to pull any remaining $^{14}CO_2$ from the Erlenmeyer flask into the CO_2 traps. The plants were then removed from the flasks and divided into shoot and root portions. Fresh weights were measured and the plants were frozen in liquid nitrogen. After freezing, the tissue was coarsely ground and stored at -10^{o} C.

Tissue was removed from the storage freezer and extraction for radioactivity was performed. Approximately 10 ml of an 80% ethanol:20% water solution were added per gram of fresh weight of tissue. The coarsely ground tissue was homogenized and centrifuged at 5500 g for 20 min. Three ml of the supernatant were collected and 1 ml placed in each of 3 scintillation vials. Five ml of Scintisol (Isolab) scintillation cocktail were added to each vial. The radioactivity of the supernatant and other samples was counted on a Beckman LS 8000 Liquid Scintillation Counter. The remainder of the supernatant was discarded and replaced with 80% ethanol. The pellet was resuspended and the extraction procedure repeated twice.

An additional group of samples was taken after the third replication of the extraction procedure. The pellet was resuspended in 80% ethanol and the volume adjusted to 25 ml. Three, 1 ml samples of the pellet suspension were removed and assayed for radioactivity as described above.

Analysis of radioactivity in the CO_2 traps was determined by removing three, 1 or 2 ml samples and placing the fluid in scintillation vials with 15 ml of Scintisol. The samples were then assayed in the liquid scintillation counter and the counts per minute (CPM) of the sample were determined.

The total amount of radioactivity in the tissue and the traps was calculated by the following formulas:

1. CPM x % Efficiency = Disintegrations Per Minute (DPM).

2. $DPM_1 + DPM_2 + DPM_3 = \overline{X}$ DPM = The mean DPM of replications 1, 2, and 3.

3. $$\frac{\overline{X} \text{ DPM} \times V}{\text{Volume of Sample}} - \text{Background DPM} = \text{Total } DPM_{AY}$$

where V is the total volume of the trap (250 ml), total volume of supernatant, or total volume of pellet suspension.

Total DPM_{AY} is the total amount of DPM found in a trap or the total amount of DPM found in extraction (A) of tissue (Y).

In determining the amount of radioactivity in the tissue, a few additional calculations were needed:

4. $$\frac{\text{Total } DPM_{AY}}{\text{Total Fresh Weight of Tissue (Y)}} = DPM_{AY}$$

DPM_{AY} is the amount of DPM calculated per gram fresh weight of tissue Y for extraction A.

5. $DPM_{AY} + DPM_{BY} + DPM_{CY} + DPM_{PY} = DPM_Y$

DPM_Y is the total DPM of the 3 replications of tissue Y plus the DPM of the pellet suspension solution of tissue Y.

6. DPM_Y x 1.96 x 10^{-5} = milligrams CO_2 recovered per gram fresh weight of tissue Y. 1.96 x 10^{-5} is a constant value that converts DPM_Y recovered to mg CO_2 recovered.

Differences in the amount of DPM and mg CO_2 per gram fresh weight tissue, based on the concentration of $^{14}CO_2$ exposed to the roots, were statistically analyzed using Fisher's LSD test at the 0.05 level of significance.

Results and Discussion

The amount of radioactivity in the shoot and root tissue of the eggplant plant varied with the concentration of $^{14}CO_2$ (Figure 5).

Roots that were exposed to the 13% CO_2 treatment had a significant increase in radioactivity extracted from the root tissue (Table 15). The 1% and 4% treatments had ^{14}C label recovery amounts that were statistically equal, 0.046 and 0.050 mg CO_2, respectively. Roots that were exposed to the 2% treatment resulted in the least amount of recovered radioactivity, 0.034 mg CO_2. The recovery rate for the 2% treatment was significantly lower than the 1% treatment.

This ^{14}C label recovery curve for the shoot tissue shows a rapid rise in the radioactivity concentration between the 1% and 2% treatments (Figure 5, Table 16). There was only a slight, non-significant, increase of 0.003 mg CO_2 recovered between the 2% and 4% treatments. The amount of ^{14}C label that was recovered from the shoot tissue for the 13% CO_2 treatment (0.012 mg CO_2) was significantly lower than the 4% treatment.

Figure 5. $^{14}CO_2$ absorption by eggplant roots. Amount of radioactivity recovered from different tissues when roots were exposed to various concentrations of $^{14}CO_2$.

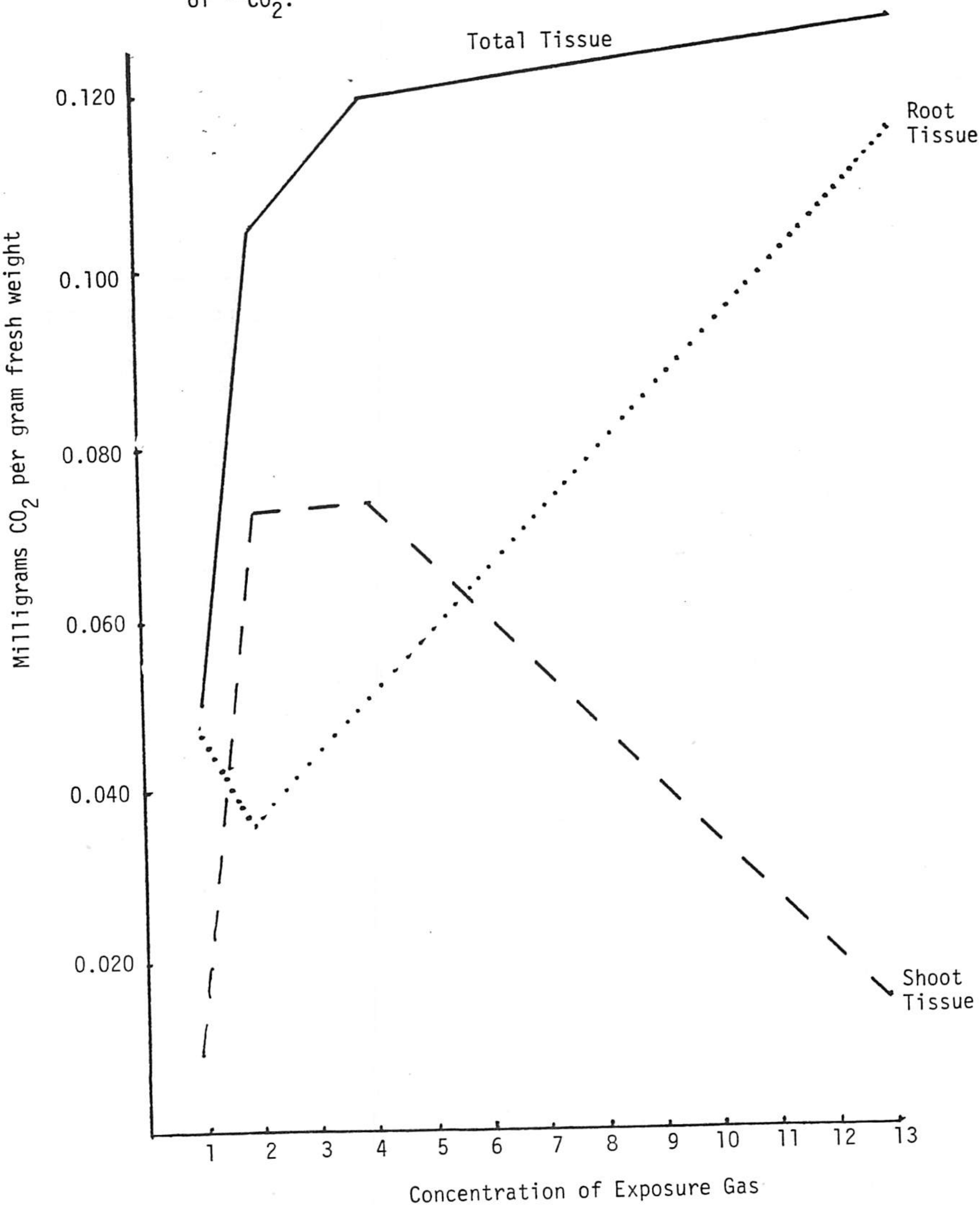


Table 15. $^{14}CO_2$ absorption by eggplant roots. Amount of radioactivity recovered per gram fresh weight of root tissue when exposed to various concentrations of $^{14}CO_2$.

Treatment	Radioactivity Recovered Per Gram Fresh Weight ($mg\ CO_2$)	Radioactivity Recovered Per Gram Fresh Weight (DPM)
13% CO_2	0.114	5,828.3
4% CO_2	0.050	2,529.3
2% CO_2	0.034	1,720.8
1% CO_2	0.046	2,354.0
LSD 5%	0.009	447.5

Table 16. $^{14}CO_2$ absorption by eggplant roots. Amount of radioactivity recovered per gram fresh weight of shoot tissue when exposed to various concentrations of $^{14}CO_2$.

Treatment	Radioactivity Recovered Per Gram Fresh Weight (mg CO_2)	Radioactivity Recovered Per Gram Fresh Weight (DPM)
13% CO_2	0.012	615.4
4% CO_2	0.071	3,626.9
2% CO_2	0.068	3,490.0
1% CO_2	0.004	208.5
LSD 5%	0.010	509.4

This drop in the radioactive material concentration in the shoot tissue is not explainable. In theory, the amount of radioactivity recovered in the shoot tissue should start low and increase to a maximum, where further increases in the $^{14}CO_2$ concentration would not change the concentration of radioactivity recovered. The maximum level is dependent on the rate of loading of assimilated carbon in the vascular system. There is a large amount of labeled carbon fixed at the 13% treatment (2.89 nanocuries per gram fresh weight--NCi/g), but only a small portion (9.6%) of the total radioactivity was found in the shoot. The 4% treatment had a total of 2.77 NCi/g ^{14}C label recovered but 58.9% was found in the shoot tissue. High levels of labeled carbon in the root tissue apparently has some effect on the transport of assimilated carbon out of the root tissue.

Total amount of radioactivity found in the plant (root and shoot tissue combined recovery) for the 1% CO_2 treatment corresponded to the low exposure concentration (Table 17). The amount of $^{14}CO_2$ label recovery significantly increased as the $^{14}CO_2$ treatment rate increased from 1% to 2% to 4%. Increasing the $^{14}CO_2$ concentration rate to 13% resulted in a further increase of recovered ^{14}C label. However, this was not significantly different from the 4% treatment.

Significant differences in radioactivity found in the plant tissue after a 30-minute exposure to various concentrations of $^{14}CO_2$ indicates that plant roots can fix CO_2. Furthermore, the amount fixed is dependent on the concentration in which the roots were exposed. These results agree with Coker and Schuber (1981), where they found that high rates of

Table 17. CO_2 absorption by eggplant roots. Amount of radioactivity recovered per gram fresh weight of tissue when exposed to various concentrations of $^{14}CO_2$.

Treatment	Radioactivity Recovered Per Gram Fresh Weight (mg CO_2)	Radioactivity Recovered Per Gram Fresh Weight (DPM)
13% CO_2	0.126	6,410.4
4% CO_2	0.121	6,156.2
2% CO_2	0.102	5,194.2
1% CO_2	0.050	2,562.5
LSD 5%	0.012	619.3

CO_2 fixation occurred when soybean roots were exposed to high concentrations of $^{14}CO_2$ (2.0-3.5%).

Salisbury and Ross (1978) estimated that temperate zone herbaceous C_3 crop plants have a maximum photosynthetic rate of 7-15 mg CO_2 fixed per gram fresh weight per hour. It was calculated that roots exposed to high concentrations (13%) of $^{14}CO_2$ were able to fix 1.7% to 3.6% of the eggplant's carbon. Roots exposed to 1% $^{14}CO_2$ had root fixation which accounted for 0.7% to 1.4% of the plant's carbon.

The percentage of CO_2 fixed by the roots in this study is small compared to the percentages reported in other studies. Arteca, et al. (1979) found that approximately 18% of the dry matter increase in potato plants that were exposed to 45% CO_2 came from the CO_2 that was assimilated through the roots. Kursanov, et al. (1952) reported that 25% of the bean plant's carbon was obtained by root fixation of CO_2.

A possible reason why the percentage of carbon fixed by eggplant roots was so low in these experiments compared to other reported experiments, was that some environmental growth conditions may have been limiting. The estimate of CO_2 fixation was based on plants having a maximum photosynthetic rate. Low light levels in the experimental area may have reduced the amount of $^{14}CO_2$ that could have been assimilated. Supplemental lighting was added to increase light levels; however, the light levels were far below those required for maximum plant photosynthesis. Limited root moisture may also have decreased the amount of carbon the roots could have fixed. Before exposure to the experimental gases, the roots were wet. At the end of the exposure period, the roots were beginning to dry out.

Root CO_2 fixation by eggplant grown under field conditions, where light levels are usually above the compensation point and moisture levels in the root rhizosphere are often adequate, plays an important role in the carbon economy of the plant.

GENERAL RESULTS AND DISCUSSION

Results of the soil CO_2 monitoring study indicate that, when a soil is covered with a polyethylene mulch, there is an increase in CO_2 concentration in the soil at the 5 cm and 15 cm depths (Tables 1 and 2). The increased levels, though not always statistically significant, were consistent throughout the experimental period, with exception of the last week. Plant roots at the 15 cm depth under the polyethylene mulch were, at times, growing in a soil that contained over 10% CO_2. Roots in the unmulched treatment existed in a soil atmosphere that usually ranged between 2% and 3% CO_2.

Evaluation of the effect of CO_2 on plant growth revealed that high levels of CO_2 in the soil influence eggplant growth. Stem diameter was significantly larger when plant roots were aerated with a gas that contained high levels of CO_2. Total leaf area and total plant dry weight were significantly increased by a soil atmosphere that contained 10% or 15% CO_2. High (15%) levels of CO_2 appeared to have a slightly toxic effect during the short, cloudy days of winter. This toxic effect was not observed when the experiment was repeated during the longer, sunny days of spring. This increased utilization of soil CO_2 when growing conditions are favorable suggest that, under summer field conditions, eggplant roots can survive and flourish in a soil atmosphere high in CO_2.

Radioactive $^{14}CO_2$ was used to determine the extent to which roots absorbed and assimilated CO_2. Increasing the concentration of $^{14}CO_2$ that was exposed to the roots increased the concentration of radioactive carbon that was recovered from the plant. A close relationship between the dose of $^{14}CO_2$ and the amount of radioactivity recovered in the tissue existed (Figure 5). In general, as the concentration of $^{14}CO_2$ increased, so did the recovered radioactivity in the root tissue. Stem tissue did not respond in a similar manner. High levels of labeled carbon were recovered from the intermediate treatments (2% and 4%), while low levels were recovered at the 1% and 13% treatments. The low level found at the 13% treatment in the stem is not explainable.

Soil aerated with ambient air results in plants with positive growth responses that are statistically similar to the growth responses exhibited by plants exposed to optimum CO_2 concentrations (10-15%). There is a significant increase in fixed ^{14}C when eggplant roots are exposed to high concentrations of $^{14}CO_2$ (13%) versus low levels (1-2%). This positive dose-response relationship would imply that the root system was not saturated by CO_2 at the 0.33% concentration and soil aeration is not totally responsible for the noted growth increases.

Carbon assimilation by eggplant roots in the laboratory comprises only a small percentage of the plant's carbon requirement. The low percentage of carbon assimilation by the roots may be due to low light and root moisture levels under the experimental conditions. Under field conditions, root fixation of CO_2 may play an important role in the carbon economy of eggplant. Any horticultural practice that will increase the CO_2 concentration in the soil would therefore have a positive effect on plant growth.

CONCLUSION

In conclusion, when soil CO_2 concentrations were monitored, higher levels of CO_2 were found under the polyethylene mulch at the soil surface, and at the 5 cm and 15 cm depths. CO_2 levels found under field conditions were artificially injected into soil-less media to determine their effect on eggplant growth.

Eggplant exhibited a positive growth response to enriched levels of CO_2 injections into the soil atmosphere. Stem diameter, leaf area, and total dry weight were all significantly increased by increasing levels of CO_2. Root dry weight was not increased, suggesting that roots fix and translocate assimilates out of the root system.

The amount of CO_2 uptake by the root system was determined by radioactive tracers. Roots exposed to high concentrations of $^{14}CO_2$ had correspondingly high levels of radioactivity recovered in its tissue. Root tissue radioactive carbon recovery amounts corresponded to treatment concentration, while high $^{14}CO_2$ treatments resulted in low ^{14}C recovery in the shoot tissue.

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APPENDIX

Table 18. CO_2 enrichment of the soil atmosphere effect on eggplant. Evaluation of fresh shoot weight measured during March 2 to March 30 experiment.

Aeration Treatment[a]	Fresh Shoot Weight (g)
15% CO_2	41.6
10% CO_2	46.0
5% CO_2	43.1
1% CO_2	38.8
Ambient Air	39.7
Control 1	41.1
Control 2	26.8
LSD 5%	4.18

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Table 19. CO_2 enrichment of the soil atmosphere effect on eggplant. Evaluation of fresh shoot weight measured during April 29 to May 21 experiment.

Aeration Treatment[a]	Fresh Shoot Weight (g)
20% CO_2	71.0
10% CO_2	56.6
5% CO_2	66.2
Ambient Air	70.6
Control 1	52.4
Control 2	43.3
LSD 5%	10.93

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Table 20. CO_2 enrichment of the soil atmosphere effect on eggplant. Evaluation of fresh root weight measured during March 2 to March 30 experiment.

Aeration Treatment[a]	Fresh Root Weight (g)
15% CO_2	29.6
10% CO_2	47.2
5% CO_2	46.5
1% CO_2	46.2
Ambient Air	43.7
Control 1	47.4
Control 2	30.7
LSD 5%	7.52

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Table 21. CO_2 enrichment of the soil atmosphere effect on eggplant. Evaluation of fresh root weight measured during April 29 to May 21 experiment.

Aeration Treatment[a]	Fresh Root Weight (g)
20% CO_2	80.2
10% CO_2	55.8
5% CO_2	70.7
Ambient Air	72.7
Control 1	54.7
Control 2	46.5
LSD 5%	12.93

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Table 22. CO_2 enrichment of the soil atmosphere effect on eggplant. Evaluation of total fresh weight measured during March 2 to March 30 experiment.

Aeration Treatment[a]	Total Fresh Weight (g)
15% CO_2	71.2
10% CO_2	93.0
5% CO_2	89.6
1% CO_2	84.9
Ambient Air	83.4
Control 1	88.5
Control 2	58.0
LSD 5%	9.67

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Table 23. CO_2 enrichment of the soil atmosphere effect on eggplant. Evaluation of total fresh weight measured during April 29 to May 21 experiment.

Aeration Treatment[a]	Total Fresh Weight (g)
20% CO_2	151.2
10% CO_2	112.6
5% CO_2	134.4
Ambient Air	143.3
Control 1	107.2
Control 2	89.9
LSD 5%	24.21

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Table 24. CO_2 enrichment of the soil atmosphere effect on eggplant. Evaluation of dry shoot weight measured during March 2 to March 30 experiment.

Aeration Treatment[a]	Dry Shoot Weight (g)
15% CO_2	5.9
10% CO_2	7.1
5% CO_2	5.8
1% CO_2	5.6
Ambient Air	5.7
Control 1	6.0
Control 2	3.8
LSD 5%	0.99

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Table 25. CO_2 enrichment of the soil atmosphere effect on eggplant. Evaluation of dry shoot weight measured during April 29 to May 21 experiment.

Aeration Treatment[a]	Dry Shoot Weight (g)
20% CO_2	19.7
10% CO_2	16.0
5% CO_2	17.4
Ambient Air	18.4
Control 1	15.5
Control 2	14.5
LSD 5%	1.74

[a]Control 1 = no aeration, polyethylene covered pot.
Control 2 = no aeration or polyethylene cover.

Table 26. $^{14}CO_2$ absorption by eggplant roots. Number of counts per minute found in root tissue supernatant and pellet when exposed to 13% CO_2.

Extraction	Number of Counts Per Minute (CPM)			
	A	B	C	Background
1	197.3	288.2	191.1	14.8
	195.4	281.7	185.4	13.2
	190.1	264.3	221.1	15.8
2	48.6	42.5	32.7	16.6
	49.7	43.3	32.1	15.6
	39.8	42.4	26.6	13.3
3	14.3	17.7	19.3	16.7
	15.3	20.4	19.4	17.0
	15.0	18.0	17.2	14.9
Pellet	30.0	46.2	27.1	10.0
	31.0	40.1	31.5	11.6
	32.5	42.9	39.6	12.0

Table 27. $^{14}CO_2$ absorption by eggplant roots. Number of counts per minute found in shoot tissue supernatant and pellet when exposed to 13% CO_2.

Extraction	Number of Counts Per Minute (CPM) A	B	C	Background
1	71.0	31.4	29.7	16.2
	65.4	30.0	25.2	15.9
	70.4	28.5	27.3	19.5
2	22.0	16.2	16.6	15.9
	22.3	15.5	17.9	15.2
	22.4	16.1	15.4	15.0
3	13.7	17.1	16.2	15.5
	17.4	14.3	15.0	14.8
	15.6	15.3	17.4	16.6
Pellet	20.0	17.4	17.2	16.7
	20.7	16.3	16.1	15.8
	19.4	15.3	15.5	19.5

Table 28. $^{14}CO_2$ absorption by eggplant roots. Number of counts per minute found in root tissue supernatant and pellet when exposed to 4% CO_2.

Extraction	Number of Counts Per Minute (CPM)			
	A	B	C	Background
1	100.6	110.1	145.4	16.8
	92.7	109.4	136.5	14.2
	91.7	103.4	138.7	16.4
2	26.4	29.8	32.2	15.9
	24.6	28.1	31.4	15.4
	24.7	29.4	32.2	13.3
3	16.3	20.0	16.3	17.4
	16.0	17.3	17.4	13.8
	16.4	15.5	18.6	14.4
Pellet	21.4	21.2	26.6	14.0
	20.0	25.0	24.3	14.0
	23.3	25.2	24.6	16.3

Table 29. $^{14}CO_2$ absorption by eggplant roots. Number of counts per minute found in shoot tissue supernatant and pellet when exposed to 4% CO_2.

Extraction	Number of Counts Per Minute (CPM) A	B	C	Background
1	214.4	263.8	52.0	17.6
	203.8	254.4	61.2	16.0
	216.4	268.1	62.9	17.4
2	57.0	70.2	27.3	15.8
	55.9	77.7	29.9	14.5
	57.6	72.1	29.1	13.4
3	28.8	31.9	17.9	14.4
	25.9	28.9	16.7	13.6
	26.4	29.2	18.2	14.8
Pellet	80.9	102.2	28.2	15.5
	80.4	91.2	28.2	16.1
	69.6	105.0	----	16.3

Table 30. $^{14}CO_2$ absorption by eggplant roots. Number of counts per minute found in root tissue supernatant and pellet when exposed to 2% CO_2.

Extraction	Number of Counts Per Minute (CPM)			
	A	B	C	Background
1	49.2	61.3	58.1	16.5
	47.8	61.4	54.0	18.1
	50.9	58.8	54.6	15.9
2	18.7	21.2	18.3	11.8
	17.7	21.6	17.1	13.1
	16.9	20.0	18.7	15.0
3	16.0	15.7	13.7	13.2
	14.5	14.9	13.2	17.0
	14.5	12.7	15.7	16.7
Pellet	18.3	15.8	20.2	12.3
	16.2	14.8	18.1	12.6
	19.0	18.7	17.9	14.4

Table 31. $^{14}CO_2$ absorption by eggplant roots. Number of counts per minute found in shoot tissue supernatant and pellet when exposed to 2% CO_2.

Extraction	Number of Counts Per Minute (CPM) A	B	C	Background
1	115.4	124.3	127.9	13.8
	105.4	124.5	128.9	16.5
	113.0	126.9	129.5	15.4
2	27.9	37.8	20.1	15.3
	27.5	33.9	16.9	17.2
	----	32.9	19.6	18.3
3	14.8	19.0	15.4	13.5
	17.4	20.4	15.6	14.5
	19.6	14.5	12.3	14.9
Pellet	40.9	45.8	37.9	14.2
	39.3	45.6	38.4	15.9
	36.7	49.6	33.0	14.0

Table 32. $^{14}CO_2$ absorption by eggplant roots. Number of counts per minute found in root tissue supernatant and pellet when exposed to 1% CO_2.

Extraction	Number of Counts Per Minute (CPM)			
	A	B	C	Background
1	82.2	165.6	147.8	16.9
	87.9	137.9	150.9	19.7
	84.0	146.8	153.8	18.6
2	23.8	47.9	35.9	17.4
	23.5	48.5	37.6	18.8
	48.9	43.8	38.0	17.6
3	16.9	22.4	21.7	13.0
	17.2	24.3	19.9	14.1
	16.7	26.5	21.8	17.7
Pellet	19.7	30.3	23.5	15.1
	21.0	30.6	24.7	14.7
	18.5	31.6	21.4	17.0

Table 33. $^{14}CO_2$ absorption by eggplant roots. Number of counts per minute found in shoot tissue supernatant and pellet when exposed to 1% CO_2.

Extraction	Number of Counts Per Minute (CPM)			
	A	B	C	Background
1	16.0	34.6	27.6	16.5
	17.5	36.2	28.8	17.9
	16.3	34.9	29.1	16.1
2	15.7	17.6	15.5	16.2
	13.9	18.6	16.5	18.6
	16.4	17.4	15.9	17.7
3	13.3	14.3	13.6	14.1
	14.2	14.2	13.5	14.4
	15.7	16.2	12.6	15.6
Pellet	14.7	21.0	14.6	15.5
	12.8	19.1	16.1	16.7
	12.5	20.2	17.0	16.1