THE EFFECTS OF OZONE ON PRIMARY DETERMINANTS OF PLANT PRODUCTIVITY

Final Report

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ABSTRACT

The Statewide Air Pollution Research Center has a continuing mission to investigate the effects of air pollutants on vegetation, and to determine the amount of losses being caused by these pollutants. The Department of Botany and Plant Sciences has a continuing mission to investigate basic and applied aspects of plant science research. To further this mission we jointly conducted the pilot project "The Effects of Ozone on Primary Determinants of Plant Productivity." The study evaluated the relationships among ozone exposure, gas exchange, chlorophyll fluorescence, and whole plant productivity responses in depth for a baseline species: spinach (Spinacia oleracea); and for four species differing in ozone sensitivity: lettuce (Lactuca sativa), corn (Zea mays), squash (Cucumis pepo), and radish (Raphanus sativus).

The study was conducted in a greenhouse using plants grown under conditions which allow for harvesting of both shoots and roots: i.e. a hydroponic system for spinach and a loose artificial media for the four other species. Spinach was exposed twice weekly for four weeks. Two patterns of exposure were used: a square wave (0.15 ppm continuously for four hours), or a triangle (0 to peak of 0.30 to 0 ppm over four hours for mean of 0.15 ppm). The four other species were exposed only to a triangle pattern with a peak of 0.24 ppm and a mean of 0.12 ppm ozone. The spinach experiment was repeated with four groups of plants, and the four species experiment was repeated with two groups. Gas exchange was measured in terms of net photosynthesis, stomatal conductance, and transpiration using a portable photosynthesis system with associated computer. Productivity was measured in terms of fresh and dry weights for shoots and roots. Gas exchange was compared to productivity in terms of a relative growth rate calculation (RGR, a measure of total dry weight gain over time as a function of initial weight).

Both plant gas exchange and productivity were affected by the relatively mild ozone stress used in this study. Spinach showed statistically significant decreases in both stomatal conductance and transpiration due to ozone for nearly all groups of plants, with the triangle pattern producing greater effects than the square wave pattern of exposure. Net photosynthesis was not affected by ozone to the same extent as the other gas exchange parameters, with a statistically significant decrease found only for one group of plants. Spinach productivity was reduced by ozone, primarily when measured as leaf or

shoot fresh weight. Spinach dry weights were reduced by ozone only for one of the four plant groups.

The four species varied in their responses to ozone. Corn and radish showed decreases in stomatal conductance, radish showed a decrease in photosynthesis, and lettuce actually showed an increase in photosynthesis in response to ozone exposure. However, these responses did not necessarily occur for both groups of plants. Transpiration was not affected by ozone for any species. Radish was the only species showing consistent ozone effects on productivity, with decreases found in both shoot, root and total fresh or dry weights.

Gas exchange was not consistently correlated with productivity (as indicated by RGR). Photosynthesis was correlated with RGR only for control plants. This occurred for two of the four groups of spinach plants and for one group each of radish and squash plants. Stomatal conductance was not correlated with RGR for any group of spinach plants, and only for the second group of squash plants (for both for control ozone exposed and second group of lettuce plants [ozone exposed]). The poor correlations between gas exchange and productivity were likely associated with the high degree of variability in plant responses, especially with ozone exposure.

The leaf sampling method for chlorophyll fluorescence determinations yielded stable, reproducible signals from control plants. However, alterations in fluorescence induced by low levels of ozone exposure were not as easily observed. The subtle ozone effects included a 10-20% lowering of the peak fluorescence and a slight distortion of secondary peaks. No changes could be seen in the initial (F_o) level of fluorescence (the so-called "dead" or baseline fluorescence unaffected by photochemistry).

This study clearly indicated that even though changes in gas exchange and plant productivity occur with ozone exposure, that the relationships between them are difficult to detect. The correlations are highly dependent on species, gas exchange parameter, and group of plants. Recommendations for further research include: more intensive gas exchange and RGR measurements to determine correlations, measurement of gas exchange and productivity in the field, conduct additional chlorophyll fluorescence research in the laboratory, and use triangle exposure patterns in future research.

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DISCLAIMER

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SUMMARY AND CONCLUSIONS

Photochemical oxidants, of which ozone is the main constituent, have resulted in severe effects on agriculture as well as native vegetation in California. Traditionally, these plant effects from ozone have been studied with (A) large field studies to evaluate ozone dose- plant yield responses, (B) greenhouse studies to evaluate ozone dose-plant growth responses, or (C) laboratory studies to evaluate the physiological bases of ozone injury at the subcellular, cellular, or single plant level under highly controlled conditions. All three types of research have yielded important information. However there has been little research regarding mechanisms by which results from yield, growth, and physiology studies can be integrated.

Field studies have required large and expensive facilities to control and monitor the air pollutant treatments and to produce a marketable yield. Yield studies have been conducted for major California crops, e.g., wheat, lettuce, rice, tomatoes, cotton, grapes, alfalfa, and navel oranges. Field studies have been limited by available resources for determination of ozone effects on many crops, especially those which may be of great local, but not statewide importance. Field measurements of physiological parameters have been limited, largely because of the expense and difficulty in obtaining repeated measurements.

Greenhouse and large scale controlled environment studies have allowed for exposure of large numbers of replicate plants for air pollutant-growth studies. Under semi-controlled greenhouse conditions plants have been grown in hydroponic solutions or loose media which allowed for harvesting of roots. These whole-plant harvests provided for a more complete picture of ozone effects on whole plant productivity than was possible in field studies where roots could not normally be measured. Thus greenhouse studies were used to indicate the effects of ozone on total plant productivity and partitioning of dry matter to different parts of plants in different species. However, greenhouse studies have not easily allowed for a normal pattern of ambient pollutant exposure and cannot provide for exposures which are of long enough duration so that yield can be determined for many crops. Thus to be an indicator of potential productivity effects in the field, greenhouse experiments incorporated

measurements of physiological parameters such as net photosynthesis and stomatal conductance to a limited extent. The most important of these studies was the recent work by Dr. Peter Reich and co-workers of the Boyce Thompson Institute, who found that low concentrations of ozone reduce net photosynthesis and affect water relations in several species.

Laboratory studies have been successful in determining the effects of ozone on plant physiological processes at subcellular, cellular, and individual whole plant levels. In general cellular molecules, membranes, and organelles were affected by ozone in various experimental systems. Net photosynthesis and other physiological processes especially were affected by ozone in studies using laboratory cuvette systems. However, most of these laboratory studies were designed to investigate basic mechanisms of action, and, thus, the ozone exposures were not representative of concentrations or environmental conditions found in the field. Furthermore, the laboratory studies were not linked to growth studies to determine if the physiological responses were directly coordinated with whole plant productivity.

Current research techniques have presented the opportunity to begin to integrate the results from field, greenhouse, and laboratory studies. Portable field infrared gas analysis systems with associated computers have enabled routine measurement of gas exchange without elaborate laboratory or greenhouse controls. Recent research has lead to a refinement of a computer controlled system for simultaneous determination of chlorophyll fluorescence, photosynthetic rates, and transpiration using leaf discs. These discs could be obtained from plants given an environmental stress such as ozone in order to determine the components of the carbon dioxide fixation process affected by the stress.

While the photosynthesis and fluorescence measuring systems hold great potential for detecting plant stress, they have not been evaluated in a comprehensive manner to determine their usefulness in determining the relationship between short-term physiological changes and long-term growth and yield effects of ozone. Thus a specific need has existed for an intensive pilot project to develop and evaluate the techniques by which these physiological measurements can contribute to procedures for evaluating losses to crops from air pollutants in California, and the metabolic basis for these losses.

Thus, a one-year pilot project was initiated with primary objectives to:

1. Document the relationships among ozone exposure, leaf gas exchange, and chlorophyll fluorescence under controlled greenhouse and laboratory conditions.

2. Determine the relationships between ozone-induced changes in whole plant physiology and subsequent productivity.

3. Determine interspecific variation in ozone-induced changes in the physiology/productivity relationships.

The study was conducted in a greenhouse using plants grown under conditions which allow for harvesting of both shoots and roots: i.e. a hydroponic system for spinach and a loose artificial media for four other species (corn, squash, lettuce and radish). Spinach was exposed to ozone twice weekly for four weeks. Two patterns of exposure were used: to a square wave (0.15 ppm ozone for four hours), or a triangle (0 to a peak of 0.30 than back to 0 ppm over four hours for mean of 0.15 ppm). The four other species were exposed only to a triangle pattern with a peak of 0.24 ppm and a mean of 0.12 ppm ozone. The spinach experiment was repeated with four groups of plants, and the four species experiment was repeated with two groups. Gas exchange was measured in terms of net photosynthesis, stomatal conductance, and transpiration using a portable photosynthesis system with associated computer. Fluorescence measurements were made on an intensive basis for spinach, and a limited basis for corn and radish. Productivity was measured in terms of fresh and dry weights for shoots and roots. Gas exchange was compared to productivity in terms of a relative growth rate (RGR) calculation (a measure of weight gain over time as a function of original weight).

Both plant gas exchange and productivity were affected by the relatively mild ozone stress used in this study. Spinach showed statistically significant decreases in both stomatal conductance and transpiration due to ozone for nearly all groups of plants, with the triangle pattern producing greater effects than the square wave pattern of exposure. Net photosynthesis was not affected by ozone to the same extent as the other gas exchange parameters, with a statistically significant decrease found only for one group of plants. Spinach productivity was reduced by ozone, primarily when measured as leaf or shoot fresh weight.

Spinach dry weights were reduced by ozone only for one of the four plant groups.

The four species varied in their responses to ozone. Corn and radish showed decreases in stomatal conductance, radish showed a decrease in photosynthesis, and lettuce actually showed an increase in photosynthesis in response to ozone exposure. However, these responses did not necessarily occur for both groups of plants. Transpiration was not affected by ozone for any species. Radish was the only species showing consistent ozone effects on productivity, with decreases found in both shoot, root and total fresh or dry weights.

Gas exchange was not consistently correlated with productivity (as indicated by RGR). Photosynthesis was correlated with RGR only for control plants. This occurred for two of the four groups of spinach plants and for one group each of radish and squash plants. Even for these groups the correlation was inconsistent, with a positive correlation between photosynthesis and RGR (both variables decreasing together) for one group of spinach and squash, and a negative correlation (one variable increasing and the other decreasing) with the other group of spinach and one group of radish. Stomatal conductance was not correlated with RGR for any group of spinach plants, and only for the second group of squash plants (for both control and ozone exposed) and second group of lettuce plants (ozone exposed). The poor correlations between gas exchange and productivity are likely associated with the high degree of variability in plant responses, especially with ozone exposure.

Conclusions

1. Both plant gas exchange and productivity are affected by a relatively mild ozone stress as used in this study, with the triangle pattern producing greater effects than the square wave pattern of exposure.

2. This study clearly indicated that even though changes in gas exchange and plant productivity occur with ozone exposure, that the relationships between them are difficult to detect. The correlations are highly dependent on species, gas exchange parameter, and group of plants.

3. The most consistent plant response to ozone was a decrease in stomatal conductance which was not associated with decreases in photosynthetic rate or growth. Thus, stomatal conductance was the most sensitive indicator of plant effects in response to ozone of all other parameters measured.

4. Whole plant productivity was correlated with photosynthetic rate for control plants for two groups of spinach and one each for squash and radish. The high degree of variability in both productivity and photosynthetic rates between plants was a primary factor in this lack of correlation.

5. There were considerable differences among species in their sensitivity to ozone, with radish being the most sensitive. For radish, ozone affected both growth and gas exchange.

6. Chlorophyll flourescence changes were not easily observed when there is only slight injury to leaves. However, with computer enhancement techniques the slight, but reproducible changes in kinetics of fluorescence were found.

RECOMMENDATIONS

1. Conduct an intensive experiment to determine the relationships among photosynthetic rate, relative growth rate (RGR), and crop commercial productivity using more repeated measurements of photosynthesis and RGR.

2. Conduct the intensive study in the field to relate the patterns of response seen in the greenhouse to ambient environmental conditions. Include an analysis of number of required measurements to determine correlations.

3. Increase the ability to detect small and subtle, but reproducible changes in chlorophyll fluorescence kinetics for plants without visible leaf injury but with ozone-induced yield reductions.

4. Show that gross chlorosis of the leaves can be as accurately and reliably quantified by chlorophyll fluorescence, as by more labor intensive chlorophyll extraction and analysis, or more qualitative visual assessments.

5. Once they are definitely documented in the greenhouse, determine whether the chlorophyll fluorescence changes with ozone also occur in the field.

6. Conduct future studies with triangle-peak patterns of exposure as they more nearly replicate ambient conditions, and because plants are more sensitive to peak than square-wave patterns of exposure.

7. Measure photosynthesis and transpiration responses versus productivity for whole plants and not just single leaves. Gas exchange measurements on a whole plant basis should be more highly correlated with productivity than measurements on a single leaf basis. This is because whole plant measurements take into account variability in response due to number of leaves, size of leaves, and differences in leaf metabolic rates while single leaf measurements do not consider these parameters.

I. INTRODUCTION

Photochemical oxidants, of which ozone is the main constituent, have resulted in severe effects on agriculture (Howitt et al. 1984), as well native vegetation in California. Traditionally, these plant effects from ozone have been studied with (A) large field studies to evaluate ozone dose-plant yield responses, (B) greenhouse studies to evaluate ozone dose-plant growth responses, or (C) laboratory studies to evaluate the physiological bases of ozone injury at the subcellular, cellular, or single plant level under highly controlled conditions. All three types of research have yielded important information. However there has been little research regarding mechanisms by which results from yield, growth, and physiology studies can be integrated.

Field studies have required large and expensive facilities to control and monitor the air pollutant treatments and to produce a marketable yield. Yield studies have been conducted for major California crops, e.g., wheat, lettuce, rice, tomatoes, cotton, grapes, alfalfa, and navel oranges (Thompson and Taylor 1969, Thompson and Kats 1976, Heck et al. 1984, Kats et al. 1985, McCool et al. 1986, Olszyk et al. 1986). However, these studies generally used only one or two cultivars and have been conducted in only two California environments. Field studies have been limited by available resources for determination of ozone effects on all crops, especially those which may be of great local, but not statewide importance. Field measurements of physiological parameters have been limited, largely because of the expense and difficulty in obtaining repeated measurements.

Greenhouse and large scale controlled environment studies have allowed for exposure of large numbers of replicate plants for air pollutant-growth studies. Under semi-controlled greenhouse conditions plants could be grown in hydroponic solutions or loose media which allowed for harvesting of roots. These whole-plant harvests provided a more complete picture of ozone effects on whole plant productivity than is possible in field studies where roots can not normally be measured. Thus greenhouse studies have been used to indicate the effects of ozone on total plant productivity and partitioning of dry matter to different parts of plants in different species (Bennett and Oshima 1976, Oshima et al. 1978, 1979, Tingey et al. 1971).

However, greenhouse studies have not easily allowed for a normal pattern of ambient pollutant exposure and cannot provide for exposures which are of long enough duration so that yield can be determined for many crops. Thus to be an indicator of potential productivity effects in the field, greenhouse experiments have incorporated measurements of physiological parameters such as net photosynthesis and stomatal conductance to a limited extent. The most important of these studies was the recent work by Dr. Peter Reich and co-workers, who found that low concentrations of ozone reduce net photosynthesis and affect water relations in several species (Reich et al. 1983, 1984, 1985a,b, Amundson et al. 1985).

Laboratory studies have been successful in determining the effects of ozone on plant physiological processes at subcellular, cellular, and individual whole plant levels. In general cellular molecules, membranes, and organelles were affected by ozone in various experimental systems (Heath 1980). Net photosynthesis and other physiological processes especially were affected by ozone in studies using laboratory cuvette systems (Heath et al. 1982, Hill et al. 1969, Carlson 1979, Ormrod 1981). However, most of these laboratory studies were designed to investigate basic mechanisms of action, and, thus, the ozone exposures were not representative of concentrations or environmental conditions found in the field. Furthermore, the laboratory studies were not linked to growth studies to determine if the physiological responses were directly correlated with whole plant productivity.

Current research techniques have presented the opportunity to begin to integrate the results from field, greenhouse, and laboratory studies. Portable field infrared gas analysis systems with associated computers have enabled routine measurement of gas exchange without elaborate laboratory or greenhouse controls. Research by Heath et al. (1985) and Harris et al. (1983a,b) has lead to the refinement of a computer controlled system for simultaneous determination of chlorophyll fluorescence, photosynthetic rates, and transpiration using leaf discs. These discs can be obtained from plants given an environmental stress such as ozone in order to determine the components of the carbon dioxide fixation process affected by the stress.

While the photosynthesis and fluorescence measuring systems have held great potential for detecting plant stress, they have not been evaluated in a comprehensive manner to determine their usefulness in determining the relationship between short-term physiological changes and long-term growth and yield effects of ozone. Thus a specific need has existed for an intensive pilot project to develop and evaluate the techniques by which these physiological measurements can contribute to procedures for evaluating losses to crops from air pollutants in California, and the metabolic basis for these losses.

Thus, a one-year pilot project was initiated with primary objectives to:

1. Document the relationships among ozone exposure, leaf gas exchange, and chlorophyll fluorescence under controlled greenhouse and laboratory conditions.

2. Determine the relationships between ozone-induced changes in whole plant physiology and subsequent productivity.

3. Determine interspecific variation in ozone-induced changes in the physiology/productivity relationships.

The specific experiments to be carried out were month-long studies involving multiple ozone exposures and sequential whole-plant harvests. Spinach (Spinacia oleracea) plants were used on an intensive basis to determine: the ozone exposure pattern to be used, physiological measurement sampling procedures, productivity sampling procedures, and correlations between physiological and productivity measurements. Four other species: lettuce (Lactuca sativa), squash (Cucumis pepo), radish (Raphanus sativas), and corn (Zea mays) were used for subsequent studies to evaluate genetically based differences in responses. Each species was studied in several separate experiments involving different groups of plants. There were four groups of plants for spinach, and two groups for each of the four species.

II. METHODS

A. Spinach Baseline Study

The spinach baseline study covered the period of approximately May 15, 1986 through May 8, 1987. The early period of May through August, 1986, primarily concerned planning of the study and planting of the first set of spinach plants. Funds were not actually available until late June to start research for the project and it took some time to start the first set of plants. Many of the methods for the spinach baseline study were also applicable for the interspecific response study. Thus only the added details particular to the interspecific study are described in II. Section B.

1. Plant Culture

All plants were grown in charcoal-filtered, glass covered greenhouses at the University of California, Riverside. The charcoal filtration allowed for production of healthy plant material without prior history of ozone exposure. Planting, exposure, and harvesting dates for the five groups of spinach [Spinacia oleracea cv. Hybrid #424 (Ferry-Morse)] plants were as shown in Table 1. The first four groups were used for the gas exchange, fluorescence, and productivity measurements. The fifth group was used only for further trial fluorescence studies.

Multiple seeds were initially sown in California #2 media in pots. This media contained a 2:1:1 ratio of soil, peat moss, and redwood shavings, as well as the following sources of major nutrients per cubic meter of media: KNO_3-1.4 kg, K_2SO_4-0.14 kg, dolomitic limestone- 2.1 kg, oyster shell lime-0.86 kg. The following micronutrients were included (all in ppm) copper-30, zinc-10, manganese-15, and iron-15.

The seedlings were transplanted to the hydroponic system after a few leaves had emerged above the cotyledons. The precise number of days between seeding and transplanting depended on the environmental conditions in the greenhouse. The first set of seedlings in July and August 1986 grew especially slowly as spinach is a cool season crop which does not grow rapidly in warm summer temperatures in Riverside. The first group of plants was kept under a black cloth from 1700 through 0800 daily from seeding to final harvest to maintain a light period of less than 13 hours. This was required to prevent early initiation of flowering in

Table 1. Planting, Exposure, and Harvesting Dates for Spinach

Group	Seeding	Trans-planting[a]	Ozone Exposure	Harvests
1	7/7/86	7/31	9/2,5,9,12,16,19,23,26	9/8,15,22,29
2	8/29/86	10/10	10/21,25,28,31 11/4,7,11,14	10/27;11/3,10,17
3	10/22/86	11/13,24	11/25,28 12/2,5,9,12,16,19	12/1,8,15,22
4[b]	12/29/86	1/22/87	2/3,6,10,13,17,20,24,27	2/9,16,23; 3/2
5[c]	2/18/87	3/12	3/3,6,10,13,17,20,24,27	3/9,16,23,30

[a]To hydroponics from media in pots.
[b]Also interspecific group 1.
[c]Also interspecific group 2. The spinach plants were for extra fluorescence measurements, with exposures continuing after 4/30/87. No regular spinach harvests were made.

spinach. After September the light period was short enough to prevent flowering without added covering.

The hydroponics system used 10 L white plastic pots equipped with lids. Each lid had two holes for plants and one hole for pressurized air delivery. Air was supplied from Gast Co. Model DOA-129 1/8 hp pumps, with air flow rates equalized between pots. Air stones were used to break up air bubbles and thus insure uniform aeration of roots. The nutrient solution was half-strength Hoaglands formulated for spinach (Table 2). The level of the nutrient solution was checked periodically and new solution was added periodically to replace that lost by evapotranspiration and to maintain nutrient concentrations near the target levels. The nutrient concentrations in the solutions were not checked during the study as the continuous replenishment of the pots with dilute solution was believed to prevent excess nutrient buildup in hydroponic systems. There were two seedlings per hole at transplanting, with the second seedling thinned out just before the first exposure for each group of plants.

Table 2. Formulation of Nutrient Solution for Spinach Hydroponics Culture

Ion	Molarity	Ion	Molarity
KNO_3	0.006	H_3BO_3	0.000046
$CaNO_3$	0.004	$MnCl_2.4H_2O$	0.000009
$MgSO_4$	0.002	$ZnSO_4.7H_2O$	0.000008
KH_2PO_4	0.001	$CuSO_4.5H_2O$	0.000003
$MgCl_2.\ H_2O$	0.004	$NaMoO_4.2H_2O$	0.000001
NaFe-EDTA	0.001		

For the first group of spinach there were a total of 14 hydroponic pots, 12 for exposures and two extra. Thus there were four pots per each of the three ozone treatments, with two plants from different pots harvested each week. For the other groups there were a total of 26 pots, 24 for exposures and two extra. There were eight pots per each of three ozone treatments, with two plants from different pots harvested each week.

Pinto beans (Phaseolus vulgaris) were used for trial runs before the spinach was ready for exposure in July and August 1986 and for pilot biochemistry studies described in Appendix A. Pinto beans were used because they are a sensitive indicator or ozone injury and were readily available. The plants were grown in U.C. #2 mix or vermiculite media in styrofoam coffee cups or 10 cm diameter plastic pots. The plants were 19 to 21 days old at the time of exposure, with a pair of fully expanded unifoliate leaves and two expanding trifoliate leaves. Exposures were either for one or two days. All greenhouse plants in pots were irrigated as necessary with fertilizer solution.

2. Ozone Exposures

Exposures were in 1.22 m diameter, Teflon covered Controlled Stirred Tank Reactor (CSTR) chambers (Rogers et al. 1977), in Greenhouse 21 of SAPRC. The temperature and humidity in the CSTR's was the same during the exposures as in the growing areas of the greenhouse. The hydroponic pots were transferred to the CSTR's one-half to two hours prior

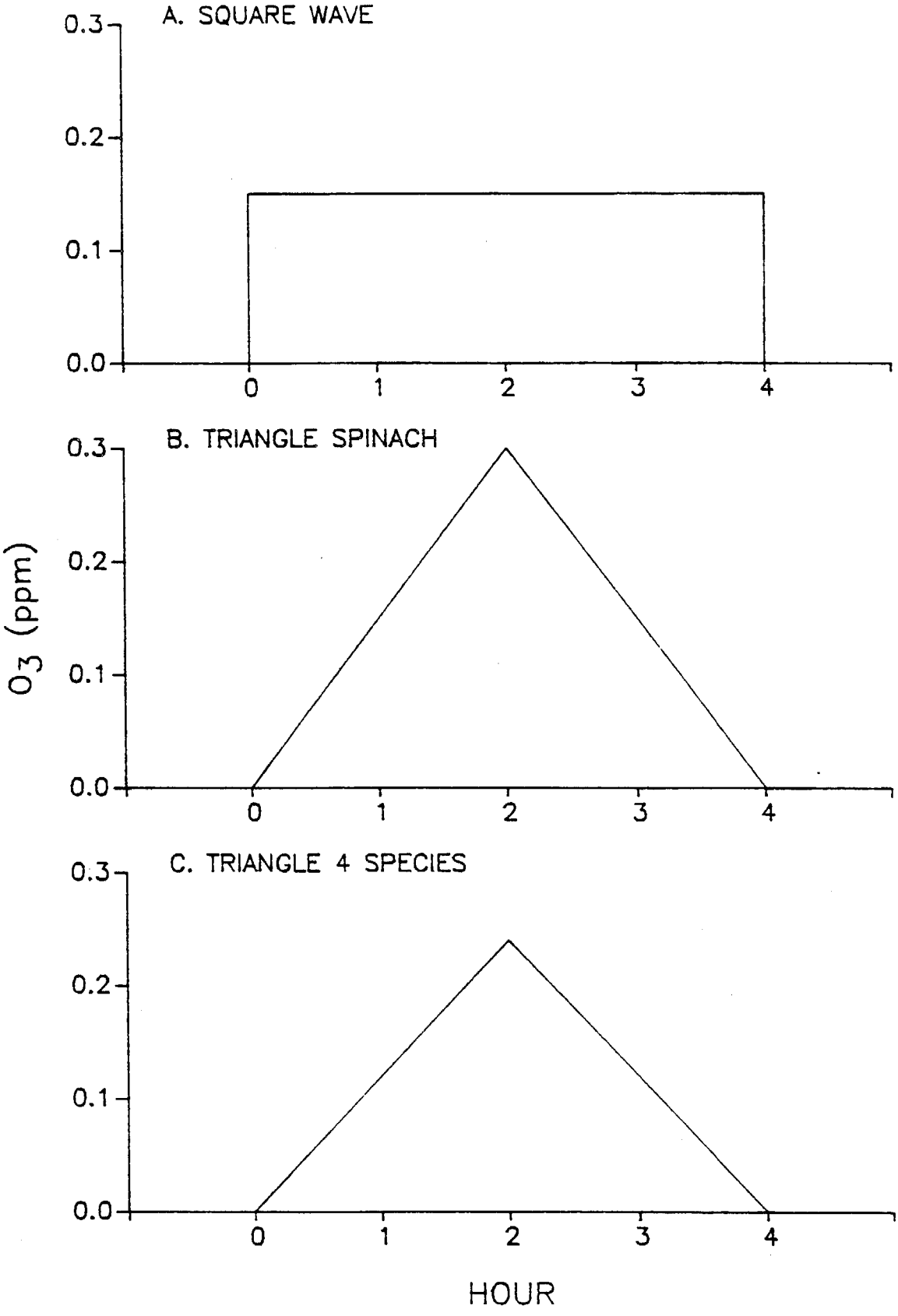

Figure 1. Patterns of ozone exposure. (A) Square wave pattern used for first three groups of spinach, (B) triangular 'peak' pattern for first three groups of spinach, and (C) triangular 'peak' pattern for last two groups of spinach and interspecific study.

to exposure and connected to the aspiration system in each chamber. Thus all pots were aspirated both during the growth and exposure portions of the study.

There were three ozone treatments for the first three groups of the spinach study (1) a no added ozone 'control', (2) a square wave pattern of exposure to 0.15 ppm ozone for four hours (Figure 1A), and (3) a triangular 'peak' pattern of exposure of from 0 to 0.30 then back down to 0 ppm ozone over four hours which resulted in a mean concentration of 0.15 ppm (Figure 1B). Thus both the square wave and triangular patterns of exposure had the same mean and total dose of ozone concentrations of 0.15 ppm and 0.60 ppm x hrs, but different maximum one hour averages of 0.15 and 0.265 ppm, respectively. The exposures were performed between approximately 0900-1300 on Tuesdays and Fridays of four successive weeks, resulting in a total of eight exposures for each group of plants. The last two groups of plants followed the same general protocol, but had only the control treatment and a triangular pattern of exposure of from 0 to 0.24 then back down to 0 ppm ozone over four hours (Figure 1C). This resulted in a mean concentration of 0.12 ppm, total dose of 0.48 ppm x hrs, and peak one hour average of 0.21 ppm ozone. All plants in a particular group were exposed at the same time. There was one chamber per treatment for the first three groups of spinach and two chambers per treatment for the last two groups as space also was needed for the other species.

Ozone was generated from tank oxygen using ultraviolet lights, with flow to chambers regulated via flowmeters. For the first group of spinach the flow was controlled manually, but for the other groups mass flow controllers connected to a computer interface and Apple® computer were used for automatic control of the triangular exposure. No computer control was necessary for the square wave exposure as the concentration remained constant in the chamber during the course of the exposure. Ozone was monitored with Dasibi® ultraviolet absorption ozone analyzers calibrated approximately quarterly with a transfer standard obtained from the California Air Resources Board office in El Monte, California.

Important environmental parameters, i.e., light (quantum) intensity, relative humidity, and air temperature were monitored during the exposures by the LI-COR® gas exchange instruments described later. Environmental

harvest date effects would be evenly distributed over the replicate plants.

On July 30, 1987, seeds of pinto beans (Phaseolus vulgaris) were planted in order to provide a second species for study. Pinto beans are a good bioindicator of ozone stress exhibiting a characteristic oxidant stipple, especially on the unifoliates which are the first leaves to emerge from the plants. The bean plants were transplanted to 3.8 L paper pulp pots on August 4, 1987, which transfered to the chambers on August 5, 1987. There were five pots per plot. The bean plants were harvested at the time of the fourth alfalfa harvest on October 6, 1987. This resulted in a total 62 days of exposure corresponding approximately to the third and fourth alfalfa harvests.

D. Plant Measurements

1. Physiology

Stomatal conductance and transpiration were measured as an indicator of ozone stress on plant gas exchange. Measurements were made using the LI-COR LI 1600 steady state porometer, on three plants per cultivar

Pigment concentrations in leaf tissue was measured before each harvest as an indicator of ozone induced leaf senescence. The pigments were extracted by a modified version of the ethanol extraction method of Knudson et al. (1977) using a Beckman DB spectrophotometer. Results were expressed on a unit of mg pigment per g dry weight basis. Five leaves were taken per chamber, per cultivar, for pigment analysis. Each leaf was from the fourth or fifth node of one stem from an individual plant. The leaf at this node was at a critical stage of development, where senescence was just beginning to develop on a stem. Lower leaves were already turning yellow and senescing in the polluted treatments, higher leaves were still green. Each leaf was measured independently for pigment concentrations, with two absorbence readings taken per leaf. These two readings were averaged to determine the pigment concentrations.

Physiological measurements made either the day before or on the day of harvest for growth and yield measurements. Physiological and biochemical measurements were not made for the fifth harvest as the personnel were not available and the cool, cloudy conditions reduced the likelihood of finding ozone effects on any parameter. The fifth harvest was extra

5. Chlorophyll Fluorescence Measurements

The kinetic transients of chlorophyll fluorescence were measured using the systems and procedures developed by Heath et al. (1985) and Harris et al. (1983a,b). The procedure consisted of taking discs from exposed leaves (3.5 cm diameter), and incubating them for various dark adaptation times while floating them on distilled water in Petri dishes in the dark. The dark adaptation time required 15-30 minutes and resulted in stable fluorescence kinetic measurements from the leaves. The leaf disc, after pre-incubation, was then placed in a Hansetec® fluorescence unit which allows for simultaneous measurements of oxygen production and chlorophyll fluorescence upon exposure to light (Delicu and Walker 1983, Walker et al. 1983). Previous experiments showed that leaf discs should be given a pre-treatment flash of light of five seconds to preset the photochemical apparatus (Heath, unpublished results). After a dark period of three minutes a sequence of flashes of light interspersed with dark periods (five seconds light, 175 seconds dark) with a final light period of 50 seconds to allow for the complete characterization of the fluorescence transients.

From these data the initial fluorescence level (F_o), the peak of fluorescence (F_p), and the rise and fall kinetics were obtained using computer methods. Previous data from Chorella and Pinto Bean have shown that fluorescent kinetic patterns change quite dramatically upon exposure to ozone, although these studies were done in a model system using algae or in a system with pinto beans and high concentrations of ozone (Schreiber et al. 1978, Heath et al. 1982). Actual fluorescence parameters were measured by irradiating with blue light (6 watts m^{-2}). Measurements were made of the fluorescence emitted at a 40^{o} angle, with a photodiode shield against the blue light consisting of a red filter that did not pass wavelengths less than 680 nm. The entire chamber was maintained at 25^{o} (Walker 1981).

All fluorescence data were taken into a Hewlett Packard 7090 measuring system and then transferred, after digitization, into a Commodore 64 computer through which all fluorescence kinetics were analyzed. The parameters were stored on floppy discs until statistical analysis was carried out. The software was developed by Dr. R. L. Heath during his sabbatical in 1983 at the University of Sheffield with Professor David Walker. Leaf

samples for fluorescence measurements were taken from separate replicate plants, with the amount of replication varying (usually 6-10) with the group of spinach plants.

B. Interspecific Response Study

1. Plant Culture

Four species were used to validate the usefulness of physiological response-productivity comparisons for different types of plants. The four species were lettuce (Lactuca sativa cv. Empire), squash (Cucumis pepo cv. Early Prolific Straightneck), corn (Zea mays cv. Bonanza), and radish (Raphanus sativus cv. Cherry Belle). These species were selected to provide a range of likely susceptible and tolerant species. Radish was expected to be the most susceptible to ozone (Reinert et al. 1972), corn was a susceptible cultivar, but of unknown sensitivity compared to the other species (Thompson et al. 1976); lettuce was expected to be tolerant (Ormrod et al. 1984); and squash was of unknown susceptibility.

All four species were grown in a one-half vermiculite, one-half perlite media which allowed for root extraction. The hydroponic system was not used as it was not adequate for growing the large number of plants needed to compare four species over a one month growing period. It would have been logistically impossible to have enough hydroponic pots for all four species or enough space for all pots. Plants for the first group of the four species were seeded directly into 10 cm diameter plastic pots on December 30, 1986. The ozone exposures and harvests corresponded to those for group four of the spinach. Plants for the second group were seeded on February 17, 1987 with ozone exposures and harvests corresponding to those for group five of the spinach.

Thirty two plants of each species were needed for each group of exposures (two treatments x four harvests x four replicate plants per harvest). Fifty and seventy-five plants per species were originally seeded for the first and second groups of plants, respectively, to provide adequate plants so that the most uniform ones could be selected prior to the exposures. Uniformity was based on visual observations of number and size of leaves. The 32 selected plants were randomly assigned to four sets to be harvested on the successive weeks, with four plants randomly assigned to the control and ozone treatments for each set.

2. Ozone Exposures

There were only two treatments for the interspecific study: filtered air control plants, and plants exposed to the triangle pattern of ozone with an average concentration of 0.12 ppm (Figure 1C). There were two chambers per treatment.

3. Gas Exchange Measurements

Measurements were made only with the LI-COR® 1600 steady state porometer during the first two weeks of exposure for the first group of plants as the leaves were too small to use with the LI-COR® 6000 gas exchange system. For the rest of the weeks of the first group and the entire second group of plants the LI-COR® 6000 was used.

4. Productivity Measurements

Fresh and dry weights of shoots and roots (hypocotyl for radish) were measured using the protocol described for spinach.

5. Chlorophyll Fluorescence Measurements

No regular chlorophyll fluorescence measurements were made for the interspecific study as intensive measurements focused only on spinach. Some pilot measurements were made on radish, corn, and squash.

C. Statistical Analysis

The results from these experiments were analyzed statistically using a general randomized complete block model as described by Steel and Torrie (1960). This model was used as the plants were assigned randomly to sets which were evaluated for physiological or productivity responses on particular days. These days were considered to be blocks. On each day identical measurements were made on individual plants which were considered to be replicates, as each plant responded according to its particular genetic makeup and microclimate. Each experiment involved a particular group of plants which was planted and maintained separately. The groups were grown sequentially over nine-months, with the environmental conditions changing over time in the greenhouse. Because the environmental conditions can substantially alter plant response to ozone (Ting and Dugger, 1968), data from each group usually were analyzed separately. However, the data also were pooled across the similar groups of plants for particular test purposes, e.g. to increase the number of replicates to detect significant differences between species responses to ozone. This pooling was only

across the groups exposed close together in time, which minimized the environmental differences.

For the spinach studies, the general statistical design included three pollutant treatments (control with filtered air, ozone-square wave, and ozone-triangle or peak). Physiological measurements were made twice per week on the days of exposure (Tuesdays and Fridays), during the last three weeks of each experiment, for a total of six days. Measurements could not be made the first week as the leaves were too small for the cuvettes. Productivity measurements were made once per week on the Monday following the two exposures. There were four replicate plants measured on each date. The normal protocol called for physiological and productivity measurements over four weeks, resulting in the partitioning of degrees of freedom for the different sources as shown in Table 3.

Table 3. General Statistical Design for Spinach Study

Source	Degrees of Freedom	
	Physiology Data	Productivity
Air Pollutant	2	2
Day of Measurement	5	3
Air x Day	10	6
Error	54	36
Total	71	47

The optimum design for spinach was modified slightly for specific groups based on particular needs such as if all four replicate plants were not available, or extra data physiological data was taken on a trial basis. For group one, there were 11 days when physiology was measured, but only two plants were measured on each date; and for productivity only two plants were measured on each harvest date. For group two, there were five days when physiology was measured. For group four, there were only two treatments-control and ozone-peak. In addition, the physiology data for each day in group one were analyzed separately because extra replicate plants were measured during the early part of the study. This day-by-day

analysis with different numbers of measurements provided the general evaluation of the number of replicates needed to detect significant differences in physiological response parameters.

Statistical analyses for the four individual species in the interspecific response study followed the same general design as for spinach. However, their were only two air treatments (charcoal filtered air and peak ozone exposure), which resulted in reduced degrees of freedom (df) as shown in Table 4. The only major exception to this design was for the first group of lettuce plants, where physiological measurements were made only on four days because the plants were too small for measuring during the first two weeks of the study. This resulted in three df for day of measurement, three df for air x day, 24 df for error, and 31 total df.

Table 4. General Statistical Design for Each Species in Interspecific Study

Source	Degrees of Freedom	
	Physiology Data	Productivity
Air Pollutant	1	1
Day of Measurement	5	3
Air x Day	5	3
Error	36	24
Total	47	31

An additional effort was made to analyze the interspecific data across all species and the two groups of plants. This was attempted because of the objective to statistically compare the species data, and both groups were used in order to increase the replicates available to detect treatment effects. Pooling the two groups appeared to be reasonable as they were grown relatively close together under similar spring environmental conditions. The partitioning of the degrees of freedom for the interspecific study with both groups of plants included is shown in Table 5.

Table 5. Statistical Design for Interspecific Response Study Across Species

Source	Degrees of Freedom	
	Physiology Data[a]	Productivity[b]
Air Pollutant	1	1
Species	2	3
Day of Measurement	5	3
Group	1	1
Air x Species	2	3
Air x Day	5	---
Air x Group	1	---
Species x Day	10	---
Species x Group	2	---
Group x Day	5	---
Air x Species x Group	2	---
Interaction Terms	--	52[d]
Error	251[c]	192
Total	287	255

[a]For corn, squash and radish data.
[b]All four species.
[c]Includes additional interaction terms: air x species x day, air x group x day, air x species x day x group.
[d]Pooled df for all interactions except air x species.

The focus was on the physiological measurements as the same responses were measured for all plants. Only corn, squash and radish were used for the analysis because there were missing data points for the first group of lettuce plants. All two factor interaction terms were tested. Nearly all three or four way interaction terms involving the day factor were included in the error term because day was not of primary interest in this study.

Pooling of data for the productivity measurements was solely as a test of the air x species effect (Table 5). Each species grew differently and partitioned material to different parts of the plant at different rates over time, so we considered all other interactions to be complex and

inevitable. Thus, all interaction terms except air x species were combined together with 52 df.

A modified relative growth rate per plant (RGR) were based on total dry weight and calculated as RGR = [(Harvest Weight - Base Weight)/Base Weight]/7 days. The base weight was determined as the average harvest weight for the four replicate plants at week one, two, or three. The harvest weight is the individual plant weight at harvest on week two, three, or four, respectively. The seven days is the time between harvests to put increases in weight on a gram/gram/day basis. The average base weights had to be used as no companion plants were available. The RGR per plant (y) was compared to stomatal conductance or photosynthetic rate (x) for the regression analysis to determine r values. The conductance or photosynthetic rate for the seven days was the average of the two measurements following the ozone exposures during the week.

No analysis of variance was carried out on the chlorophyll fluorescence data due to its pilot nature: measurements were carried out only on occasional plants and days. Analysis of fluorescence data was by comparing means and standard deviations of treatments.

III. RESULTS AND DISCUSSION

The text for this report contains the most pertinent data from the experiments, i.e., the results from the statistical analysis and figures representing time course changes for the gas exchange and productivity data. The treatment means and standard deviations for each species, group, and response parameter are shown in Appendix B.

A. Ozone Effects on Gas Exchange

Changes in gas exchange parameters were readily found across the different species, experiments, and days of measurement. The baseline measurements on spinach clearly indicated that ozone significantly affected gas exchange, especially in terms of stomatal conductance. Ozone also affected radish in the interspecific study, but had little effect on corn, squash, or lettuce.

1. Stomatal Conductance

Spinach. As shown in Table 6, stomatal conductance always was significantly reduced in spinach exposed to ozone compared to control plants when data were considered across all weeks of each experiment (group). The triangle or 'peak' pattern of exposure generally reduced conductance more than the square wave pattern even though both patterns had similar average ozone concentrations over the four hours of exposure.

For group one, stomatal conductance was affected by ozone when the data was analyzed across all measurement days. However, plants exposed to only the peak pattern of ozone were significantly different from the controls (Table 5). Two of the available replicates on each day were used for the across-group evaluation as the number had to be balanced each day to run the analysis of variance program. Only two replicates were used as this was the maximum number of plants remaining toward the end of the experiment after the other plants had been harvested. Additional statistical significance emerged when results from each day were analyzed separately (Table 7). For example, on 9/2 and 9/5/87 all three treatments were different from each other. This likely was related to the larger number of replicates of ten and eight on 9/2 and 9/5/87, respectively.

Table 6. Results from Statistical Analysis for Spinach Gas Exchange Measurements Across all Days of Each Experiment[a]

Group #	Treatment #	Days #	Reps. #	Stomatal Conductance	Net Photosynthesis	Transpiration
One	3	11[b]	2	*[c]	ns	*[c]
Two	3	5	4	***[d]	ns	***[d]
Three	3	6	4	***[d]	*[c]	***[d]
Four	2	6	4	**[c]	ns	ns

[a] *, **, and *** indicate significant difference at $p<0.05$, 0.01, and 0.005 levels, respectively.
[b] Out of 12 total days.
[c] Significant difference between control and peak treatments.
[d] Significant differences among all paired contrasts for control, square wave, and peak treatments.

Table 7. Results from Statistical Analysis for Spinach Group One, Gas Exchange Measurements for Individual Days[a]

Day #	Reps. #	Stomatal Conductance	Net Photosynthesis	Transpiration
9/2/86	10	***[b]	ns	***[b]
9/5/86	8	***[b]	*[c]	***[b]
9/8/86	2	ns	ns	ns
9/9/86	6	*[d]	*[d]	*[e]
9/12/86	6	ns	ns	ns
9/15/86	2	ns	ns	*[d]
9/16/86	4	ns	ns	ns
9/19/86	4	ns	ns	ns
9/22/86	2	ns	ns	ns
9/23/86	2	ns	ns	ns
9/26/86	2	ns	ns	ns
9/29/86	2	ns	ns	ns

[a]Values followed by *, **, or *** are significantly different at $p<0.05$, 0.01, and 0.005 levels, respectively, according to analysis of variance.
[b]Significant differences among all paired contrasts between control, square wave, and peak treatments.
[c]Significant difference between control or square wave, as compared to peak.
[d]Significant difference between control, as compared to square wave or peak.
[e]Significant difference between control and peak.

Stomatal conductance was also significantly reduced by ozone exposure for groups two through four when four plants were routinely measured on each day (Table 6). The peak pattern of exposure produced the greatest reduction in conductance, with a smaller reduction for the square wave pattern (Figures 3A and 4A). A large reduction in conductance due to ozone exposure also was seen for group four when only the control and peak pattern were present (Figure 5A).

Interspecific. There was a great deal of variability in stomatal responses among species as reported by other researchers (Tingey and Taylor, 1982). Conductance rates were reduced for corn in both experimental group and radish in the first group, but not for squash or lettuce (Table 8).

All four species showed considerable variability in conductance on the different exposure days as shown in Figures 6A and 7A for corn, 8A and 9A for squash, 10A and 11A for radish, and 12A and 13A for lettuce. Radish had the highest stomatal conductance rate of the four species examined in both groups of plants, which is a likely reason for its greater sensitivity to injury from O_3.

2. Net Photosynthesis

Spinach. Net photosynthesis was not consistently affected by O_3 exposure across all days and groups of plants. There was occasionally a trend for reduced photosynthesis with but O_3 exposure, but the reduction was statistically significant only with the peak exposure pattern for group three (Table 6) There was some evidence for reduced photosynthesis with 6 or 8 replicate plants on 9/5 and 9/9/86, but not across all during group one exposures (Table 7, Figure 2B).

Interspecific. Only radish showed reductions in photosynthetic rates with exposure to O_3 (Table 8, Figures 10B and 11B). Corn (Figures 6B and 7B) and squash (Figures 8B and 9B) were not affected by O_3, and lettuce actually had a trend toward increased photosynthetic rate with ozone exposure which was statistically significant for group 2 (Figures 12B and 13B).

3. Transpiration

Spinach. The effect of O_3 on transpiration paralleled the effect on conductance (Tables 6 and 7). Transpiration was reduced with O_3 exposure for groups two and three (Figures 3C and 4C), and early during

Table 8. Results from Statistical Analysis for Interspecific Gas Exchange Measurements[a]

Species	Group #	Factor	Stomatal Conductance	Net Photosynthesis	Transpiration
Corn	1	Treatment	*	ns	ns
	2	Treatment	*	ns	ns
Squash	1	Treatment	ns	ns	ns
	2	Treatment	ns	ns	ns
Radish	1	Treatment	*	***	ns
	2	Treatment	ns	*	ns
Lettuce	1	Treatment	ns	ns	ns
	2	Treatment	ns	*	ns
Corn, Squash, Radish	1&2	Treatment	*	**	ns
		Species	***	***	***
		T x S	ns	***	ns

[a]Two treatments (control, peak O_3), and six measurement days except for five for lettuce in group 1), and four replicate plants. *, **, and *** indicate significant difference at $p<0.05$, 0.01, and 0.005 levels, respectively. All changes due to treatment are decreases, except for increase in photosynthetic rate for lettuce, group two.

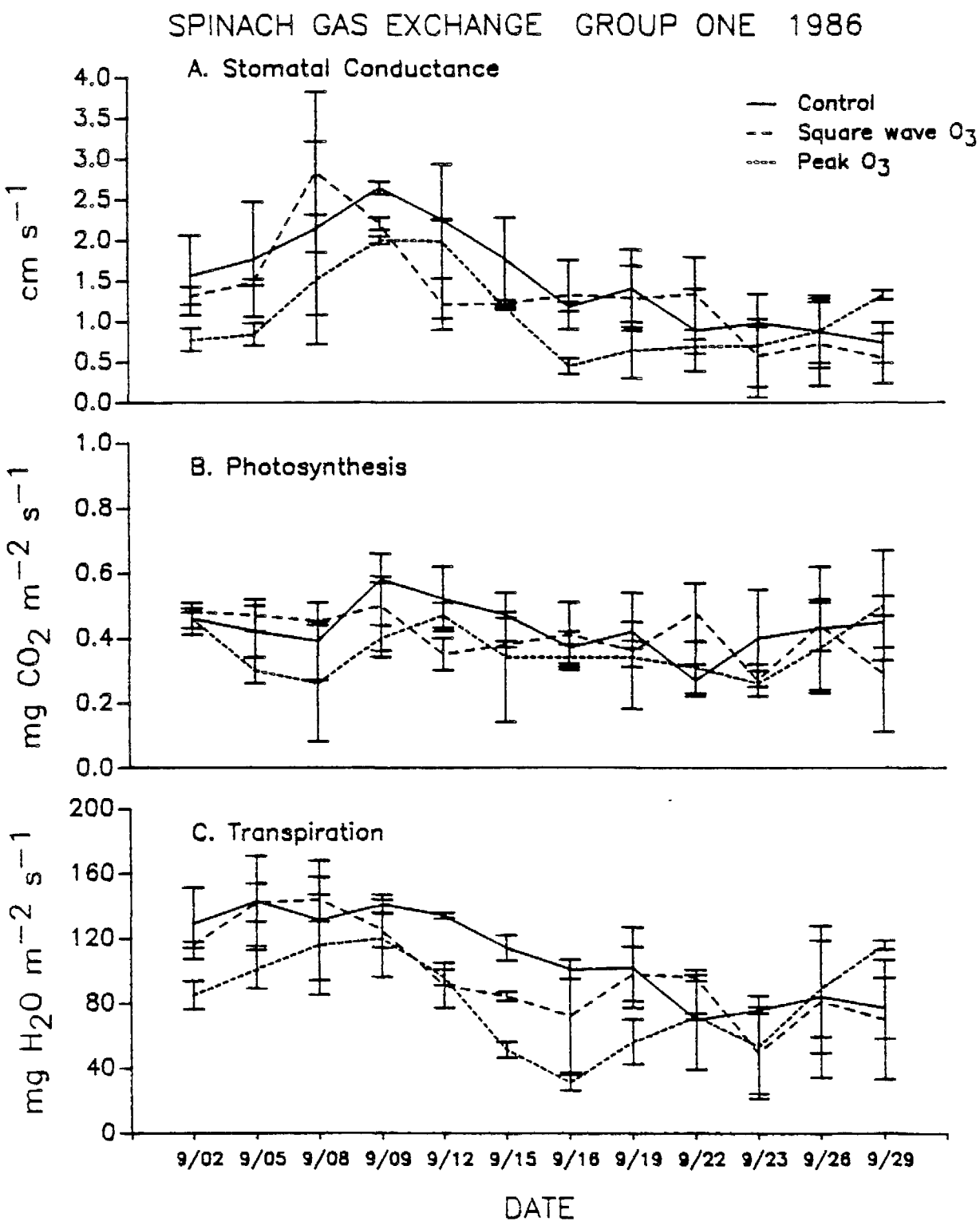

Figure 2. Effects of ozone on spinach gas exchange, group one. Data are for (A) stomatal conductance, (B) photosynthesis, and (C) transpiration. Values are means ± SD for two single plant replicates.

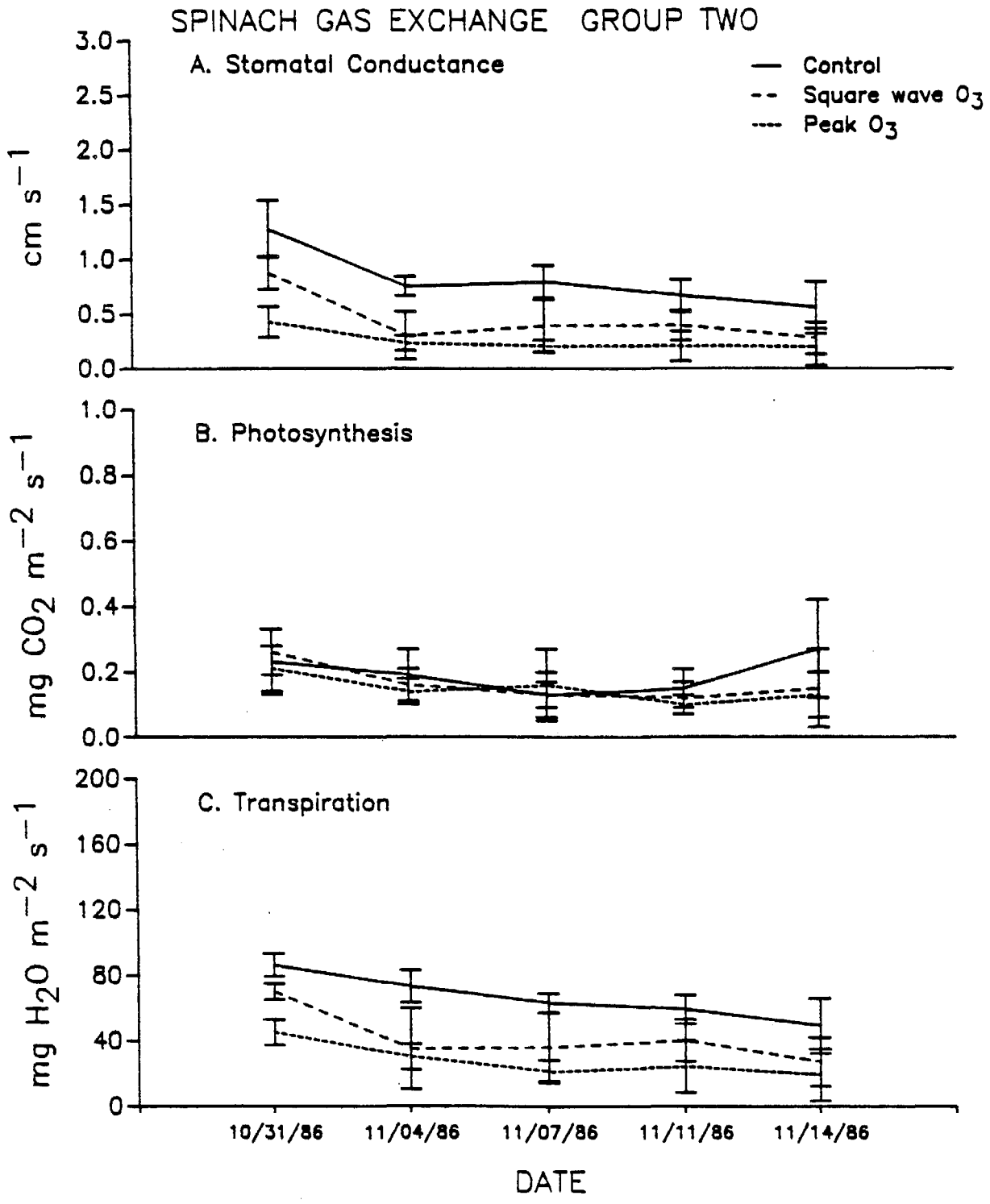

Figure 3. Effects of ozone on spinach gas exchange, group two. Data are for (A) stomatal conductance, (B) photosynthesis, and (C) transpiration. Values are means ± SD for four single plant replicates.

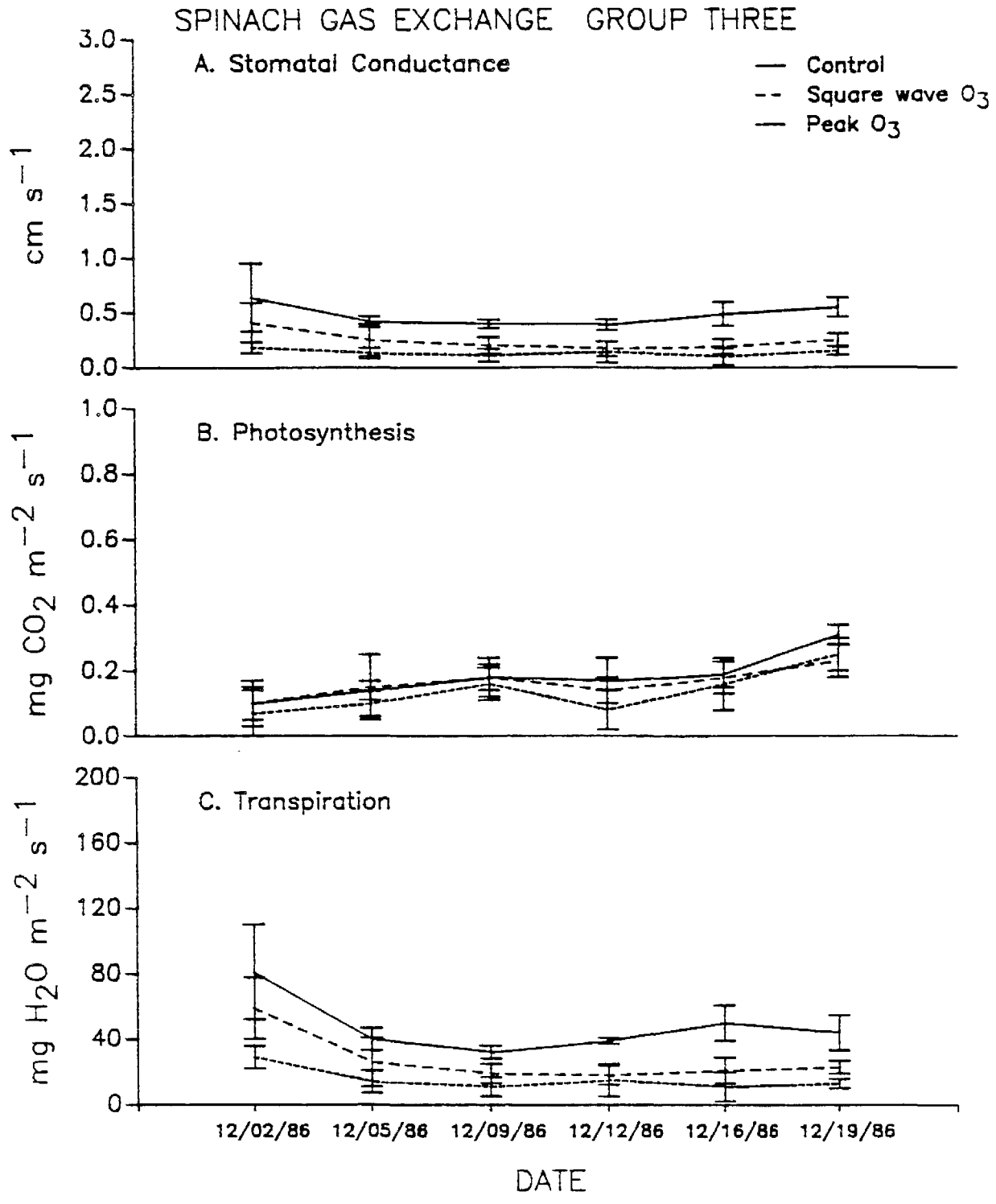

Figure 4. Effects of ozone on spinach gas exchange, group three. Data one for (A) stomatal conductance, (B) photosynthesis, and (C) transpiration. Values are means ± SD for four single plant replicates.

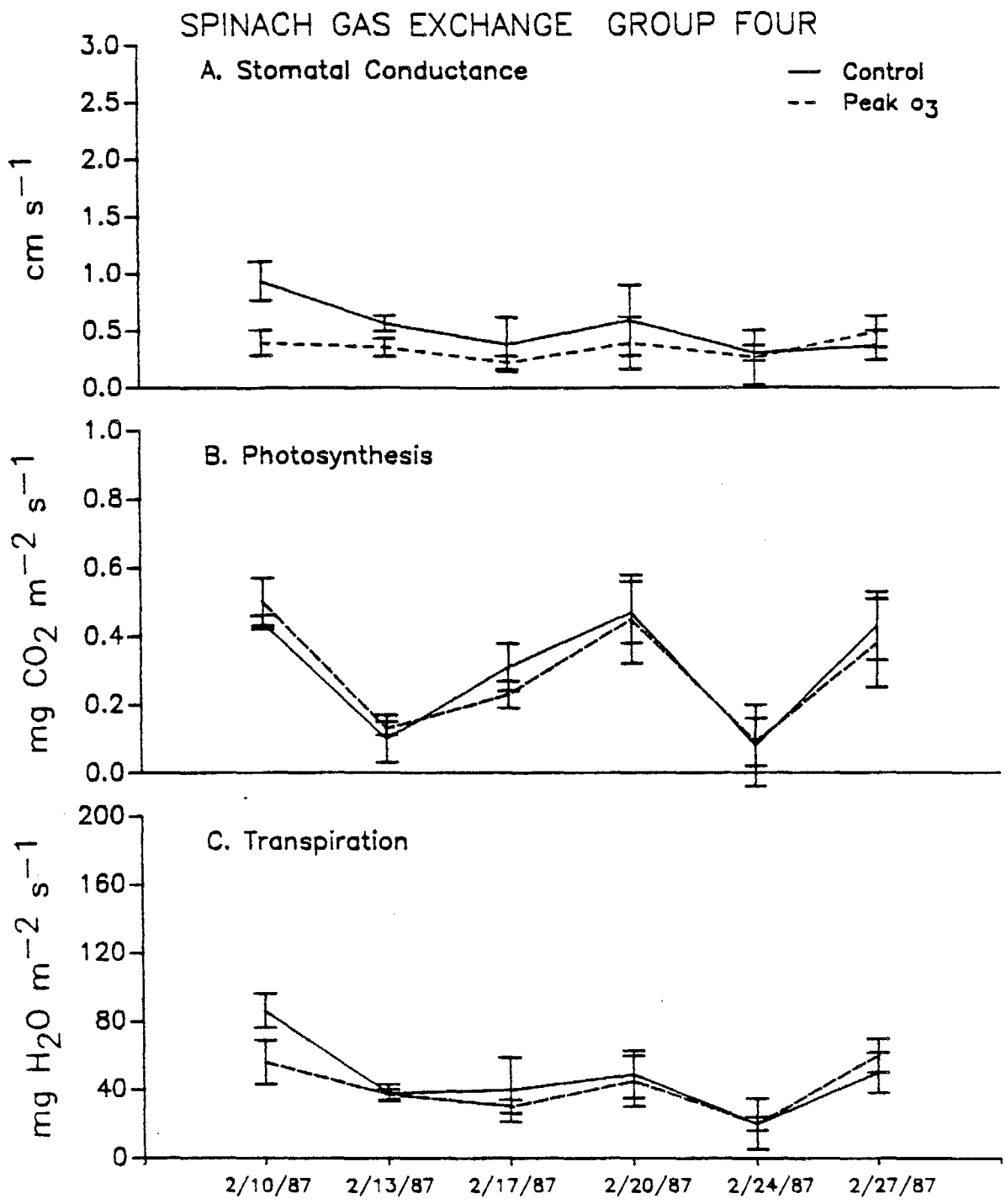

Figure 5. Effects of ozone on spinach gas exchange, group four. Data are for (A) stomatal conductance, (B) photosynthesis, and (C) transpiration. Values are means ± SD for four single plant replicates.

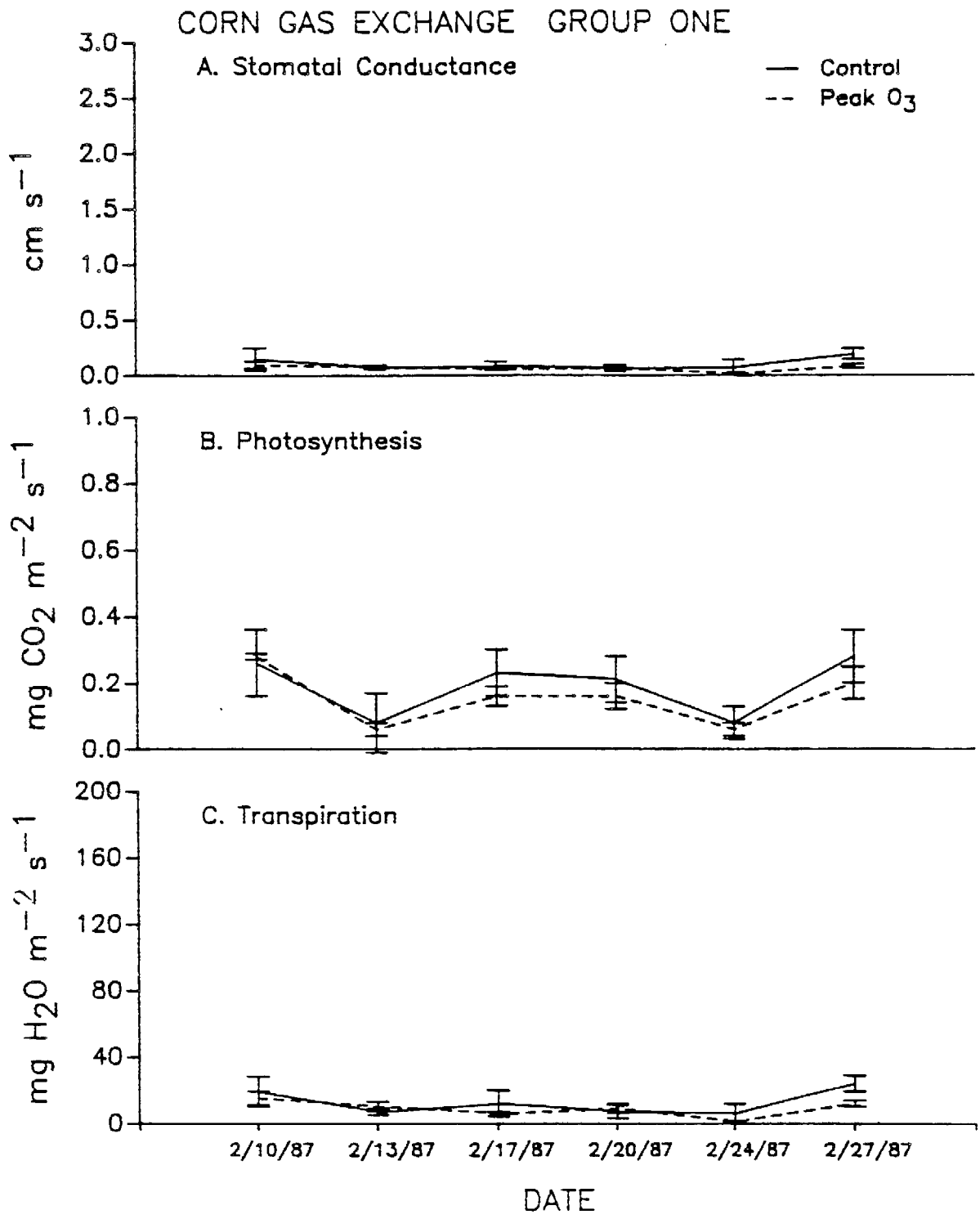

Figure 6. Effects of ozone on corn gas exchange, group one. Data are for (A) stomatal conductance, (B) photosynthesis, and (C) transpiration. Values are means ± SD for four single plant replicates.

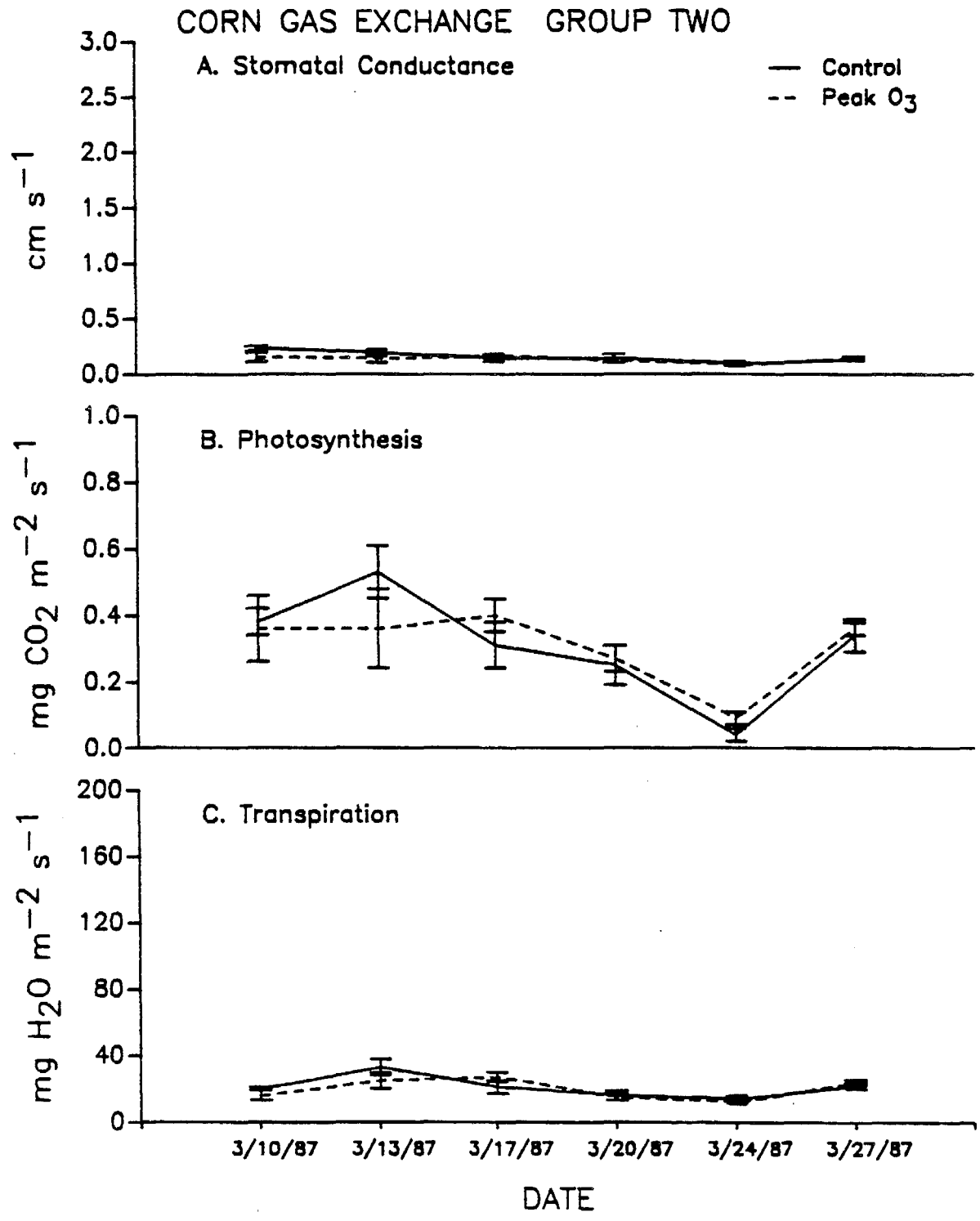

Figure 7. Effects of ozone on corn gas exchange, group two. Data are for (A) stomatal conductance, (B) photosynthesis, and (C) transpiration. Values are means ± SD for four single plant replicates.

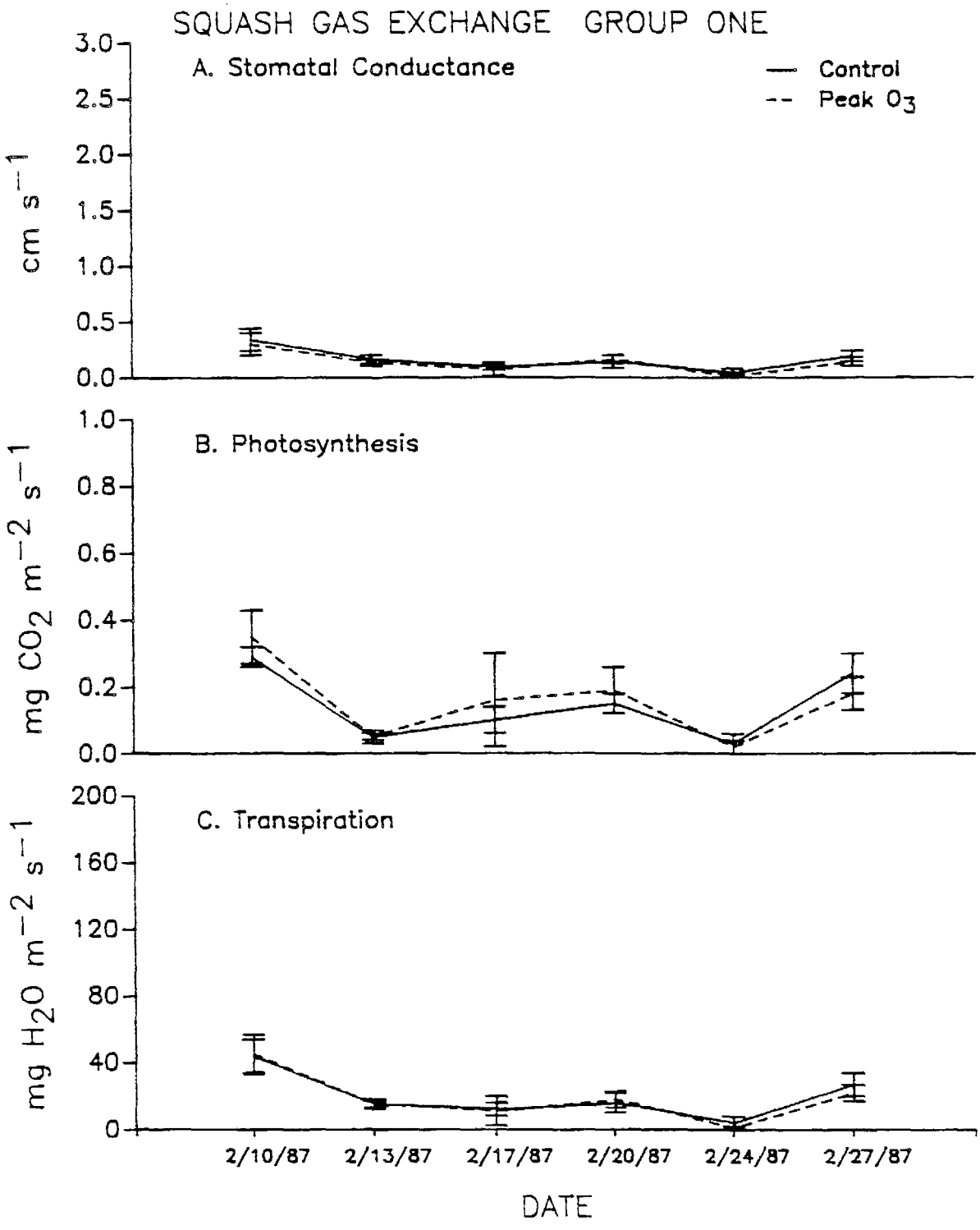

Figure 8. Effects of ozone on squash gas exchange, group one. Data are for (A) stomatal conductance, (B) photosynthesis, and (C) transpiration. Values are means ± SD for four single plant replicates.

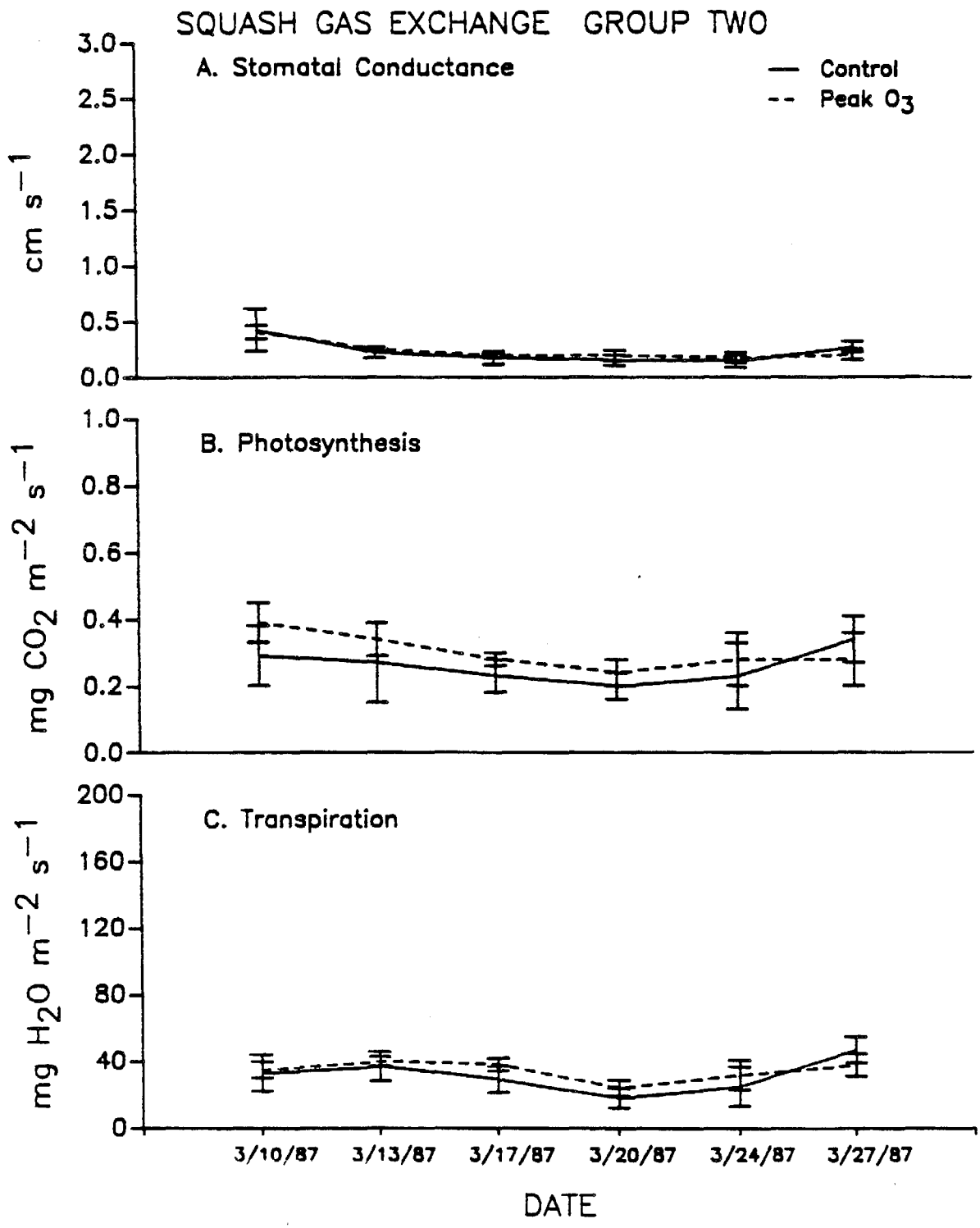

Figure 9. Effects of ozone on squash gas exchange, group two. Data are for (A) stomatal conductance, (B) photosynthesis, and (C) transpiration. Values are means ± SD for four single plant replicates.

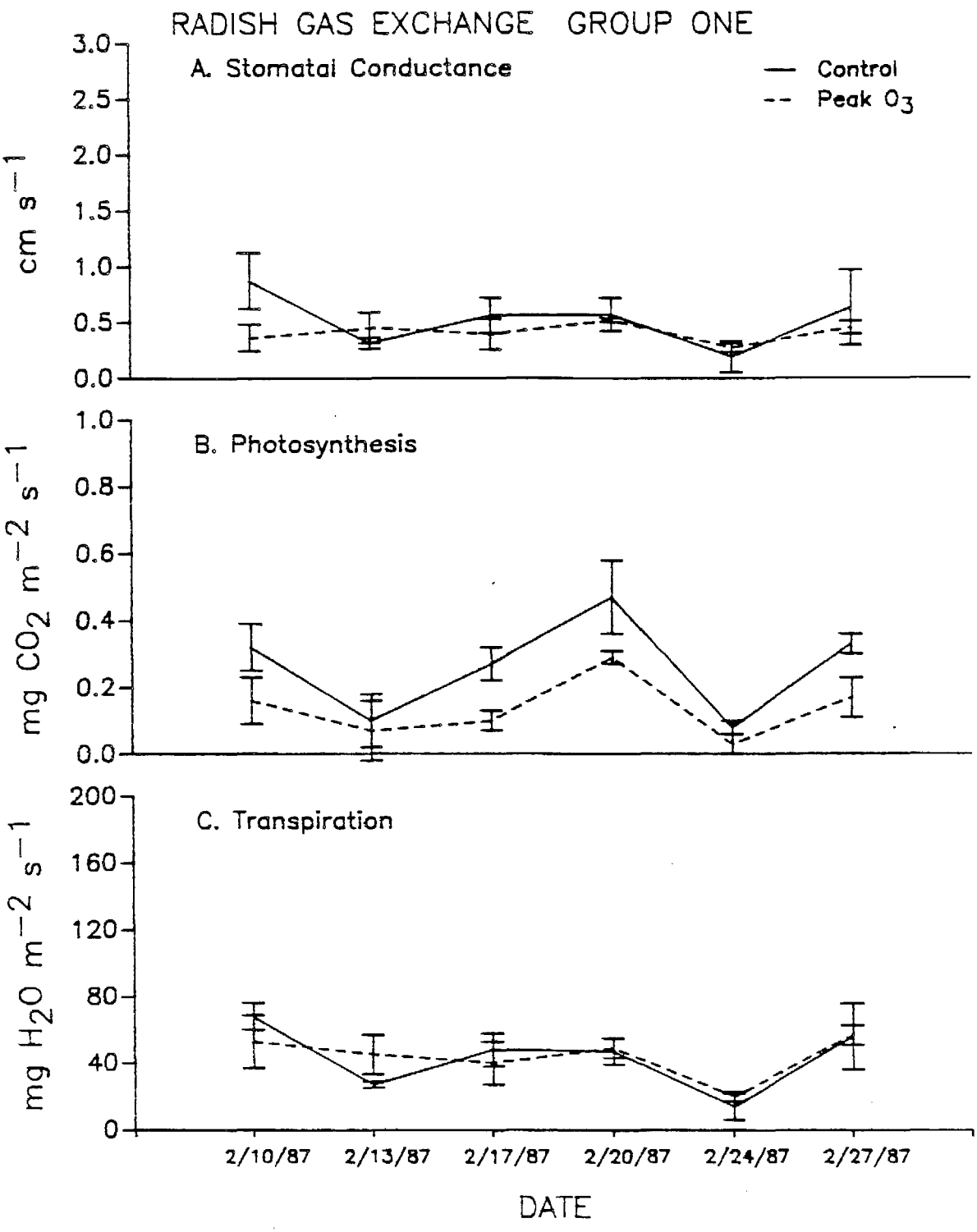

Figure 10. Effects of ozone on radish gas exchange, group one. Data are for (A) stomatal conductance, (B) photosynthesis, and (C) transpiration. Values are means ± SD for four single plant replicates.

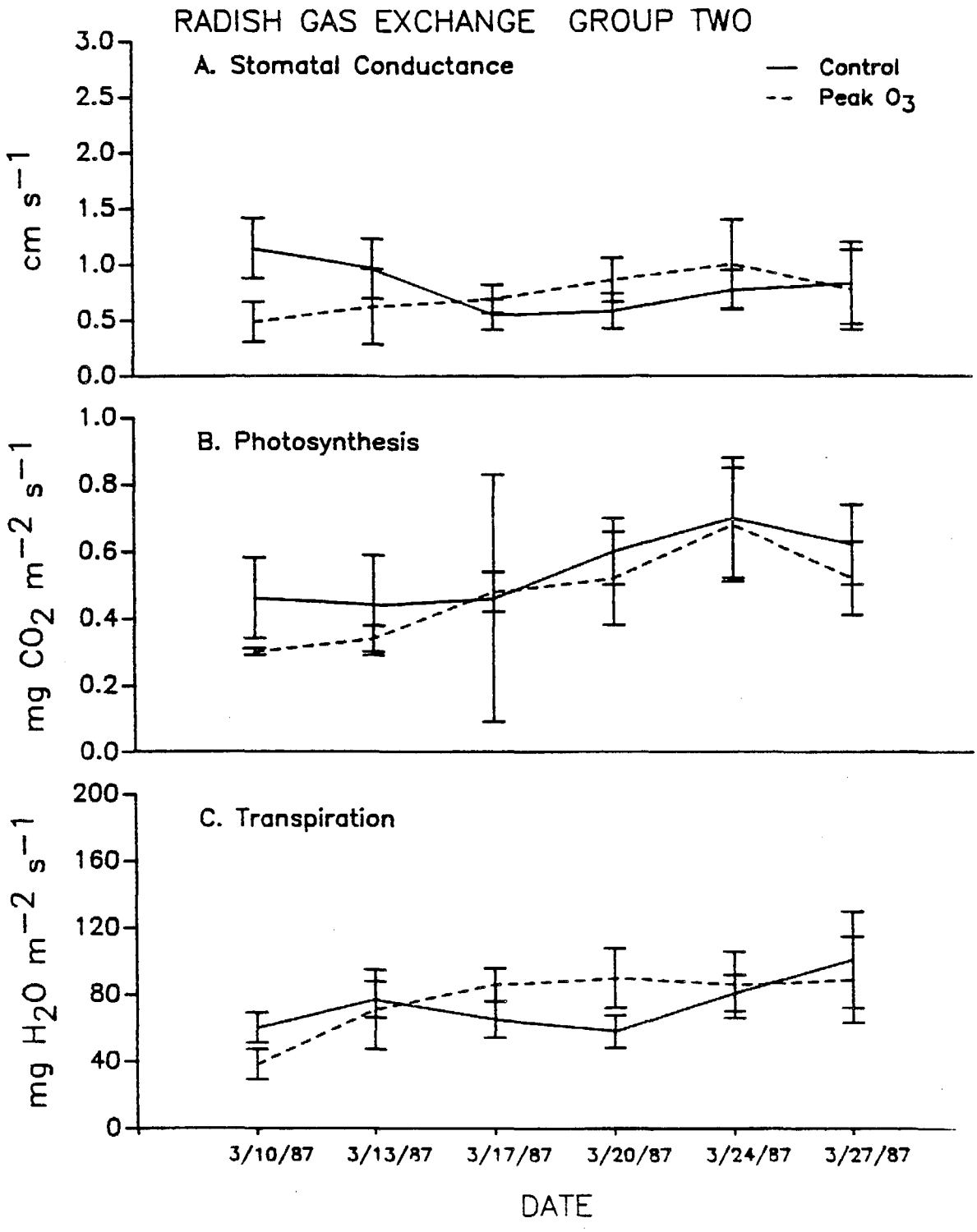

Figure 11. Effects of ozone on radish gas exchange, group two. Data are for (A) stomatal conductance, (B) photosynthesis, and (C) transpiration. Values are means ± SD for four single plant replicates.

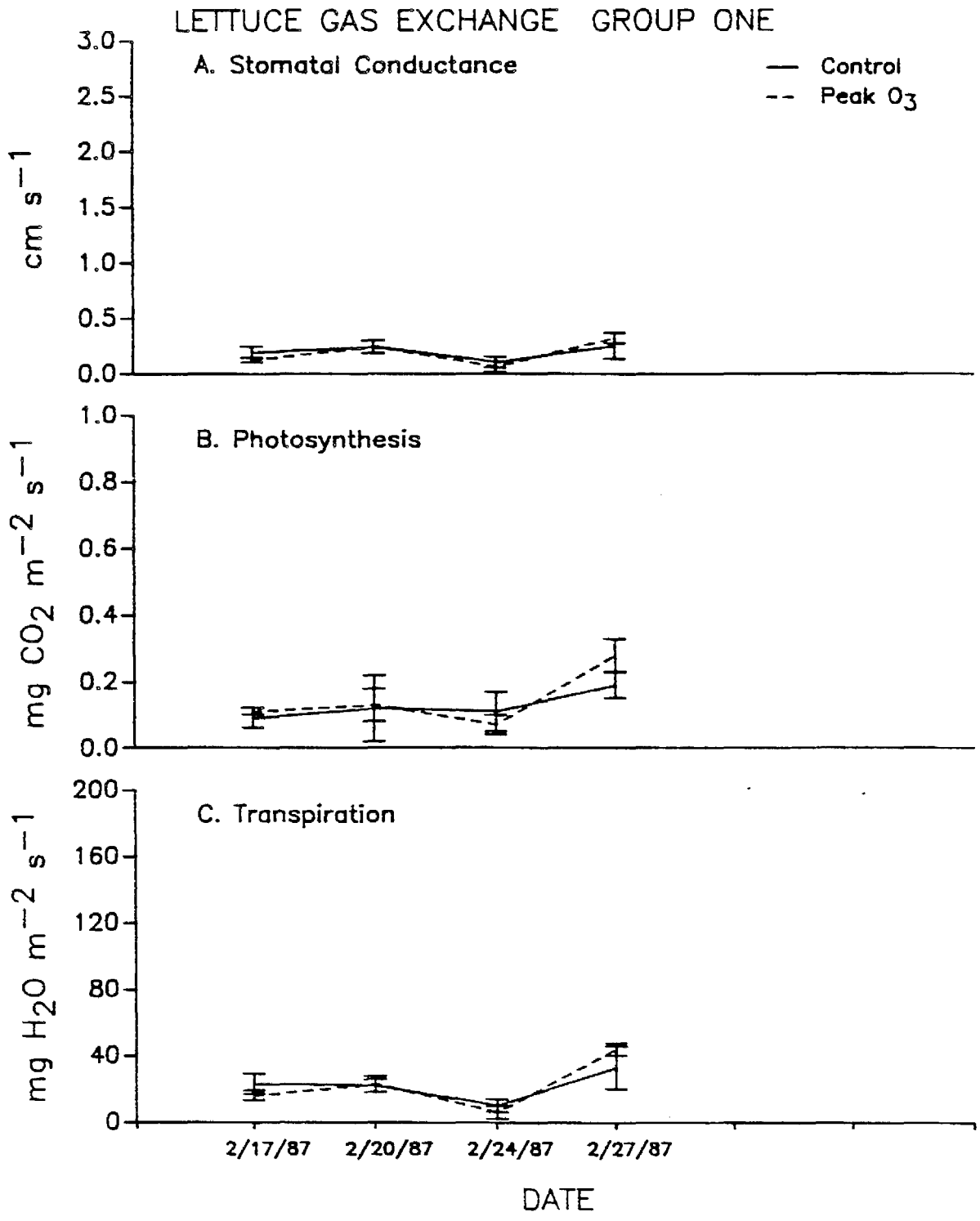

Figure 12. Effects of ozone on lettuce gas exchange, group one. Data are for (A) stomatal conductance, (B) photosynthesis, and (C) transpiration. Values are means ± SD for four single plant replicates.

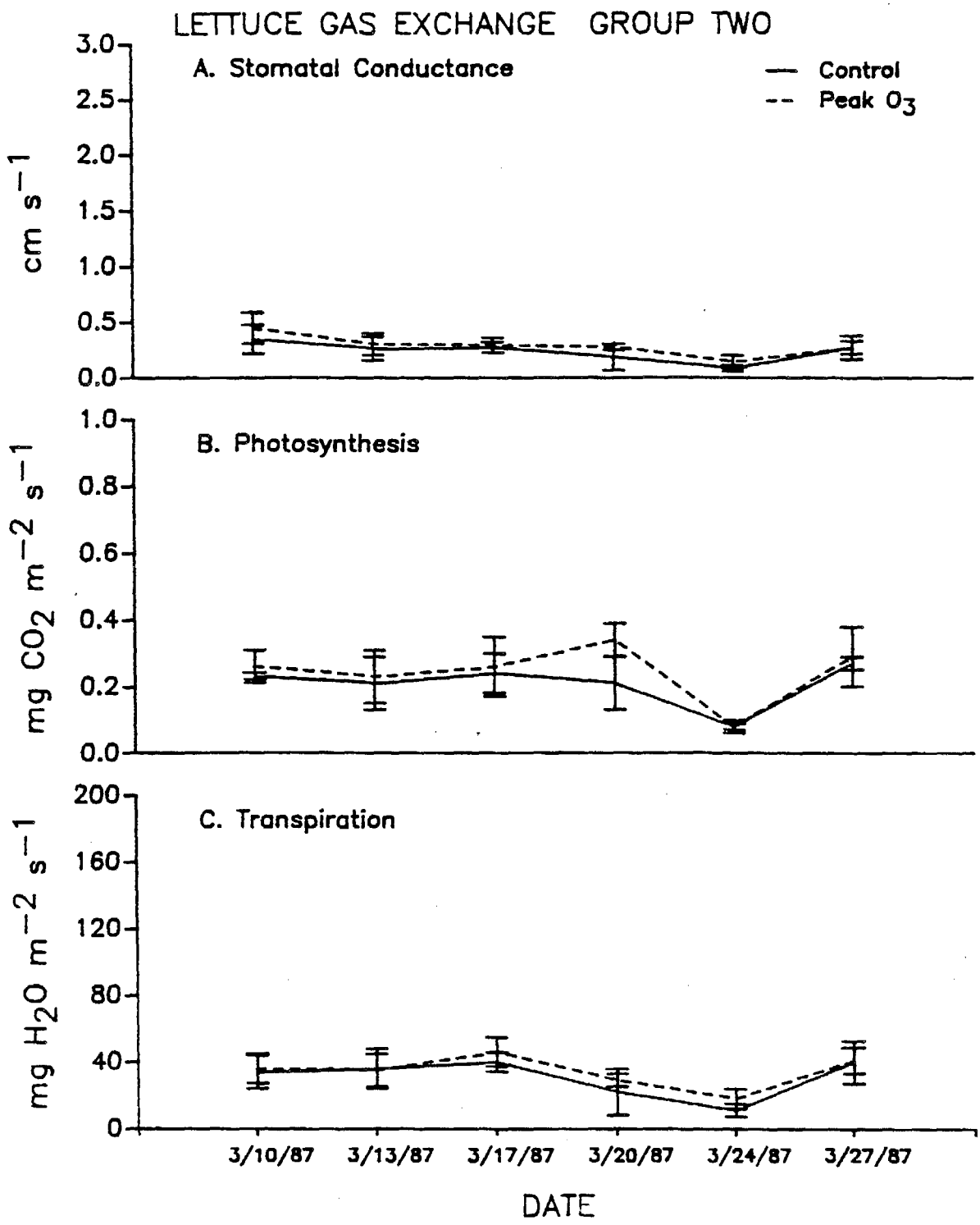

Figure 13. Effects of ozone on lettuce gas exchange, group two. Data are for (A) stomatal conductance, (B) photosynthesis, and (C) transpiration. Values are means ± SD for four single plant replicates.

group one (Figure 2C). Transpiration was not significantly reduced over the course of exposure for group four (Figure 5C). The close relationship to stomatal conductance was expected as the water loss due to transpiration is a factor in the calculations for conductance. However, transpiration does not include correction of the water loss for the relationship between leaf temperature, air temperature, or ambient humidity; and, thus, is not as direct a measurement of actual plant metabolic response.

Interspecific. Transpiration was not affected significantly by O_3 for any species (Table 8). Ozone tended to reduce transpiration, but the effect was not great enough to be statistically significant (Figures 6C-13C).

B. Ozone Effects on Productivity

Ozone tended to reduce the fresh and dry weights of the plants, however, the results were not consistent over all species, weeks, and experimental groups. In large part, this may have been because a relatively moderate ozone stress was used in these studies. The relatively low mean concentrations of 0.15 an 0.12 ppm ozone for four hour exposures with spinach and the four crops, respectively, were intended to produce only slight visible injury. These concentrations were within the range of 0.13 ± 0.02 ppm give a typical ozone concentration which would produce 5% leaf injury for intermediately sensitive crops exposed for four hours (USEPA, 1978). Only slight injury was desired to insure that physiological responses could be studied without the large amount of cellular death associated with higher ozone concentrations. The objective of having little visible injury was fulfilled for spinach which showed slight necrosis that occurred only after the first one or two exposures for each group of plants. No new necrosis was observed after most of the remaining six or seven exposures. Lettuce, squash and corn exhibited essentially no visible injury from ozone. Only radish showed extensive visible injury, but the majority of the symptoms occurred after the initial exposure as observed for spinach.

Spinach. Ozone caused a significant reduction in shoot fresh weight for all experimental groups (Table 9). The reduction was for either the square or peak pattern of exposure and control plants. There did not appear to be any additional reduction in growth for peak vs. square wave

patterns of exposure to correspond to the greater effects of the peak pattern on gas exchange. The reduction in spinach shoot fresh weight was due primarily to reduction in leaf weight as shown by the significant reduction for all three exposure groups where it was measured. There was more variability in response to ozone for stems, roots, total fresh weights, and shoot/root ratio; compared to the response for leaves and shoots. Root and fresh weights were affected only for group two. Total fresh weight was affected only for groups two and three.

The fresh weight shoot/root ratio was affected by ozone only for group three; where a decrease in the ratio was observed with either the square or peak pattern of ozone compared to control plants. This was surprising as it was expected that ozone may have preferentially caused an increase in the ratio as photosynthate was expected to be preferentially allocated to the shoots in ozone exposed plants which should have resulted in an increase in the ratio (Bennett and Oshima, 1976).

Spinach dry weights were not affected by ozone to the same degree as fresh weights (Figures 14A, 15A, 16A, 17A). This was important as it was expected that dry weights may have been more sensitive to any changes in photosynthetic rates due to ozone over the course of an exposure. This greater sensitivity of fresh weights may have been related to the more obvious effects of ozone on stomatal conductance which are indicative of effects on plant water relations and possibly water content.

Ozone affected spinach dry weights only for group two (where fresh weights also were most affected) (Table 8). Shoot and total dry weights were affected by ozone, but not root weights. The dry weight shoot/root ratio was not affected by ozone for any group of spinach (Figures 14B, 15B, 16B, and 17B).

Interspecific

On an individual species and group basis, ozone produced statistically significant effects for a few parameters (Table 10). No ozone effects were found on corn for either group or any response parameter (Figures 18 and 19). The only O_3 effect on squash was a decrease in root dry weight for group one and shoot fresh weight for group two. No significant effects were observed for any other parameter including total dry weight and shoot/root ratio (Figures 20 and 21). Radish showed many statistically significant O_3 effects on both fresh and

Table 9. Results from Statistical Analysis for Spinach Productivity Measurements for Ozone

Group	Replicates	Leaf	Stem	Root	Shoot	Total	Shoot/Root
				Fresh Weight			
One	2	*	ns	ns	*	ns	ns
Two	4	***	**	*	**	**	ns
Three	4	**	ns	ns	*	*	*
Four[c]	4	--	--	ns	*	ns	ns
				Dry Weight			
One	2	ns	ns	ns	ns	ns	ns
Two	4	**	*	ns	**	**	ns
Three	4	ns	ns	ns	ns	ns	ns
Four[b]	4	--	--	ns	ns	ns	ns

[a]Over four weeks. *, **, and *** indicate statistical significance at $p<0.05$, 0.01, and 0.005 levels, respectively, according to analysis of variance. Significant differences between control, as compared to square wave or peak treatment.
[b]Leaves and stems inadvertantly not separated at harvest.

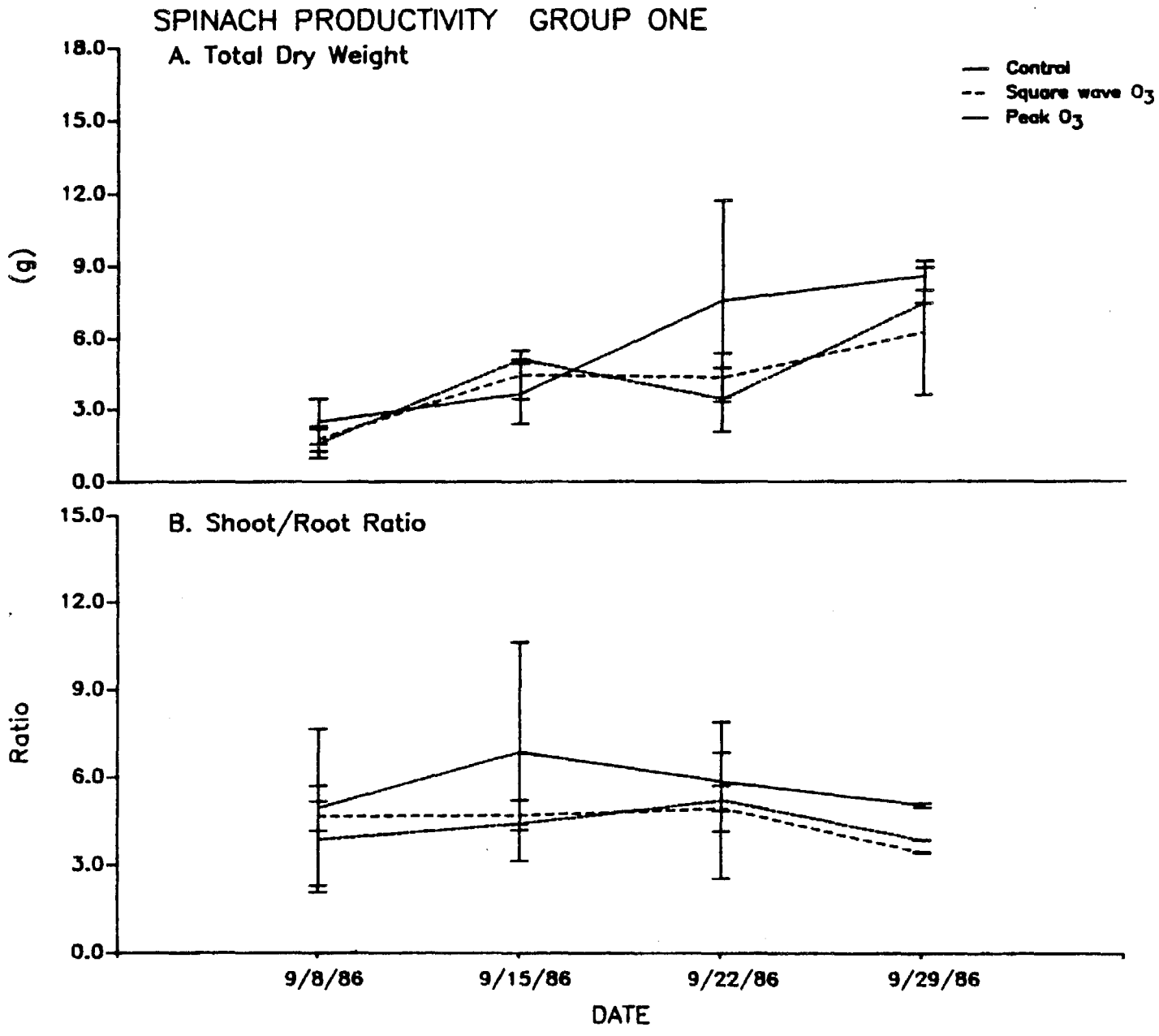

Figure 14. Effects of ozone on spinach productivity, group one. Data are for (A) total dry weight, and (B) dry weight shoot/root ratio. Values are means ± SD for two single plant replicates.

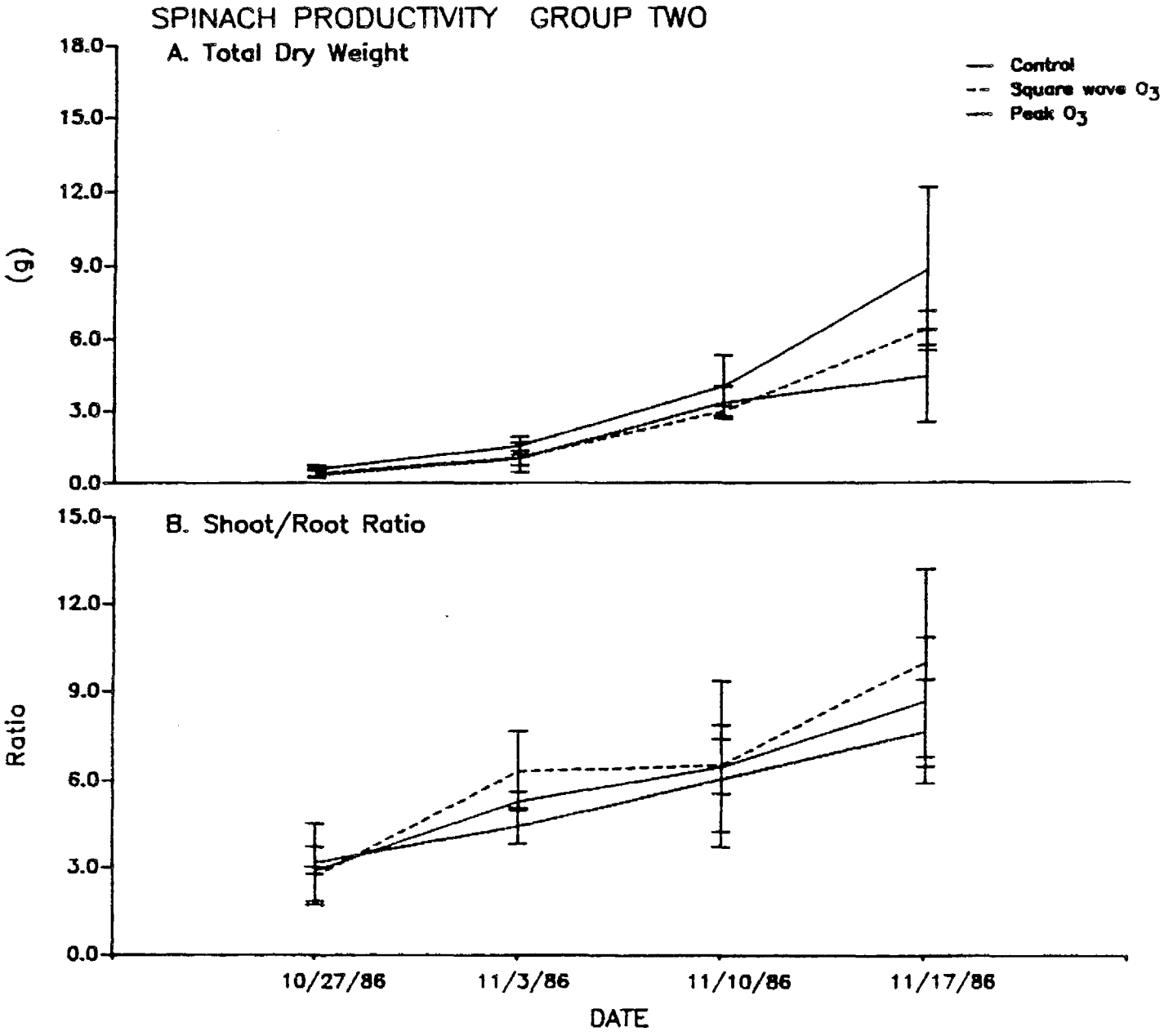

Figure 15. Effects of ozone on spinach productivity, group two. Data are for (A) total dry weight, and (B) dry weight shoot/root ratio. Values are means ± SD for four single plant replicates.

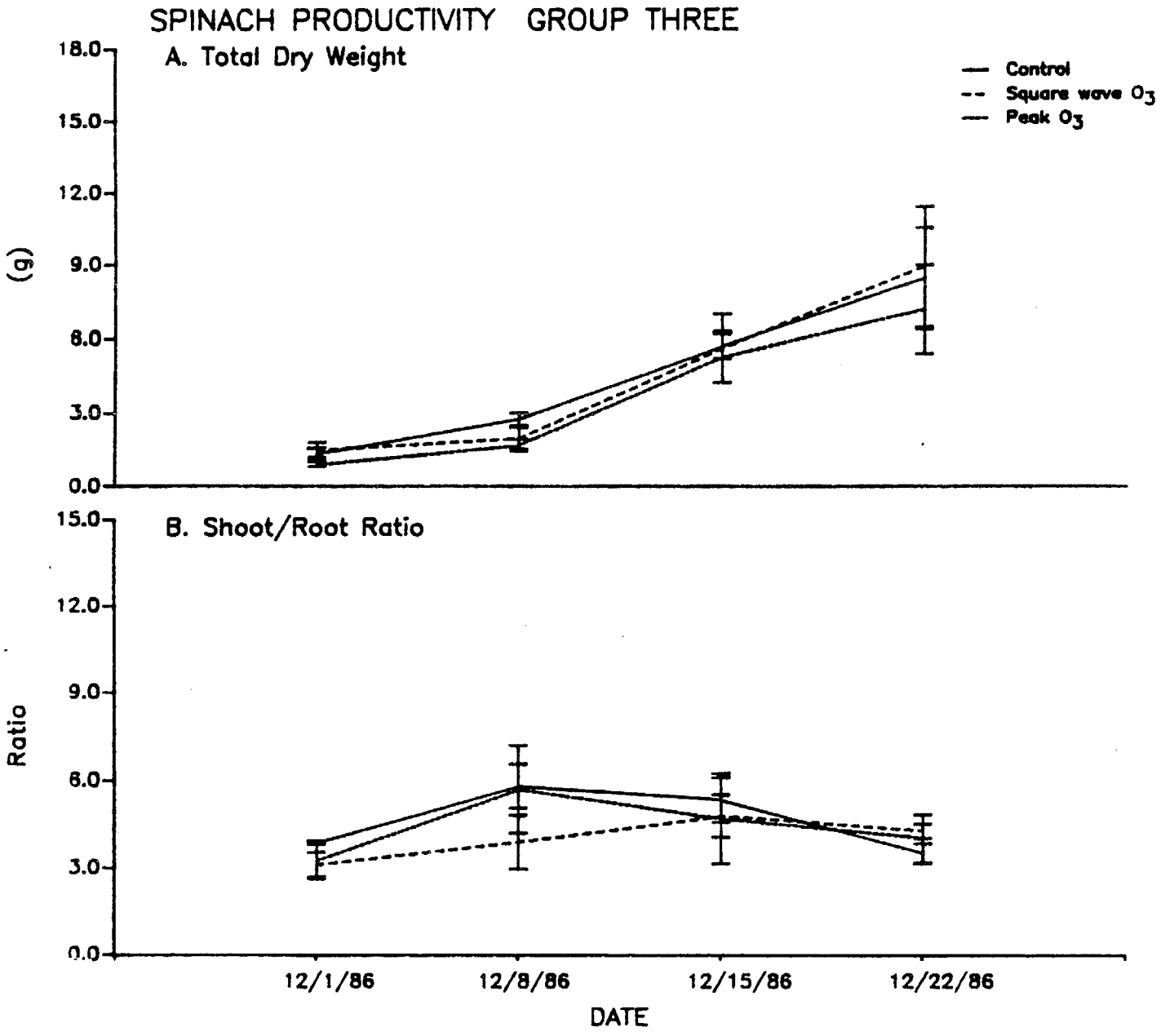

Figure 16. Effects of ozone on spinach productivity, group three. Data are for (A) total dry weight, and (B) dry weight shoot/root ratio. Values are means ± SD for four single plant replicates.

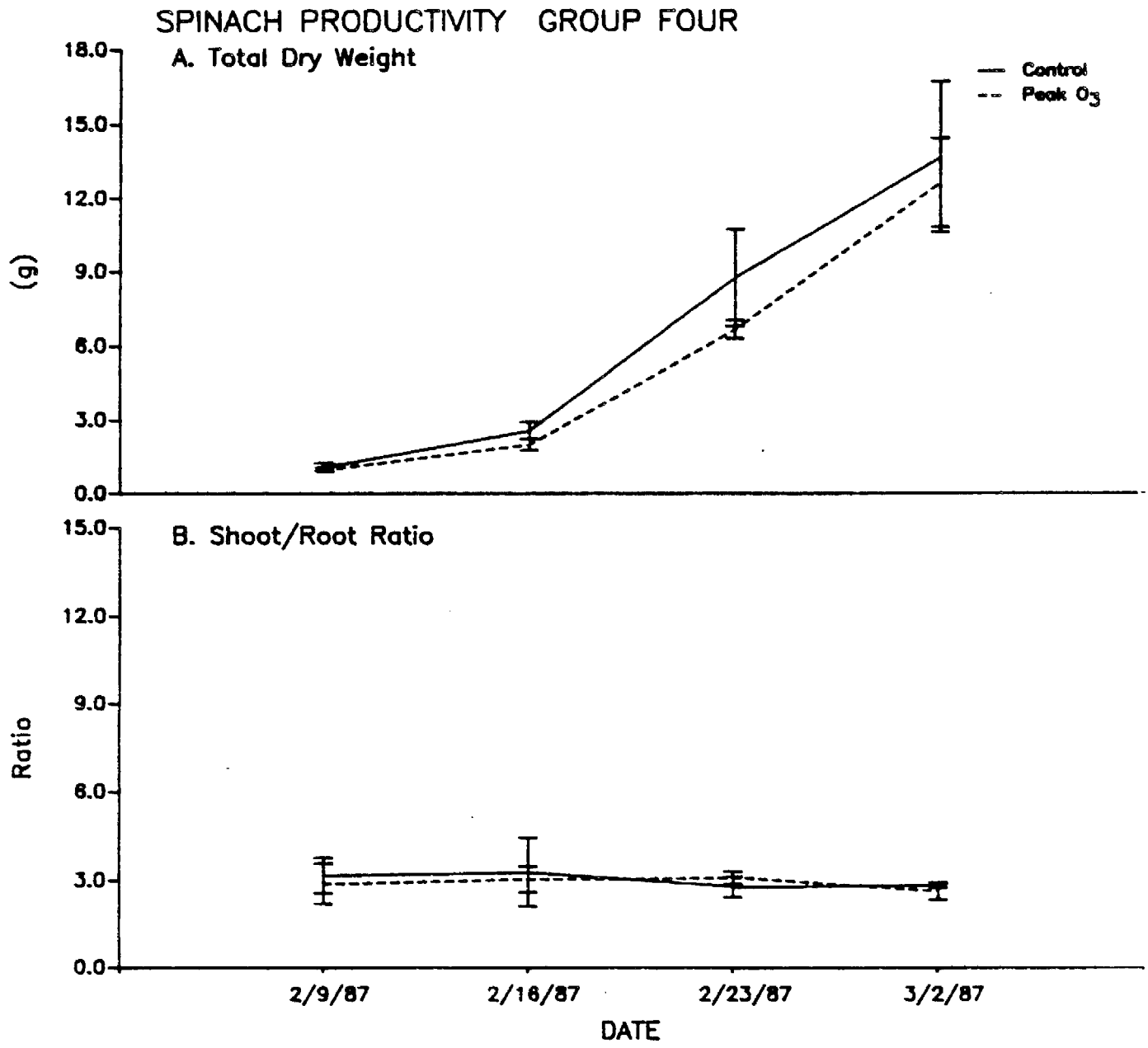

Figure 17. Effects of ozone on spinach productivity, group four. Data are for (A) total dry weight, and (B) dry weight shoot/root ratio. Values are means ± SD for four single plant replicates.

Table 10. Results from Statistical Analysis for Individual Species Productivity Measurements[a]

Species Group		Fresh Weights				Dry Weights			
		Shoot	Root	Total	Shoot/ Root	Shoot	Root	Total	Shoot /Root
Corn	1	ns	ns	ns	ns	ns	ns	ns	ns
	2	ns	ns	ns	ns	ns	ns	ns	ns
Squash	1	ns	ns	ns	ns	ns	*	ns	ns
	2	*	ns	ns	ns	ns	ns	ns	ns
Radish	1	**	***	***	ns	ns	***	***	ns
	2	ns	*	*	*	ns	**	*	***
Lettuce	1	*	ns	*	ns	ns	ns	ns	ns
	2	ns	ns	ns	ns	ns	ns	ns	ns

[a]Two treatments (control, peak O_3), across four measurement weeks and four single plant replicates per experiment. *, **, and *** indicate significant difference between treatments at $p<0.05$, 0.01, and 0.005 levels, respectively. All changes due to O_3 treatment are decreases, except for increase in shoot/root ratios for radish fresh and dry weights in experiment two.

dry weights. Of particular importance were the reductions in total dry weight for both groups of plants (Figures 22A and 23A), and increase in shoot/root ratio for group two (Figure 23B). Apparently, there was a reduction in the transport of photosynthate to the storage hypocotyl organ for repair of the O_3-damaged leaf tissue. For lettuce, fresh weights of the shoot and total plant were reduced by ozone for group one. No other statistically significant effects were observed (Figures 24 and 25).

Ozone had a large effect on fresh and dry weights when the data was analyzed across all species, weeks, and experimental groups (Table 11). However, the effect was due primarily to the effects of ozone on radish which overshadowed the general lack of ozone effects on the other species. This was expected as radish was very sensitive to ozone in previous studies (USEPA, 1978), and interspecific differences in response to ozone have been well documented (Tingey and Taylor, 1982).

Table 11. Results from Statistical Analysis for Interspecific Productivity Measurements[a]

Factor	Fresh Weights				Dry Weights			
	Shoot	Root	Total	Shoot/ Root	Shoot	Root	Total	Shoot/ Root
Air	**	ns	**	*	***	ns	ns	***
Species	***	***	***	***	***	***	***	***
A x S[a]	***	ns	***	**	***	ns	*	***

[a]Interaction consists of statistically significant effect for control vs. O_3 for radish, but not the other three species. There was an air effect only for radish.

C. Correlations Between Gas Exchange and Productivity

Regression analysis was carried out to determine whether plant productivity was directly related to the rates of gas exchange measured immediately after the O_3 exposures. Relative growth rates (RGR's) were used to correct biomass production for the amount of plant material present at the start of each weekly period, as described in the statistical analysis portion of the methods. Only stomatal conductance and photosynthesis were used for the analysis. Transpiration was not used as it was not as sensitive to O_3 exposure as stomatal conductance in these studies (Tables 6 and 8), or as directly related to potential productivity as photosynthetic rate.

Spinach. Net photosynthesis but not stomatal conductance was correlated with plant productivity. Table 12 indicates the correlation coefficient (r) values for RGR vs. conductance and photosynthesis calculated for individual treatments for groups two though four, and for pooled data from groups one through three. Individual treatments were not evaluated for group one as there were only six replicates. Data from group four were not included in the pooled analysis because the square wave treatment was not present.

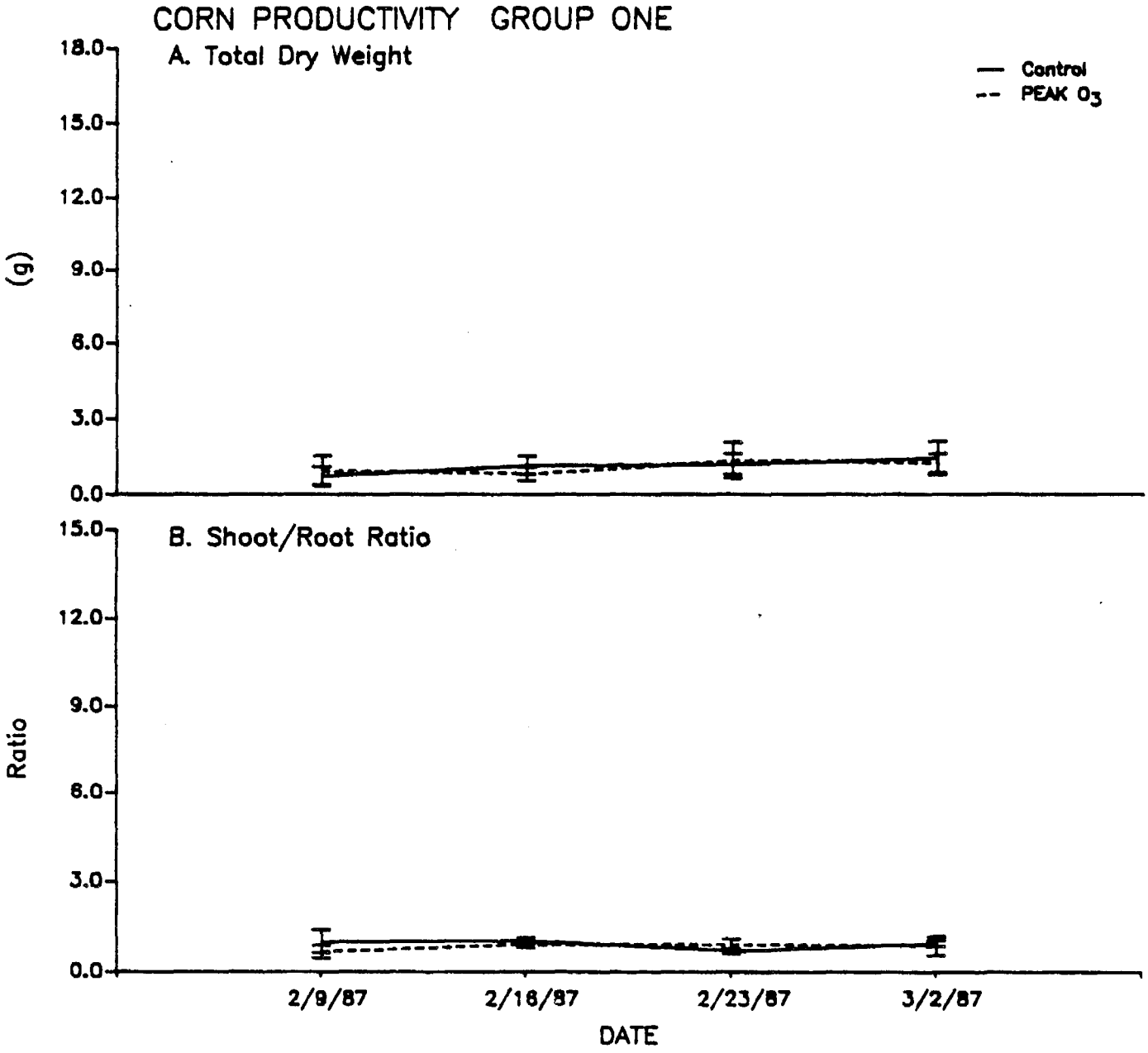

Figure 18. Effects of ozone on corn productivity, group one. Data are for (A) total dry weight, and (B) dry weight shoot/root ratio. Values are means ± SD for four single plant replicates.

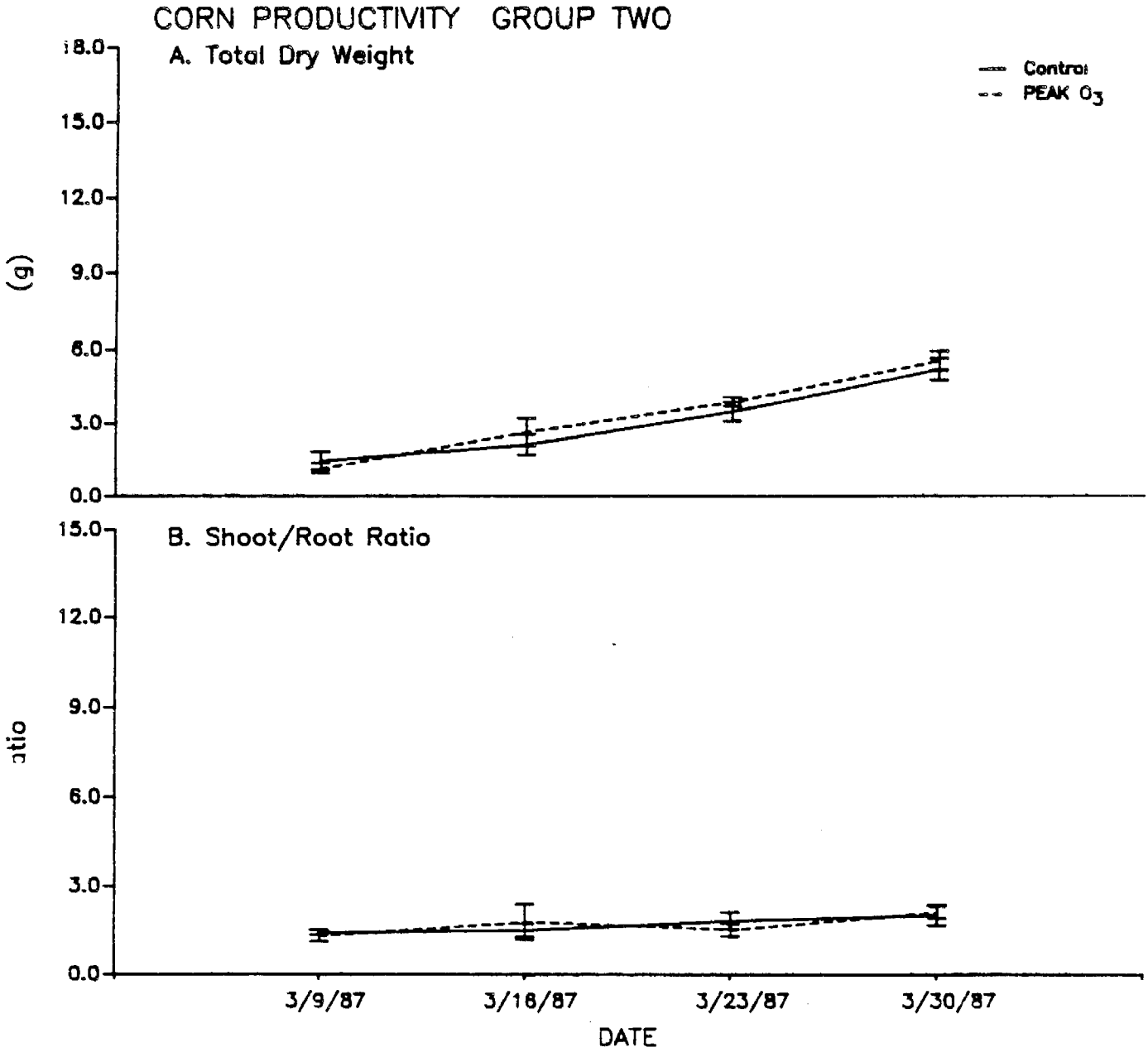

Figure 19. Effects of ozone on corn productivity, group two. Data are for (A) total dry weight, and (B) dry weight shoot/root ratio. Values are means ± SD for four single plant replicates.

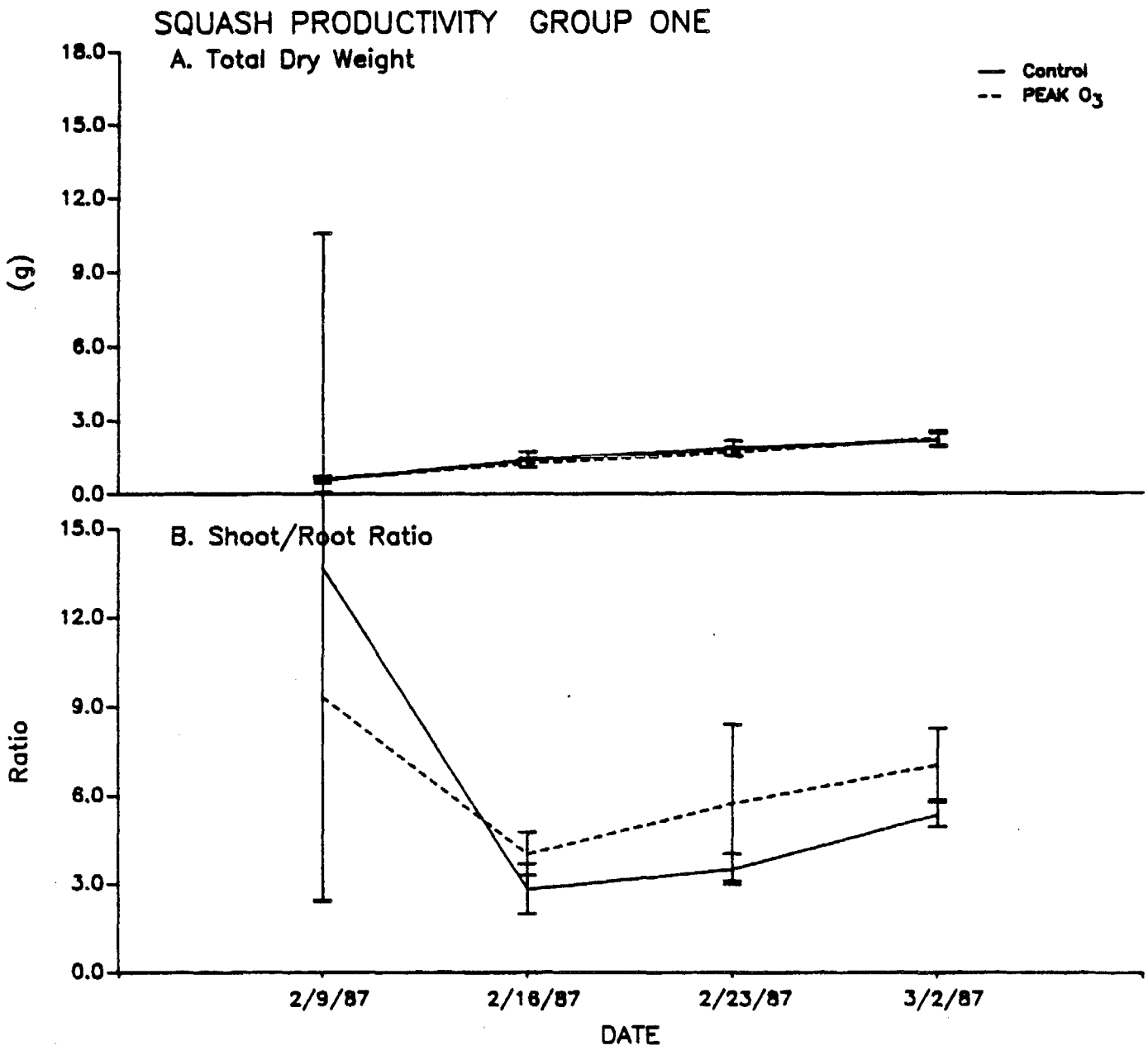

Figure 20. Effects of ozone on squash productivity, group one. Data are for (A) total dry weight, and (B) dry weight shoot/root ratio. Values are means ± SD for four single plant replicates.

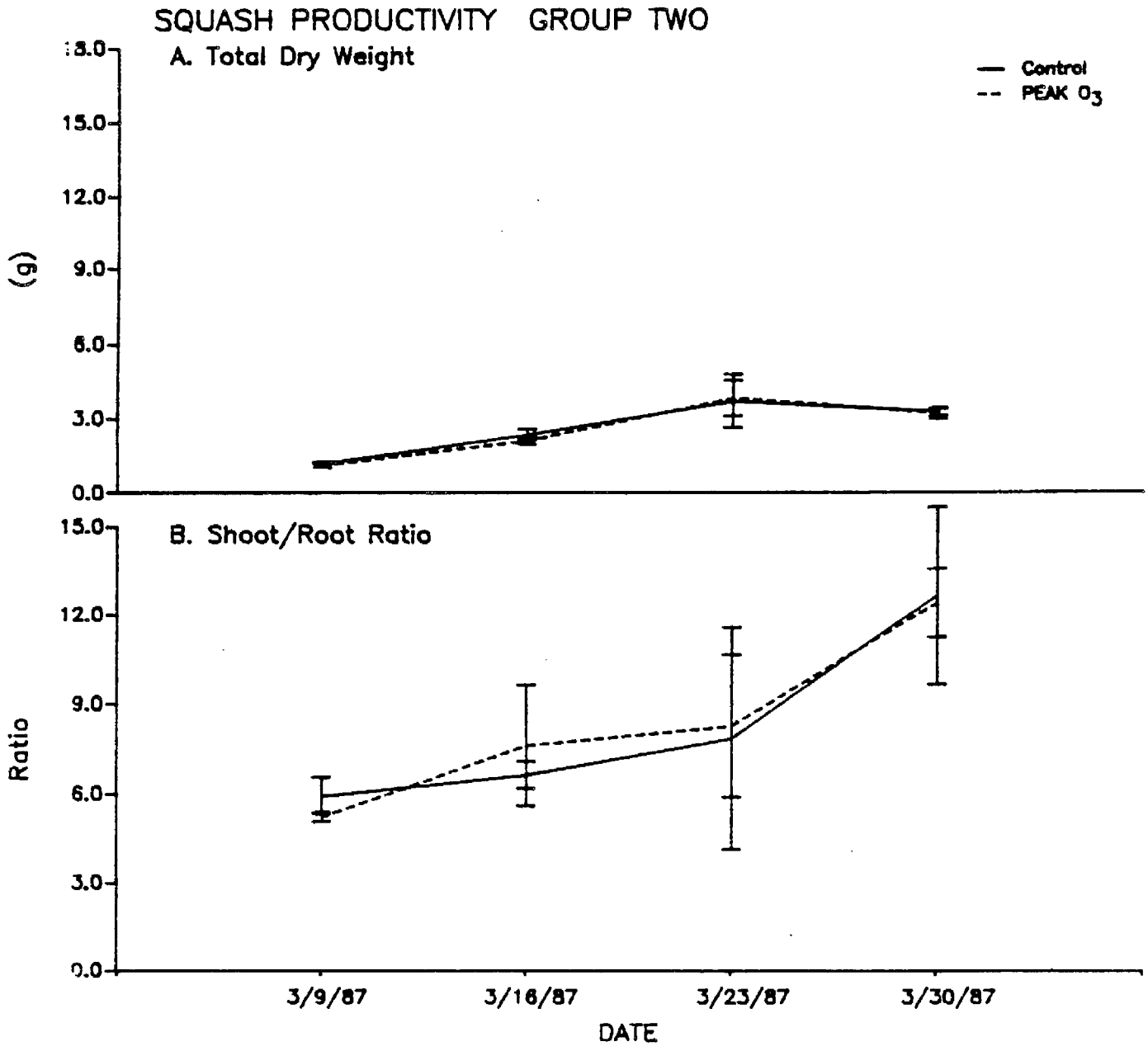

Figure 21. Effects of ozone on squash productivity, group two. Data are for (A) total dry weight, and (B) dry weight shoot/root ratio. Values are means ± SD for four single plant replicates.

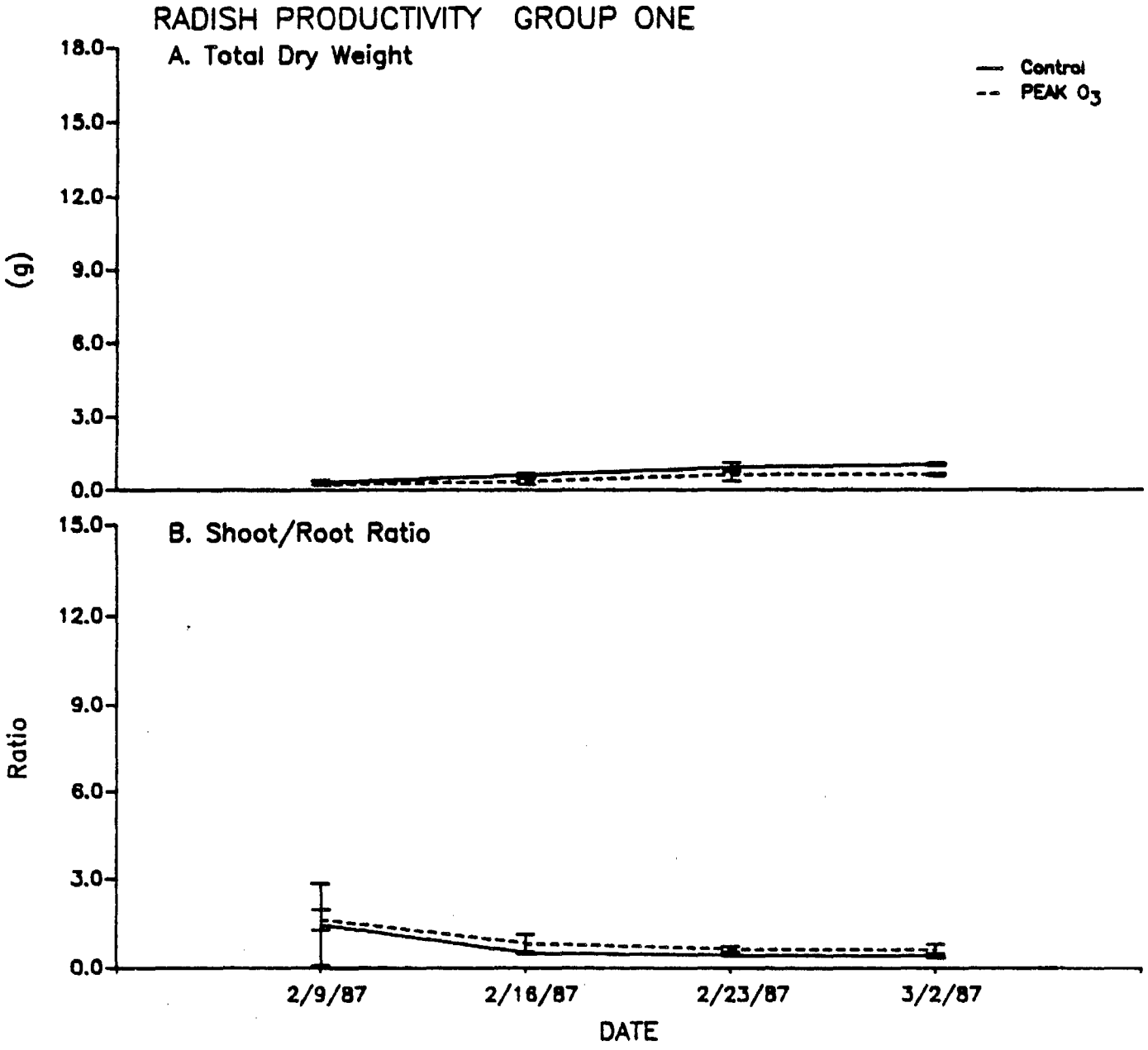

Figure 22. Effects of ozone on radish productivity, group one. Data are for (A) total dry weight, and (B) dry weight shoot/root ratio. Values are means ± SD for four single plant replicates.

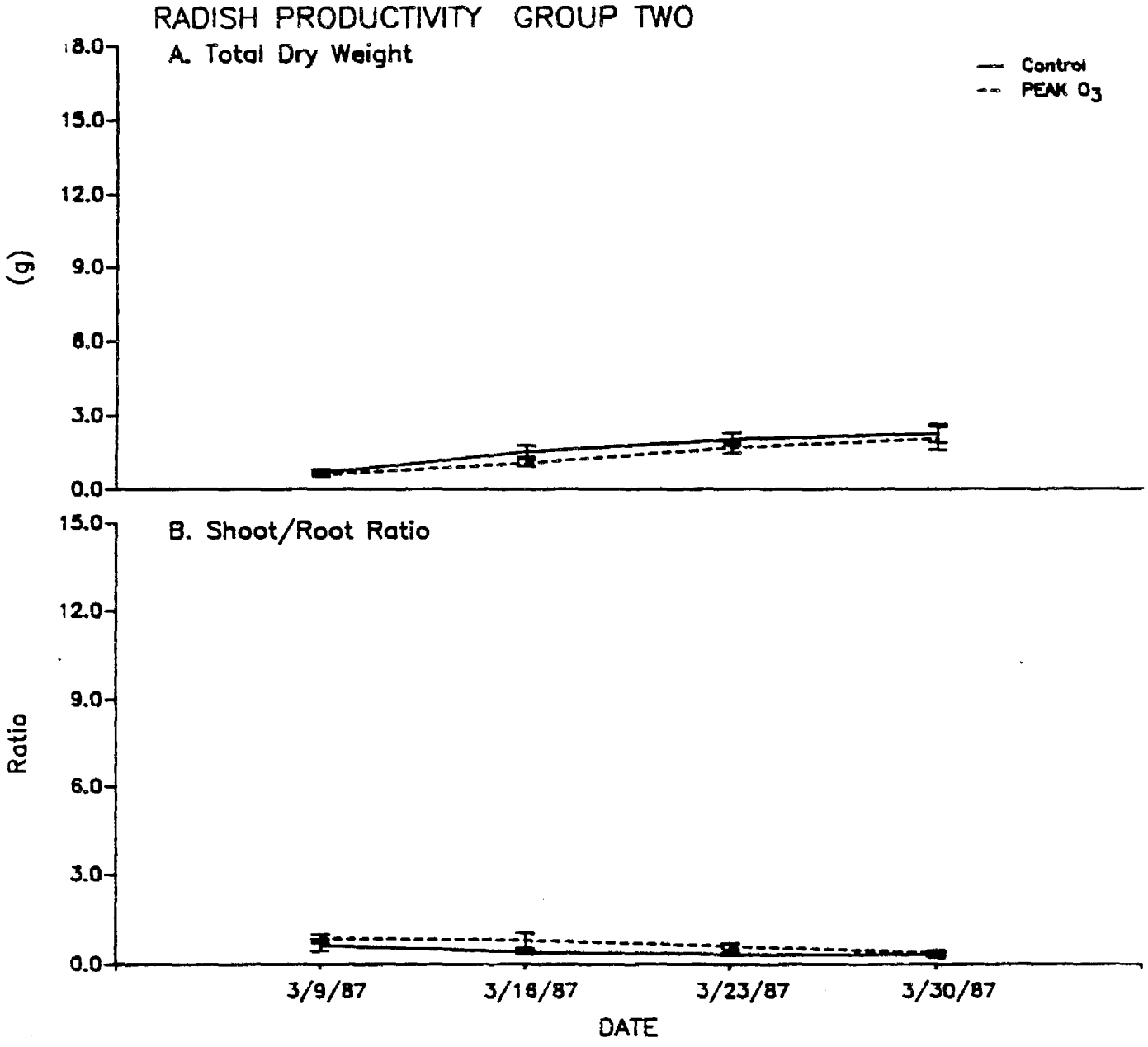

Figure 23. Effects of ozone on radish productivity, group two. Data are for (A) total dry weight, and (B) dry weight shoot/root ratio. Values are means ± SD for four single plant replicates.

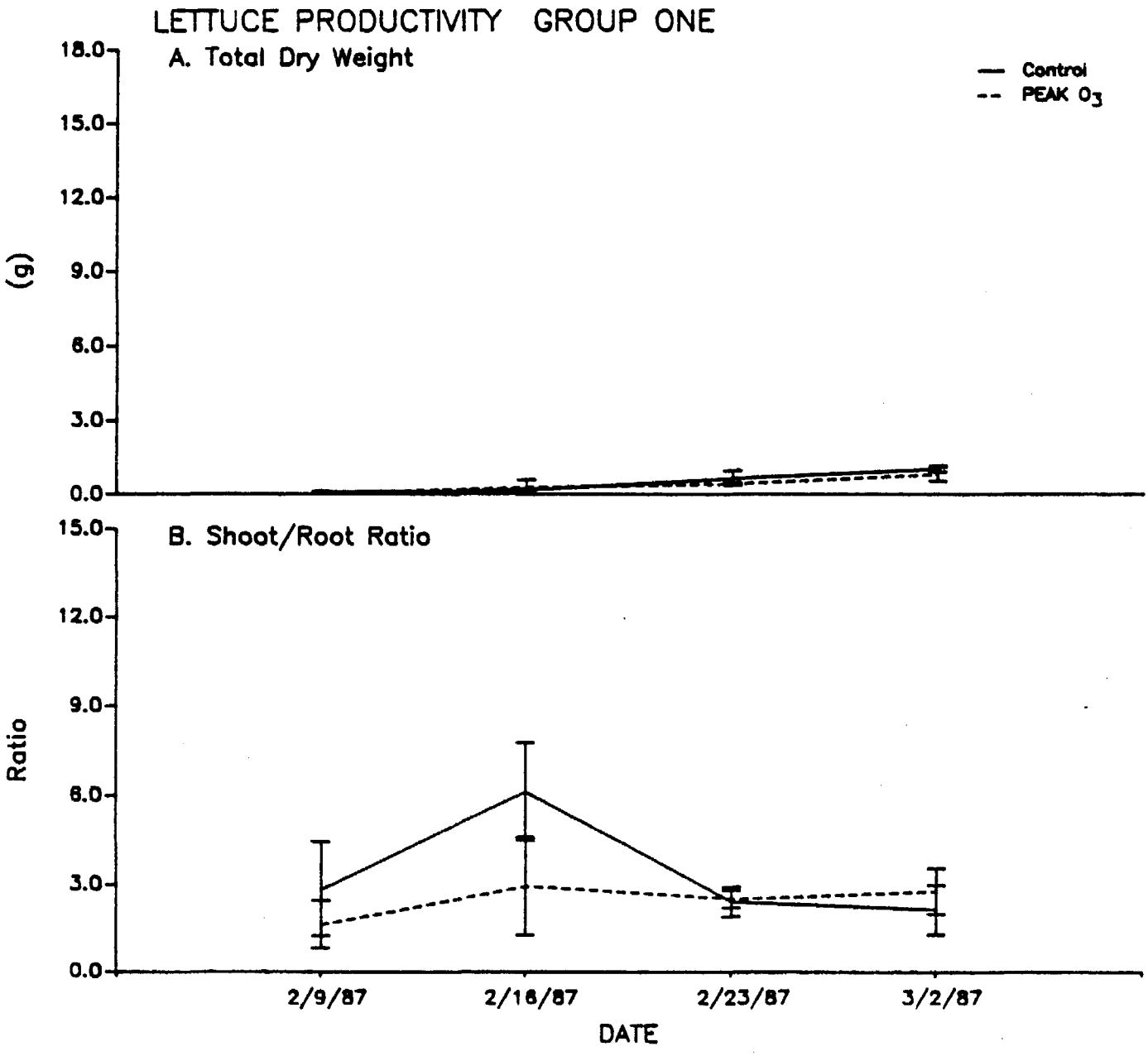

Figure 24. Effects of ozone on lettuce productivity, group one. Data are for (A) total dry weight, and (B) dry weight shoot/root ratio. Values are means ± SD for four single plant replicates.

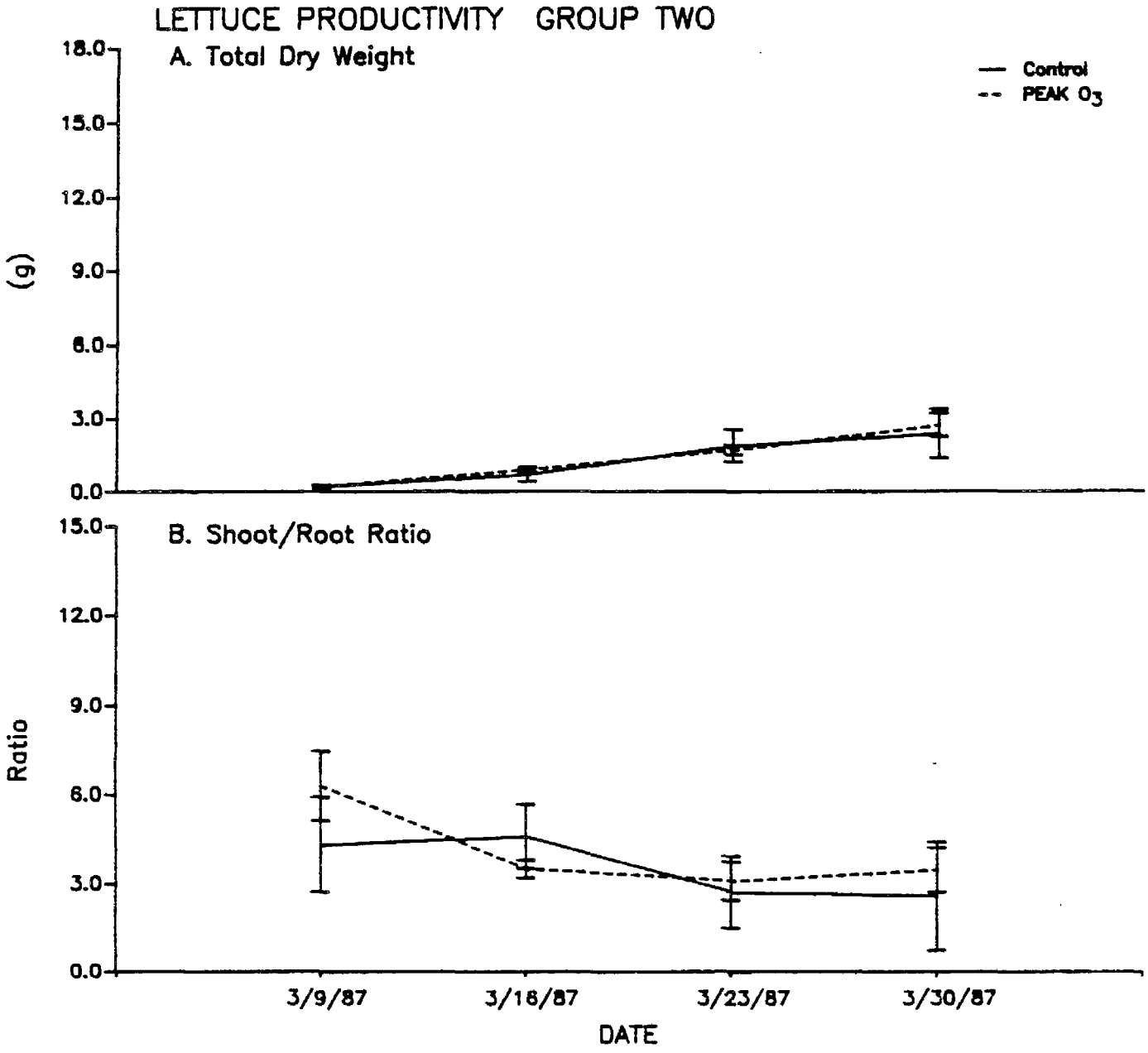

Figure 25. Effects of ozone on lettuce productivity, group two. Data are for (A) total dry weight, and (B) dry weight shoot/root ratio. Values are means ± SD for four single plant replicates.

The correlation between RGR and photosynthesis was significant only for control plants as shown for groups three and four (Table 12). No RGR-photosynthesis correlations were found with ozone treatment. Even though photosynthesis was correlated with RGR for two groups of control plants, the relationships for the two groups were contradictory. There was a positive relationship for group four (Figure 26). This indicated that RGR increased as photosynthetic rate increased, as was intuitively expected. However, the strong correlation for group three actually was negative, with RGR increasing as photosynthetic rate decreased.

Interspecific The relationships between RGR and conductance or photosynthesis were even more variable for the four species than for spinach (Table 13). There was a significant correlation between photosynthetic rate and RGR for squash group one, and radish group two. In both cases the correlation was for control plants and not ozone treated plants - which was the same relationship previously found for spinach. However, there also was a significant correlation between conductance and RGR for some groups of squash and lettuce. Furthermore, for squash group two and lettuce group two the correlation occurred without a similar correlation between photosynthesis and RGR. For lettuce group two, the correlation was for the ozone-treated but not the control plants - which was contrary to the correlation seen for control plants for spinach.

There are several possible reasons why the correlation coefficients were so low for spinach and the four other species. First, physiological measurements were not made for five of the seven days in each week prior to each harvest. Thus, there was ample time for the plants to recover and possibly compensate for any physiological process rate depressions due to ozone. Second, there are many other steps in allocation of carbon fixed by photosynthesis before dry matter is formed in plants. If some of these rate limiting steps were more important than photosynthetic rate or stomatal conductance, than dry matter may not have been affected by changes in those gas exchange parameters due either to ozone or general environmental conditions which determined the productivity of each plant. However, the general scatter in both physiological and productivity response data was likely the primary reason why the correlations were so poor between RGR and the physiological parameters.

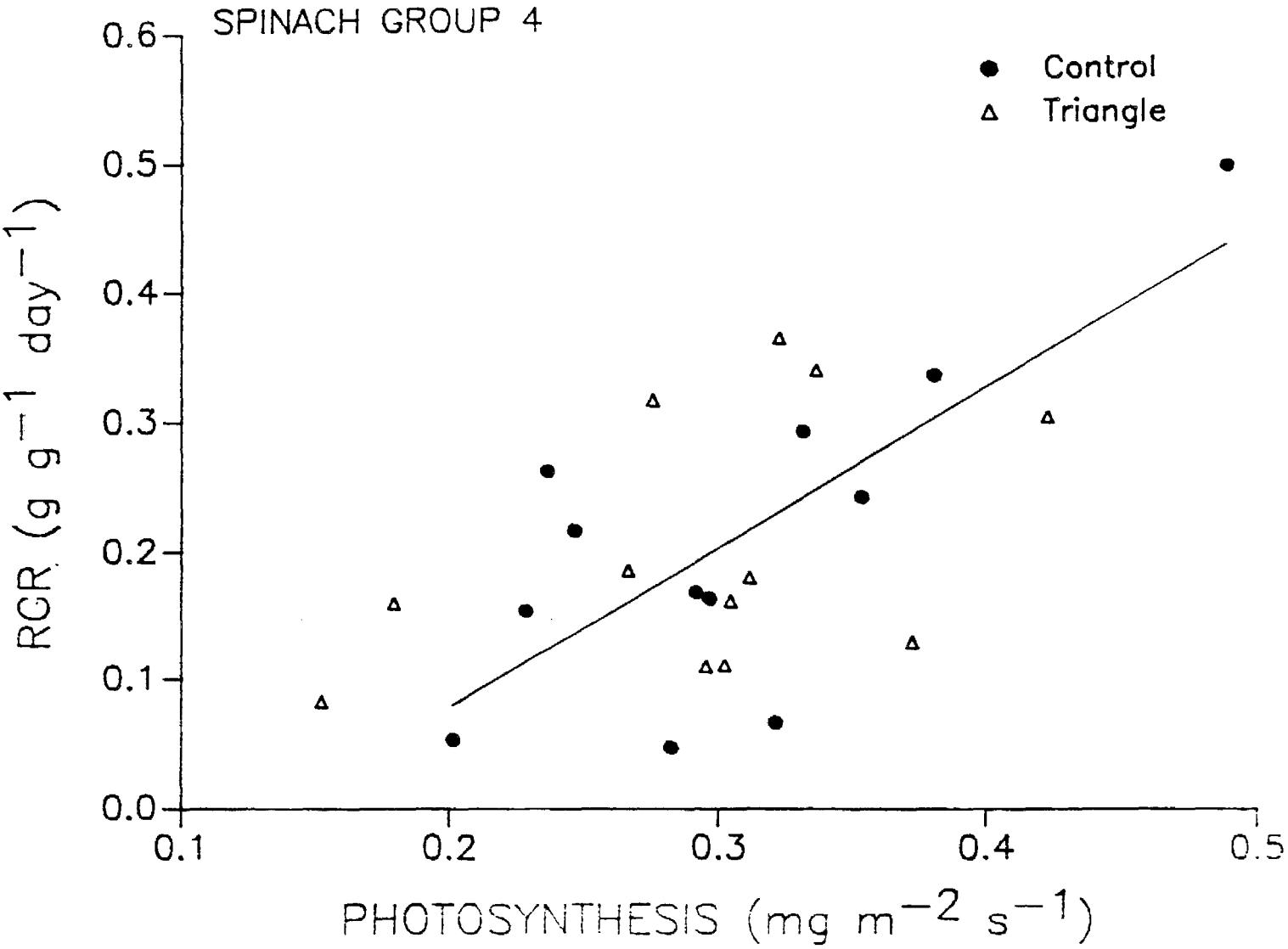

Figure 26. Correlation between photosynthetic range and relative growth rate (RGR) for spinach, group four across both control and ozone exposed (triangle-peak) plants. RGR = (Photosynthesis x 0.9371)-0.0754, where df=24.

Table 12. Correlation Coefficients Between Spinach Relative Growth Rate (RGR) and Stomatal Conductance or Net Photosynthesis[a]

Group	Treatment	nb	r^2 for RGR vs. Conductance	r^2 for RGR vs. Photosynthesis
1-3	All three	88	-0.004	-0.033
	Control	30	-0.007	-0.111
	Square Wave	30	-0.006	-0.027
	Triangle-Peak	28	0.010	-0.001
2	Control	12	0.050	0.179
	Square Wave	12	-0.005	-0.001
	Triangle-Peak	12	0.038	0.303
3	Control	12	-0.253	-0.623***
	Square Wave	12	-0.048	-0.022
	Triangle-Peak	12	-0.049	-0.185
4	Both	24	0.011	0.382***
	Control	12	0.101	0.561**
	Traingle-Peak	12	-0.221	0.196

[a] r^2 followed by ***,**, or * are statistically significant at $p<0.005$, 0.01, and 0.05 respectively.
[b] df = n-2.

Table 13. Correlation Coefficients Between Relative Growth Rate (RGR) and Stomatal Conductance or Net Photosynthesis for the Interspecific Study[a]

Species	Group	Treatment	n[b]	r for RGR vs. Conductance	r for RGR vs. Photosynthesis
Corn	1	Control	12	0.222	0.243
		Triangle-Peak	12	-0.026	0.005
	2	Control	12	-0.003	0.004
		Triangle-Peak	12	-0.008	-0.002
Squash	1	Control	12	0.317	0.343*
		Triangle-Peak	12	0.270	0.125
	2	Control	12	0.510**	0.037
		Triangle-Peak	12	0.521**	0.270
Radish	1	Control	12	0.096	0.058
		Triangle-Peak	12	-0.066	0.203
	2	Control	12	0.202	-0.432*
		Triangle-Peak	12	-0.007	0.248
Lettuce	1	Control	8	0.046	0.084
		Triangle-Peak	8	-0.070	0.159
	2	Control	12	0.012	0.004
		Triangle-Peak	12	0.419*	0.017

[a] r followed by ***,**, or * are statistically significant at $p<0.005$, 0.01, and 0.05 respectively.
[b] df = n-2.

D. Ozone Effects on Chlorophyll Fluorescence

The fluorescence level from chlorophyll in green plants is not constant. Upon exposure to constant light, the level jumps immediately to a base line level called F_o, which is the so-called "non-variable" or "dead" fluorescence level. The fluorescence level then changes in time dependent fashion depending upon species. Generally, there is a small time period where the fluorescence level rises either slightly or rises and then falls slightly (within the first 100-200 milliseconds). The chlorophyll fluorescence then rises to a maximum after about one second of illumination (to the peak level or F_p, which is 2-3 times as large as F_o). The chlorophyll fluorescence level then falls, exhibiting several shoulders or secondary peaks for the next 10 to 20 seconds. The level of chlorophyll fluorescence finally levels off within one minute to a low level, very close to that of the initial or F_o level.

The studies in the literature on chlorophyll fluorescence suggested that chlorophyll fluorescence could be used as a monitor of ozone injury. Unfortunately, the literature demonstrates changes of green plant fluorescence only when the plant has been exposed to severe levels of ozone and and the leaf exhibits visible injury. In this study, we wanted to determine whether chlorophyll fluorescence could be used as an indicator of early or mild ozone injury to the plants. We expected that the F_o level would be relatively unaffected by the exposure and that only the kinetics would demonstrate changes. In general, that is what we have found, although the effects are much smaller and more subtle that we expected.

The work was divided into two phases. First of all, we found that the control level of spinach fluorescence was slightly variable, giving a 5% to 10% variation in both F_o and F_p values when many leaves from a variety of plants, unexposed to ozone, were used. (Table 14 and later data). These results indicated that we would be forced to use a large number of plants and leaves in order to determine subtle effects on either F_o or F_p. However, these studies did show that this approach could be used to determine the kinetics with relatively good precision.

We determined that the effects upon chlorophyll fluorescence induced by ozone exposure were subtle under the conditions used here. We saw only very small changes, generally in the F_p and the secondary peaks upon exposure. These effects were difficult to quantify and highly variable.

This variability meant that we could not determine the peak changes induced by exposure by the normal graphic methods which we had previously employed. Thus, in phase two, we were forced to rewrite our computer programs to allow a better evaluation of each individual curve (this is a process which is continuing).

As described in the methods section, we cut leaf discs from control plants or plants which had been exposed ozone. These discs were then taken back to the laboratory in the dark and maintained, floating on distilled water in a closed, darkened petri dish, for approximately 30-90 minutes following the protocol illustrated in Appendix C. This period was determined to allow the photochemistry and CO_2 fixation levels to reset to a standardized level (Table 14 and Figure 27). Placing the leaf disc into the Hansatec fluorometer, we exposed the disc to a five second pulse of light to begin all experiments from a standardized level. This was then followed by a series of light-dark cycles, the timing of which allowed the photochemistry to "de-adapt" in a uniform manner so that the measurements at each cycle could be superimposed upon each other. The four cycles used to determine the chlorophyll fluorescence are shown in Figure 28A for spinach. The top left-hand corner shows the first 50 milliseconds of the chlorophyll fluorescence kinetics, including time before the light goes on, to indicate a base line reference ("zero light"). The initial six milliseconds (from the second to the third line, roughly) shows the shutter opening for our illumination system. There is, as you can see, a small 120 cycle "beat frequency" due the line current picked up the instrument. In the lower left figure, the first half second of illumination and its effect upon chlorophyll fluorescence is shown. In spinach we can see a small rise within the first 50-100 milliseconds followed by a slight lag and then a linear rise to a final level, which is nearly reached at the end of the half-second illumination period. In section 3 (upper right-hand graph), we see the first 5 second of illumination in which the peak, or F_p level, is easily ascertained followed by the decline to a lower level. In the lower right-hand figure (-4) we see the full 50 seconds illumination for spinach. The peak which occurs within the first one-second, is obvious, followed by the secondary shoulder occurring at about 5-10 seconds. This secondary shoulder gradually declines to a final, very low level of chlorophyll fluorescence occurring after about 30 seconds of illumination. In this particular

trial we see a slight subtle rise occurring to about 50 seconds. (In other experiments this small "M" peak reaches a maximum at about 60 seconds and decays away to a lower baseline level at about 1 to 2 minutes more). This "M" peak is very difficult to study due to its small size, its variability, and its interaction with photosynthesis is not clearly understood.

In Figure 28B we see the effects of the chlorophyll fluorescence for exposed leaves (compare this to Figure 28A). No effects can be determined by the eye in this exposure sequence. However, you will note that there are two printed values on the curve, designates F_o and F_p in the lower right-hand corner. These are the values of F_o and F_p determined for the pre-conditionary flash. From these values, we see that F_o and F_p do vary somewhat (compare Figure 28A and B). If we are to determine the changes in the variable fluorescence which seems to be more of an indication of the CO_2 fixation rates, we must divide the peak fluorescence level by the initial fluorescence level, denoted as the variable (VAR.) F level (Fv) on the two curves. In this particular experiment we note that the exposure results in a slight lowering in the variable F level by a few percent. We will return to this point later.

If this was the whole story, we could then take a very close look at the variable chlorophyll fluorescence kinetics. However, we do get variations which are shown in Figure 28C and D. For all intents and purposes, these are experiments on two similar leaves from similar plants but done a day later (compared to Figure 28A and B). Unfortunately, the kinetics are more variable here with the variable F levels being lower and the secondary shoulder (which occurs at several seconds after the illumination is turned on) being much more pronounced. Fortunately, this particular sequence of kinetics was not often observed and Figure 28A and B are more usual for spinach. We do not know why these levels change, but most probably they are due to either developmental age and/or environmental condition during the growth period of that particular leaf.

In Table 15 we tabulate the F_o, F_p, and F_v, as well as the ratio of F_p versus F_o for a variety of experiments that we have done. The average of F_o and F_p are very similar for both control and exposed plants. The general variation in F_p from experiment to experiment is large. The time period which the F_p occurs (Tr) likewise is similar in the exposed and

Table 14. Variation of Initial Fluorescence (F_o) With Light Cycles: I. Variation of F_o With Leaf, II. Variation of F_o with Dark Absorption Time[a]

Experiment I. - Leaf Variation[a]		Experiment II. - Dark Absorption[b]	
Leaf Sample	F_o (relative)	Time (min)	F_o (relative)
1	53.7 ± 2.1	30	57.8 ± 4.8
2	66.1 ± 2.3	60	54.5 ± 4.8
3	51.1 ± 1.6	90	56.4 ± 6.9
4	55.9 ± 2.2	120	54.5 ± 5.3
5	54.8 ± 1.0	150	56.1 ± 5.3
6	52.6 ± 0.8	180	52.8 ± 1.5

[a] The average F_o (± SD) for discs from six spinach leaves was determined over five total light periods (pre-incubation plus four cycles, see Methods Section -II.B.).

[b] The average F_o (± SD) for discs from four spinach leaves was determined over the first three cycles of light-dark periods (see Methods Section -II.B.). The dark adaption was the period that the leaf discs were floating on water in the dark.

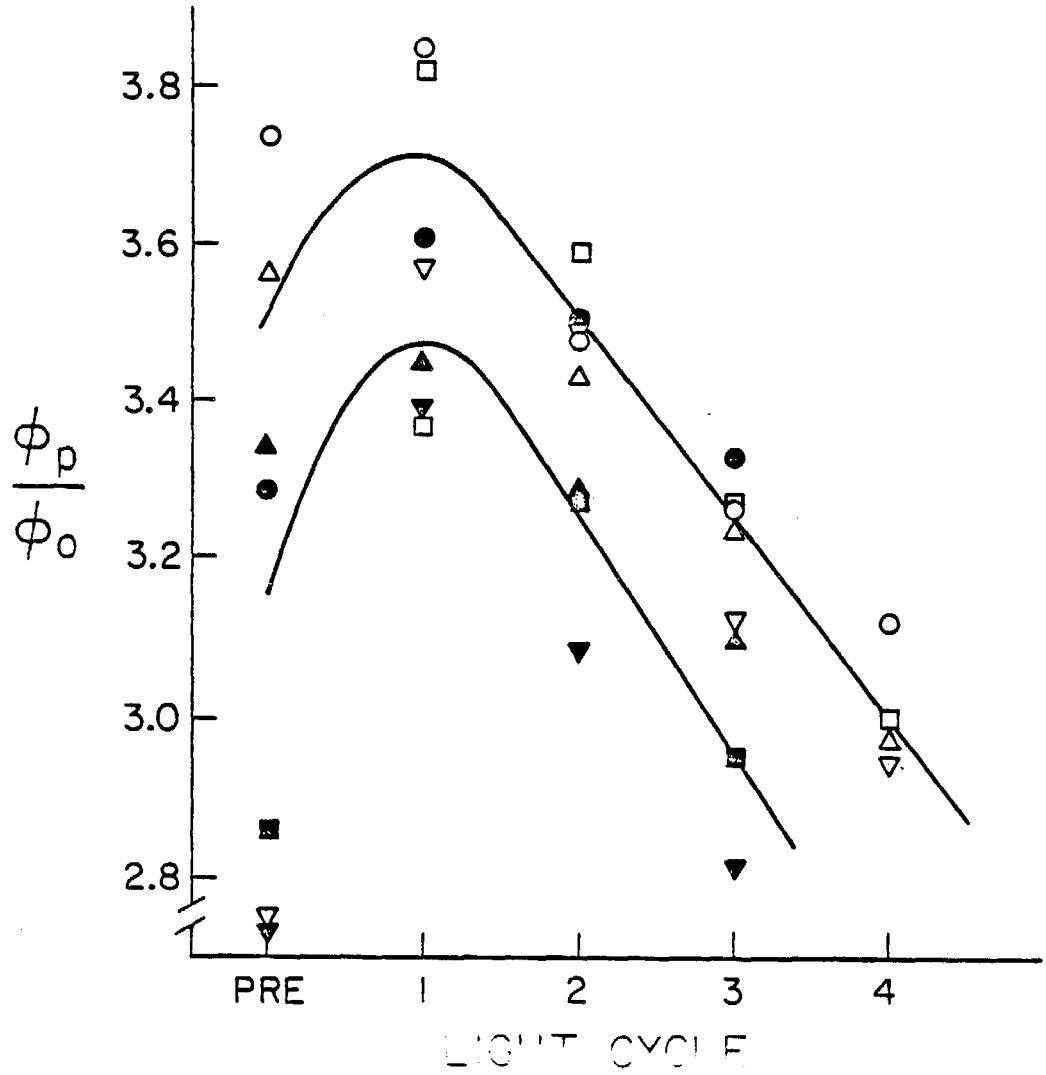

Figure 27. Variation of Variable Peak Fluorescence During Light-Dark Cycles. The fluorescences from discs from eight leaves of spinach plants were studied by light-dark cycling as described in the Methods Section (II.B.). The peak fluorescence ($\phi_p = F_p$) was normalized by the initial fluorescence ($\phi_o = F_o$) to yield the variable fluorescence. The preconditioning pulse of light (PRE) yielded more variable results. The cycle series yielded a drop in peak as the sequence progressed. The lines are visible fits of the data.

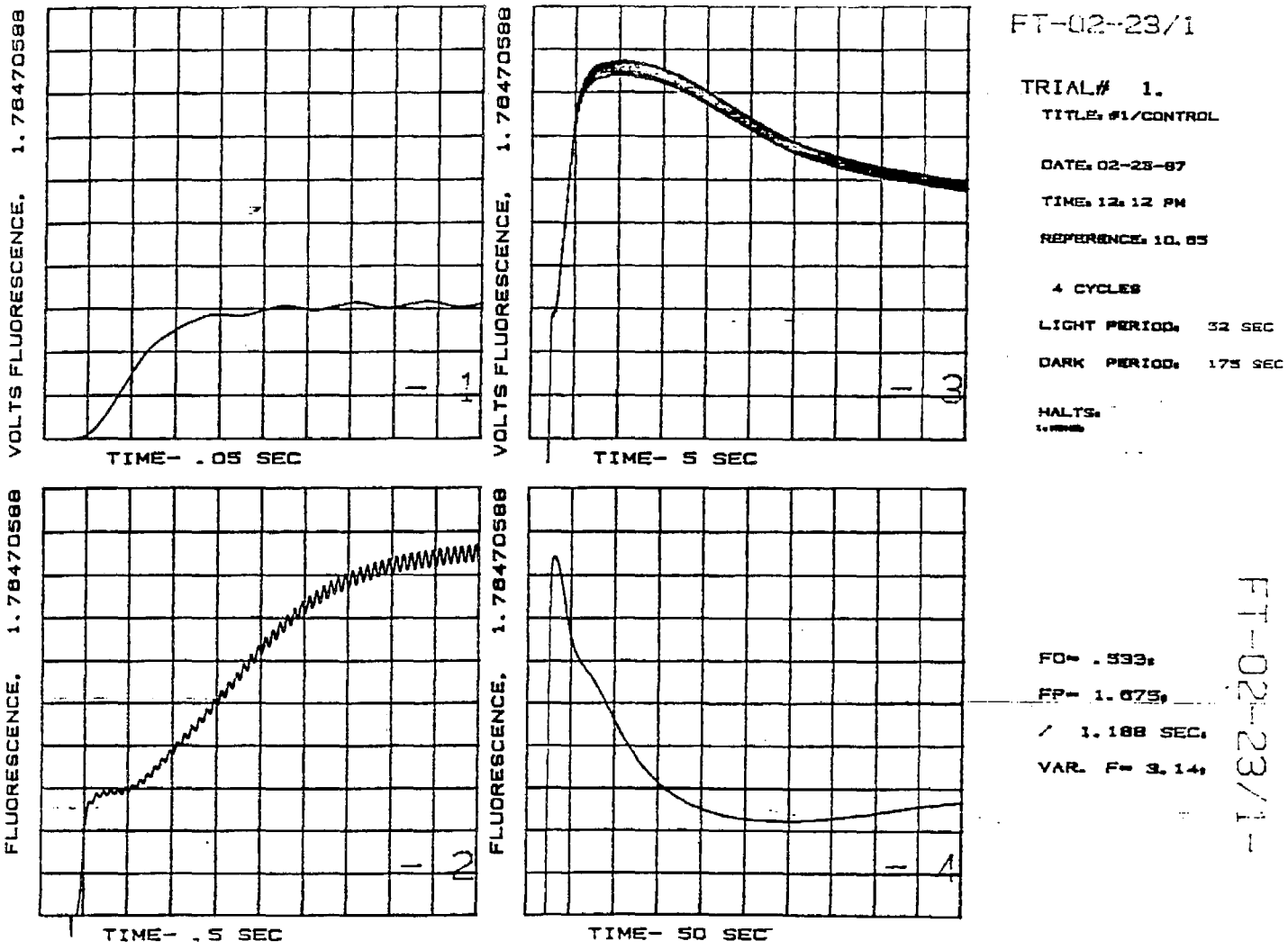

Figure 28. Chlorophyll fluorescence kinetics for spinach. (A) Trial 1, Control

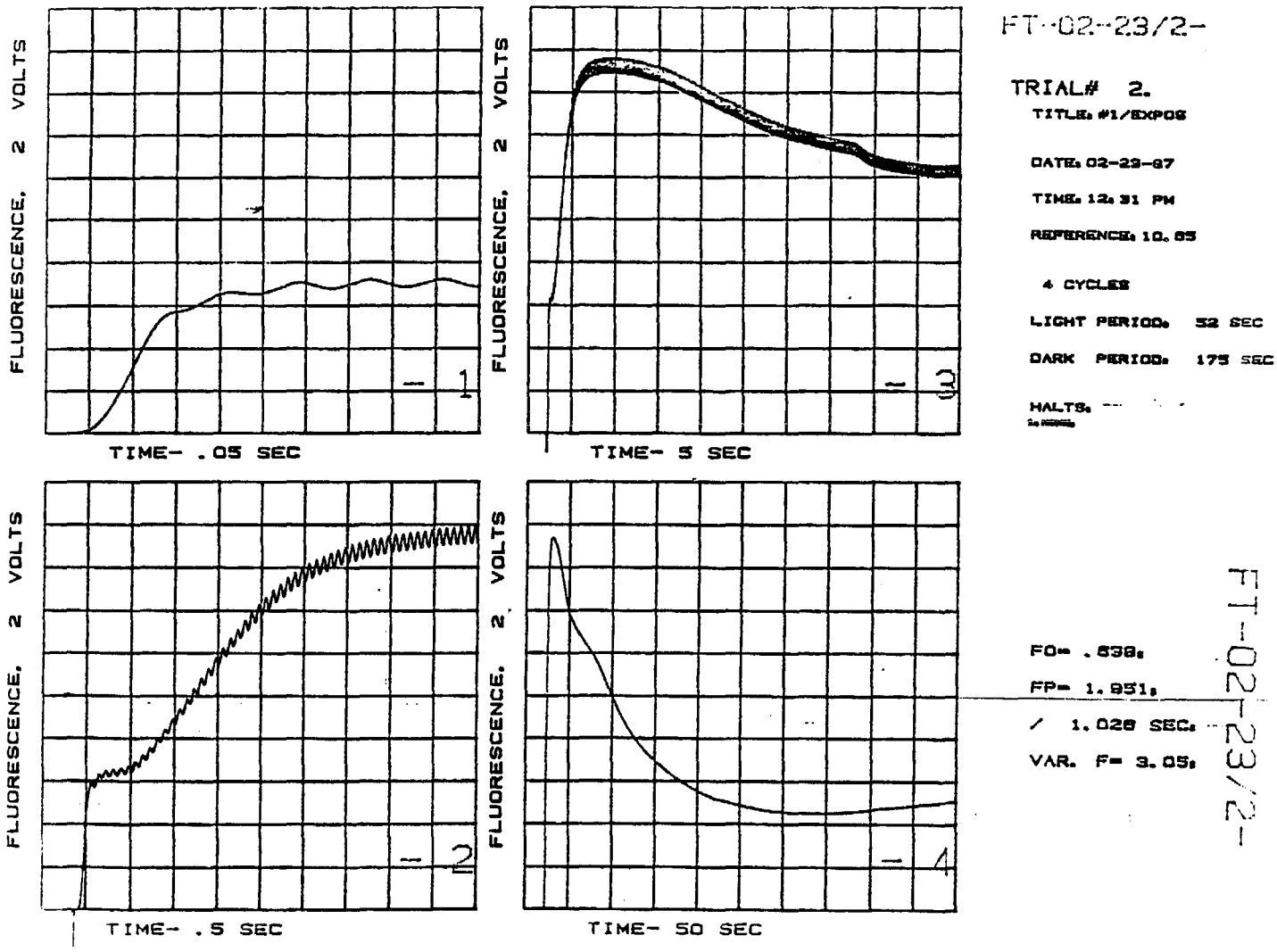

Figure 28. Chlorophyll fluorescence kinetics for spinach. (B) Trial 2, Ozone

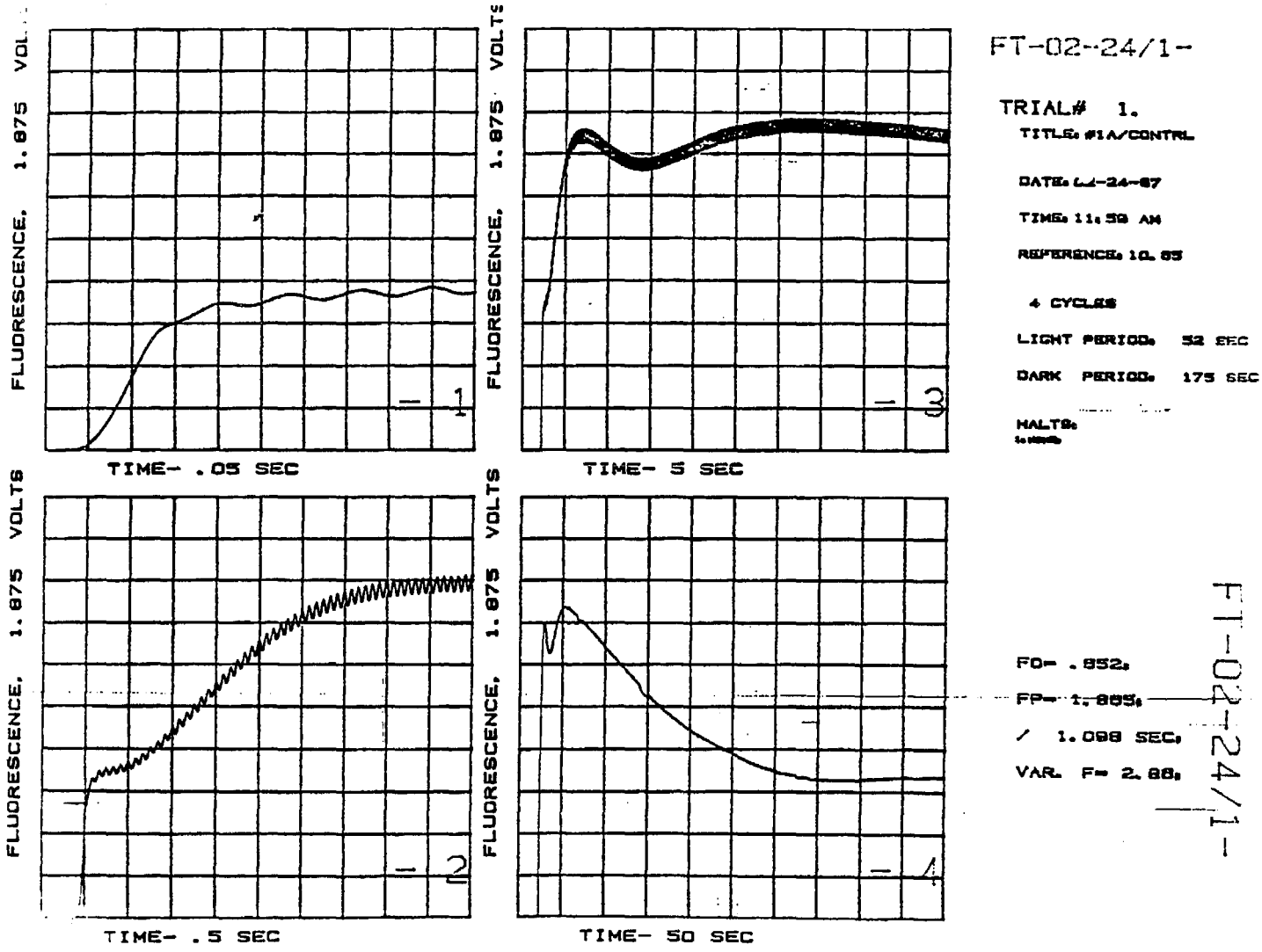

Figure 28. Chlorophyll flourescence kinetics for spinach. (C) Trial 1A, Control

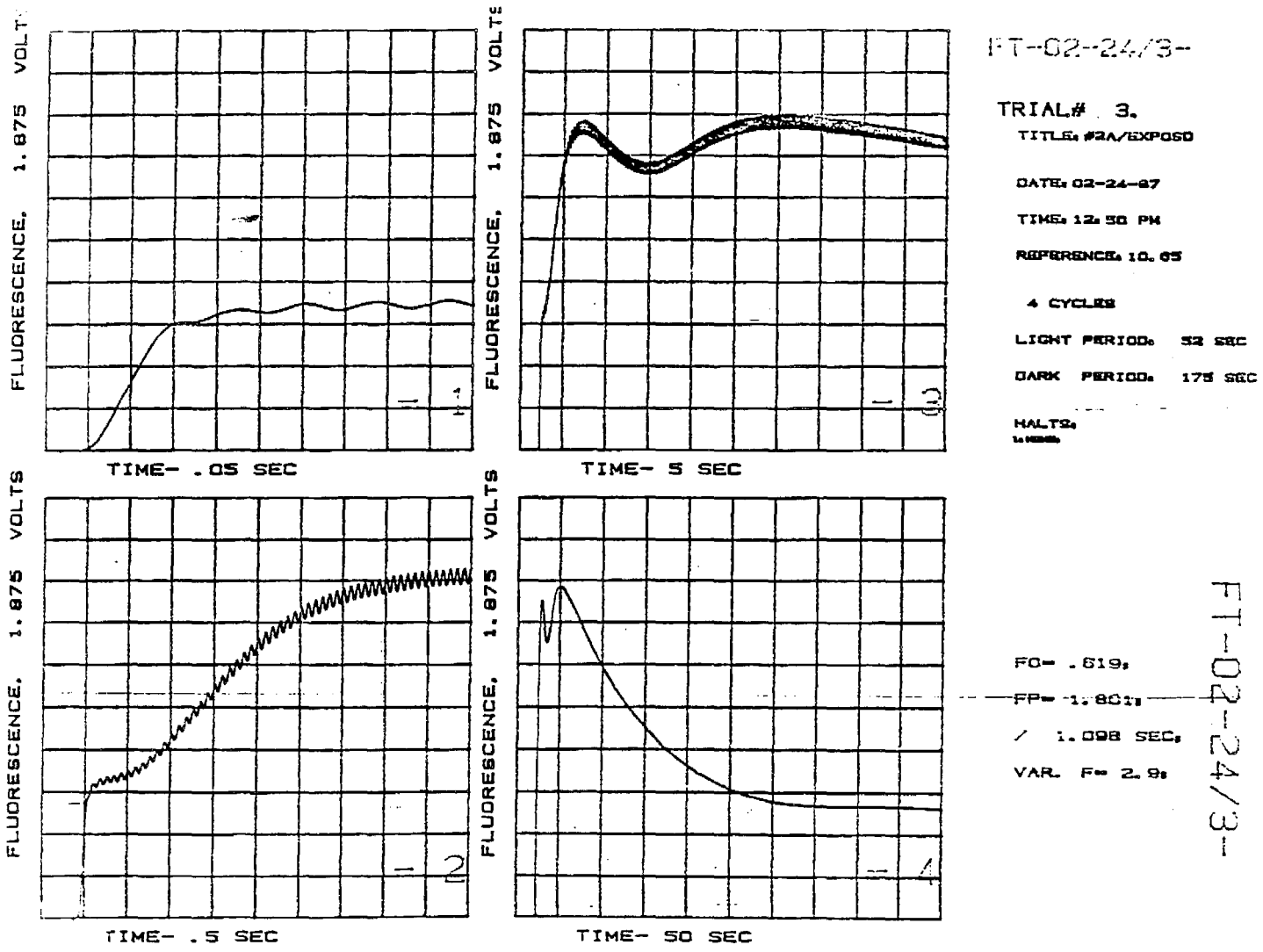

Figure 28. Chlorophyll flourescence kinetics for spinach. (D) Trial 3, Ozone

Table 15. Fluorescence Parameters (F_o and F_p) for Spinach Exposed to Filtered Air or Ozone[a]

Trial	Control - Filtered				Ozone - (Triangular Peak)				Control / Ozone		
	F_o	F_p	Tr	Fr	F_o	F_p	Tr	Fr	F_o	F_p	Fr
					Set I						
1	0.733	2.172	1.158	2.963	0.858	2.488	1.084	2.900	0.85	0.87	1.02
2	0.740	2.135	1.120	2.885	0.831	2.143	1.290	2.579	0.89	1.00	1.12
3	1.169	3.036	1.548	2.597	1.112	3.030	2.150	2.725	1.05	1.00	0.95
4	1.132	3.055	1.220	2.699	1.217	2.774	1.130	2.279	0.93	1.10	1.18
5	1.021	3.053	1.244	2.990	1.021	2.973	1.724	2.912	1.00	1.03	1.03
6	0.953	2.994	1.252	3.142	1.011	2.950	1.118	2.918	0.94	1.01	1.08
7	1.044	3.100	1.130	2.969	1.112	2.977	1.112	2.677	0.94	1.04	1.11
8	0.196	0.494	1.346	2.520	0.213	0.527	1.196	2.474	0.92	0.94	1.02
9	0.375	1.077	1.200	2.872	0.422	1.001	1.140	2.372	0.89	1.08	1.21
					Set II						
1	0.574	1.617	1.070	2.817	0.349	1.459	1.054	4.181	1.64	1.11	0.67
2	0.629	1.614	1.116	2.566	0.547	1.492	1.028	2.728	1.15	1.08	0.94
3	0.617	1.426	1.080	2.311	0.553	1.577	0.920	2.852	1.12	0.90	0.81
4	0.631	1.731	0.906	2.743	0.573	1.517	0.946	2.647	1.10	1.14	1.04
5	0.574	1.676	1.212	2.920	0.636	1.713	0.904	2.693	0.90	0.98	1.08
6	0.387	1.314	1.134	3.395	0.398	1.079	1.018	2.711	0.97	1.22	1.25
7	0.384	1.117	0.965	2.909	0.419	1.302	1.090	3.107	0.92	0.86	0.94
8	0.366	1.345	1.120	3.675	0.407	1.392	1.070	3.420	0.90	0.97	1.07
9	0.412	1.395	1.144	-----	0.444	1.391	1.060	-----	----	----	----
Ave.	0.678	1.939	1.166	2.881	0.687	1.906	1.175	2.834	1.01	1.02	1.03
SD	0.310	0.880	0.146	0.326	0.328	0.854	0.302	0.435	0.18	0.10	0.14
% SE	7.5	21.3	3.5	7.9	8.0	20.7	7.3	10.5	4.4	2.3	3.4
Max.	1.169	3.100	1.548	3.675	1.217	3.030	2.150	4.181	1.65	1.22	1.25
Min.	0.196	0.494	0.906	2.311	0.213	0.527	0.904	2.279	0.85	0.86	0.67

[a]Fluorescence parameters for the pre-illumination period only (five sec. light) are: F_o, initial level of fluorescence immediately when light goes on (volts); F_p, peak level of fluorescence (volts) reached after illumination time of Tr (sec.); F_p/F_o, the ratio of peak to initial fluorescence which is indicative of the variable fluorescence component. Plants were exposed to filtered air or ozone over four weeks for each set and were assayed at varied times during the four weeks. Two sets of plants were run, and plants were duplicated in 6A and 6B. The grand averages and SD for the data are shown on the bottom of the table. The data were taken for the pre-incubation time as described in the methods section II.B.

control plants. This ratio of the control to the exposed for each individual sample shown in the right-hand columns, shows very little variation in F_o and a slight elevation in the F_o ratio. This indicated that the peak was slightly depressed for the exposed plants. Again, variation is severe and in order to determine whether or not any given exposure does alter the F_p levels, many samples would need to be taken.

Similar experiments were conducted on the flourescence level of the other species that were exposed (except for lettuce which had leaves that were too small to include in the Hansatec chamber). The results are shown in the next series of figures (Figures 29-30). The most dramatic effects, but still small, were shown by radish which exhibited both a productivity decline and visible injury patterns upon the leaves when exposed to the triangular pulse of ozone. Figure 29A and B show the effects of exposure upon radish. The interesting changes that were noticed in chlorophyll fluorescence of the control plants, compared with spinach, were that the initial rise was followed very rapidly by a small depression easily seen in 29 A-2 graph and a prolonging of the primary peak of fluorescence (the decay of the peak fluorescence was slower). Again, comparing Figures 29A and B for control and exposed, we noticed no obvious changes. The values for the variable fluorescence (variable F in figures) shows a decline of the peak with exposure for the radish. This decline in the peak value is easily seen in the data for radish shown in Table 16. Again the F_o level shows little variation and is similar for control and exposed, while the F_p level possesses a slightly larger variation and a lower level for the exposed tissue. Again, looking at the ratio of control to exposed for each individual day, we find that the F_o is only slightly higher in the control level, while the F_p is more strongly lowered by exposure to ozone. However, again, the results are variable and each individual exposure cannot demonstrate whether or not the leaf has been injured.

We also examined both corn (Figure 30A and B) and squash (data not shown). The traces again showed no obvious changes between exposed and control plants for either corn or squash. Furthermore, there was no statistical difference in the levels of F_o or F_p for either corn or squash. There were, however, small differences in the time course of the chlorophyll fluorescence for the control plants for both corn and squash which have not (as yet) been reported in the literature.

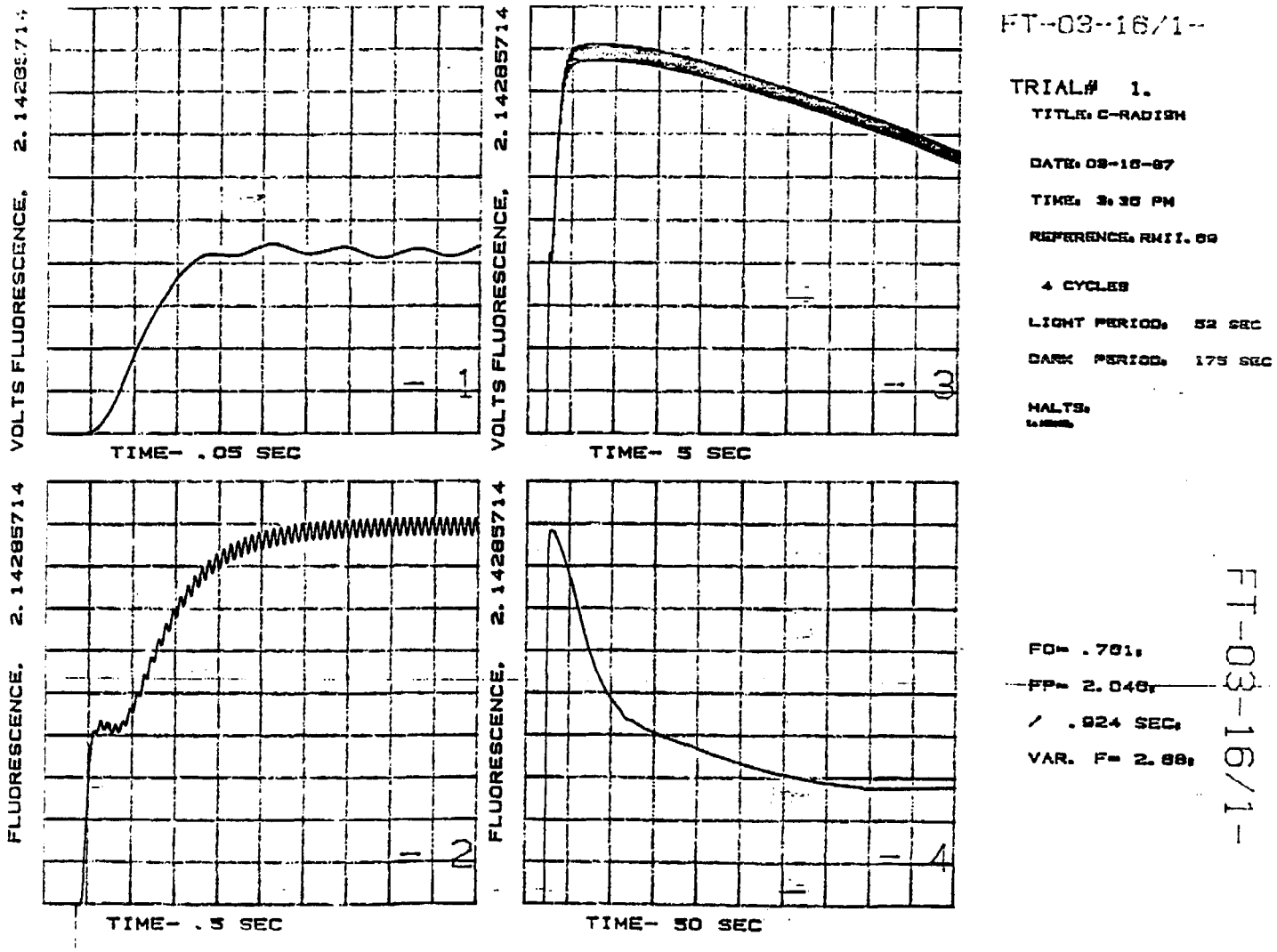

Figure 29. Chlorophyll Fluorescence Kinetics for Radish. (A) Trial 1, Control

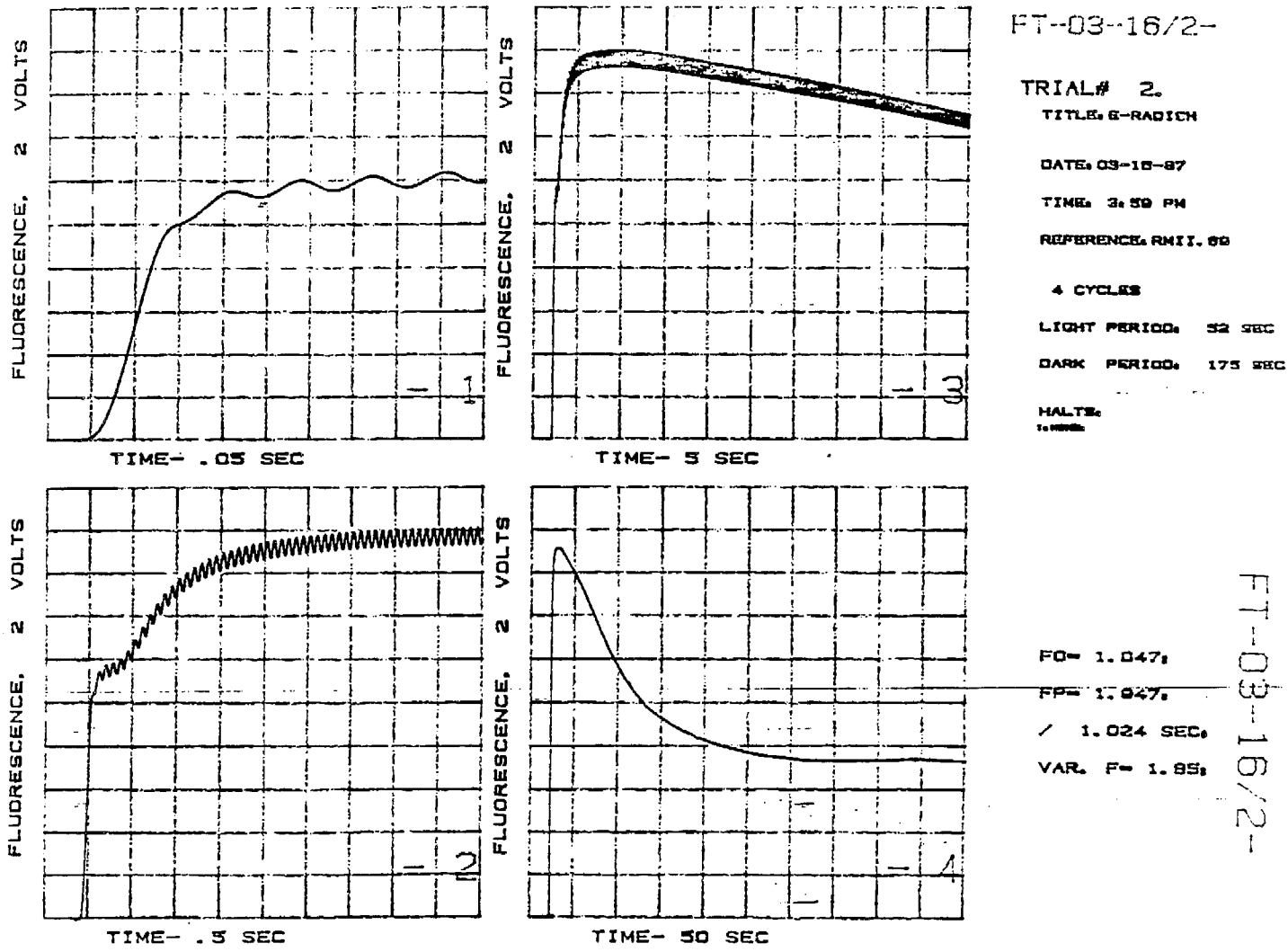

Figure 29. Chlorophyll fluorescence kinetics for radish. (B) Trial 2, Ozone

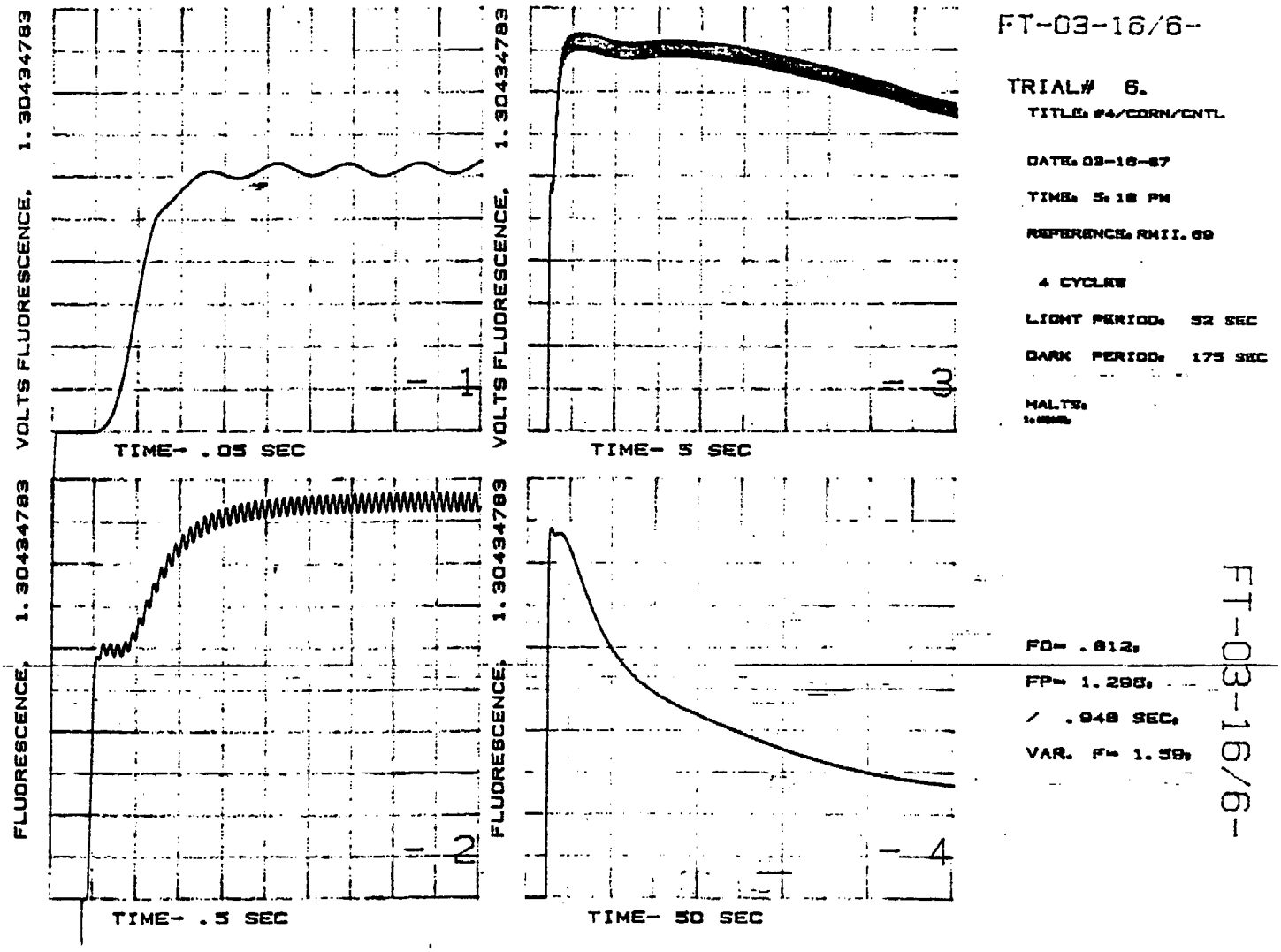

Figure 30. Chlorophyll fluorescence kinetics for corn. (A) Trial 6, Control

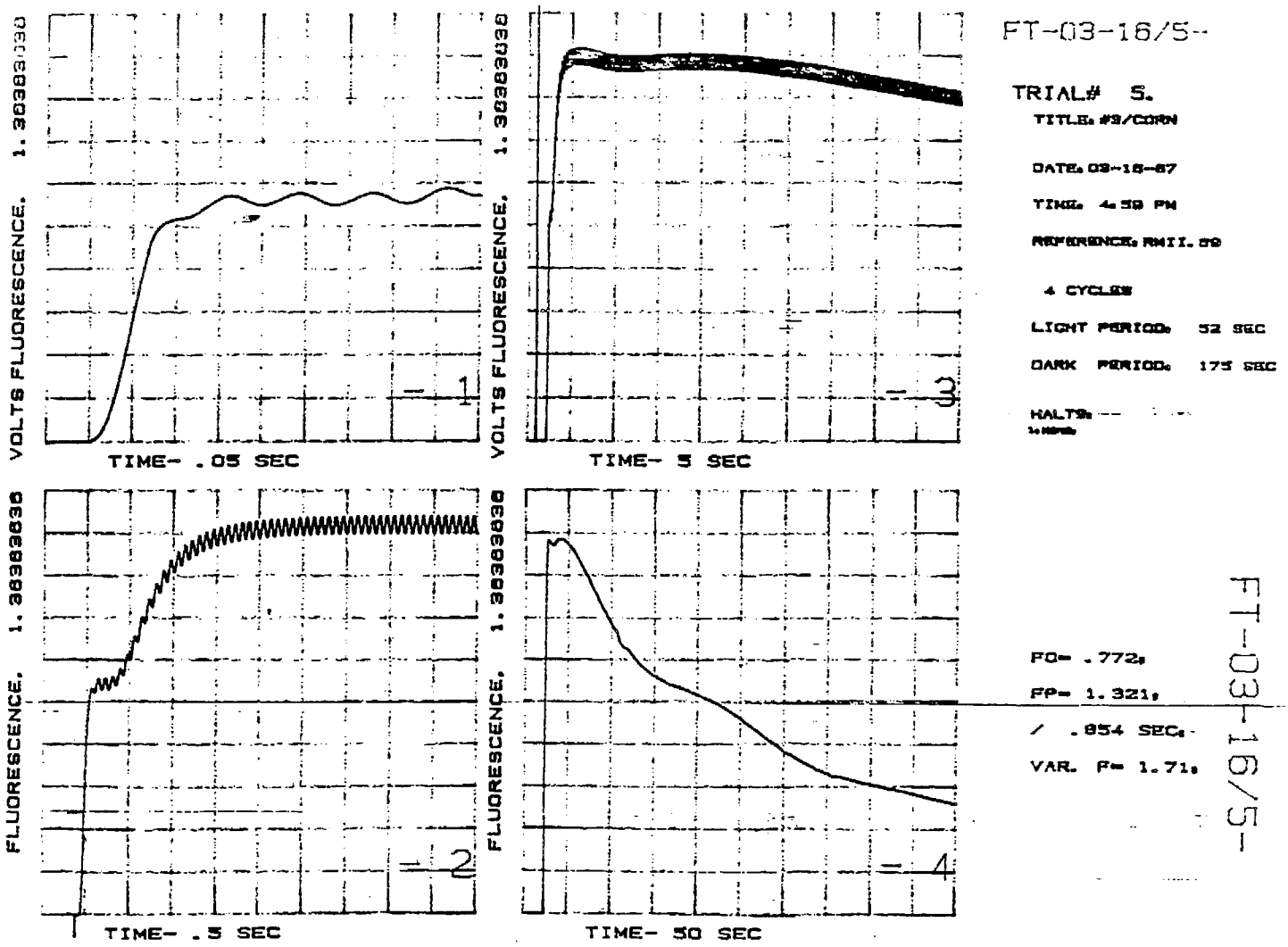

Figure 30. Chlorophyll fluorescence kinetics for corn. (B) Trial 5, Ozone

Table 16 Fluorescence Parameters (F_o and F_p) for Radish Exposed to Filtered Air or Ozone[a]

Trial	Control - Filtered				Ozone - (Triangular Peak)				Control /Ozone		
	F_o	F_p	Tr	Fr	F_o	F_p	Tr	Fr	F_o	F_p	Fr
					Set I						
1	0.761	2.482	0.924	2.691	1.047	1.947	1.024	1.860	0.72	1.05	1.45
2	0.818	1.813	1.600	2.216	0.667	1.605	2.020	2.406	1.23	1.13	0.92
3	1.050	1.970	-----	1.876	0.653	1.500	1.080	2.297	1.61	1.31	0.82
4	0.489	1.916	1.824	3.918	0.775	1.777	2.014	2.293	0.63	1.08	1.71
5	0.643	1.652	1.220	2.569	0.815	1.852	0.952	2.272	0.79	0.89	1.13
6A	0.956	1.840	0.982	1.925	0.598	1.424	1.000	2.380	1.60	1.29	0.81
6B	0.728	1.520	0.958	2.088	0.713	1.584	0.924	2.222	1.02	0.96	0.94
7	1.205	2.664	0.940	2.211	0.915	2.403	1.198	2.626	1.32	1.11	0.84
					Set II						
1	0.384	1.178	1.254	3.068	0.408	1.178	1.096	2.887	0.94	1.00	1.06
2	0.415	1.384	1.188	3.335	0.441	1.372	1.080	3.111	0.94	1.01	1.07
3	0.404	1.141	1.258	2.824	0.581	1.318	1.322	2.269	0.70	0.87	1.24
4	0.502	1.214	1.320	2.418	0.387	0.713	0.956	1.842	1.30	1.70	1.31
5	0.448	1.115	0.996	2.489	0.587	1.185	1.252	2.019	0.96	0.94	1.23
6	0.535	1.454	0.940	2.718	0.433	1.278	0.950	2.952	1.24	1.14	1.92
7	0.410	1.284	0.978	3.132	0.435	1.091	1.060	2.508	0.94	1.18	1.25
8	0.544	1.319	0.836	2.425	0.486	1.360	1.064	2.798	1.12	0.97	0.87
9	0.482	1.318	0.956	2.734	0.460	1.182	1.480	2.570	1.05	1.12	1.06
10	0.498	1.289	0.778	2.588	0.525	1.100	1.000	2.095	0.95	1.17	1.24
Ave.	0.672	1.562	1.115	2.624	0.607	1.437	1.193	2.411	1.05	1.11	1.04
SD	0.236	0.398	0.381	0.499	0.182	0.375	0.323	0.352	0.28	0.19	0.24
% SE	5.6	9.4	9.2	11.8	4.3	8.9	7.6	8.3	6.6	4.4	5.5
Max.	1.205	2.664	1.824	3.918	1.047	2.403	2.020	3.111	1.61	1.70	1.71
Min.	0.384	1.115	0.778	1..876	0.387	0.713	0.924	1.842	0.63	0.87	0.81

[a]Fluorescence parameters for the pre-illumination period only (five sec. light) are: F_o, initial level of fluorescence immediately when light goes on (volts); F_p, peak level of fluorescence (volts) reached after illumination time of Tr (sec.); F_p/F_o, the ratio of peak to initial fluorescence which is indicative of the variable fluorescence component. Plants were exposed to filtered air or ozone over four weeks for each set and were assayed at varied times during the four weeks. Two sets of plants were run, and plants were duplicated in 6A and 6B. The grand averages and SD for the data are shown on the bottom of the table. The data were taken for the pre-incubation time as described in the methods section II.B.

In the second phase of the study, we rewrote programs so that we could subtract away the F_o value and expand the time scale. Furthermore, we removed most of the beat frequency noise which was exhibited in the previous figures. The first figure (Figure 31A,B,C,D) is the same study that was done in Figure 28. The top traces show the same time course for the four sections in the previous figure. The bottom section shows a different plot which gives all time information in a much more coherent manner. The variable fluorescence is plotted as a function of the logarithm of the time of illumination. This allows the superimposition all the kinetic parameters and a clear picture of where changes are occurring. This program for altering the time scale and superimposing each individual cycle of illumination is not yet finished and so the curves do not match perfectly. Yet, we can see on the left-hand side the initial rise and/or fall of the fluorescence, in the center of the curve, the peak fluorescence, and towards the right-hand side the secondary fluorescence humps or shoulders. It is still difficult to see changes in the pattern of the fluorescence kinetics induced by ozone; however, by superimposing the curves of the control and exposed plants, we do see that the total peak declines somewhat and that this increases the apparent shoulder of secondary rise. There seems to be no change in the initial rise of the fluorescence (within 100 msec.).

In Figure 31C and D we show the curves that correspond to Figure 28A and B for spinach where the secondary peak was considerably enlarged. Very little change in the overall pattern of the two curves is seen, but the two peaks become very clear upon this type of plot.

When we carry out the same analysis for radish (Figure 32), we see that the initial rise within the first few tens of milliseconds is roughly the same for both exposure conditions. Furthermore, we see that the primary peak and the secondary peak blend together to make a very large peak occurring between half a second and two seconds. And, finally, we see that the exposure does lower the primary peak with no change in the initial peak (within the first few tens of milliseconds). This type of analysis would allow us to subtract the data of two peaks and analyze further on the variation within these experiments.

For corn and squash we reevaluted the same plants (Figure 33 and not shown) and found that there was no obvious change in the logarithmic

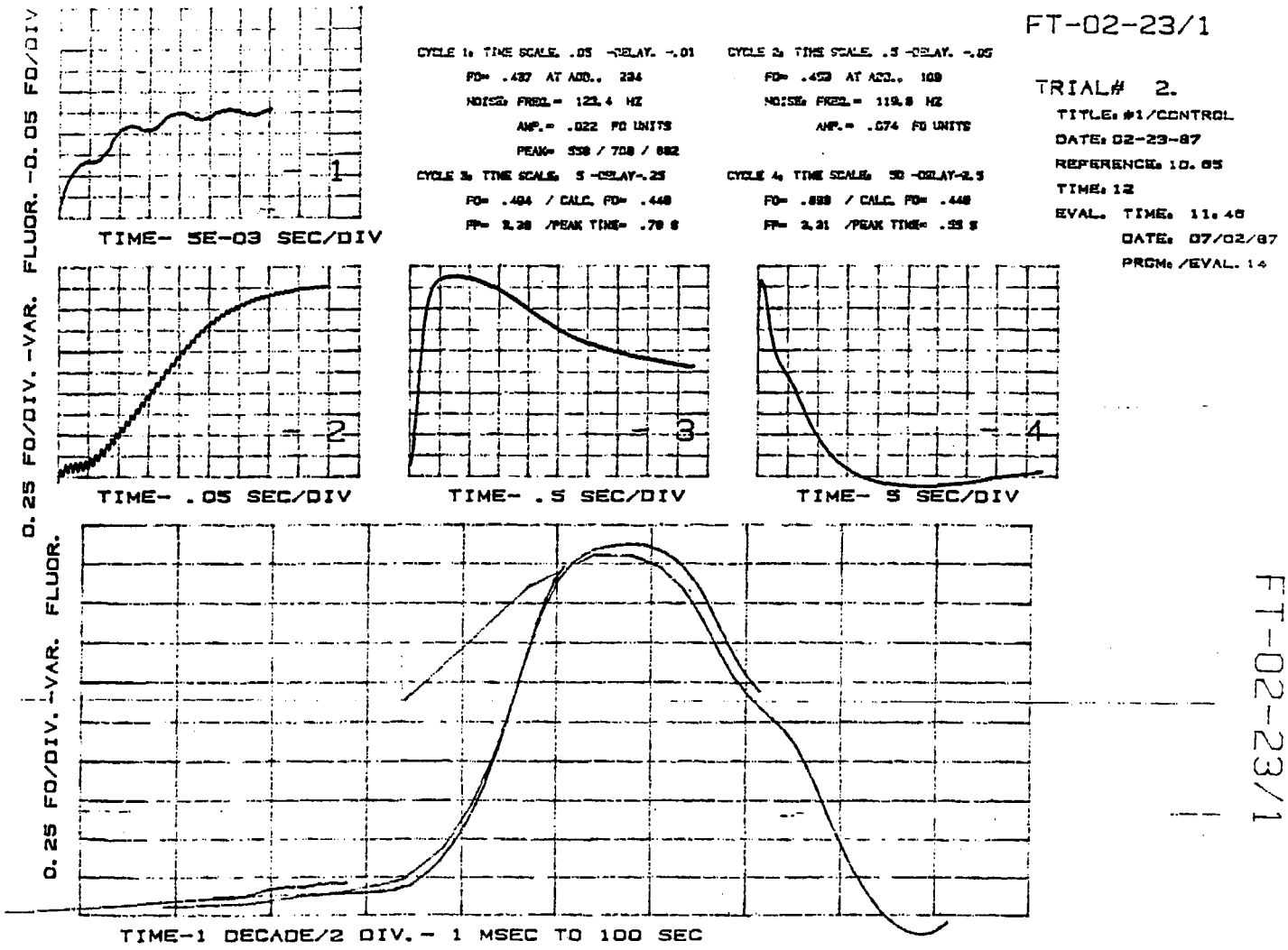

Figure 31. Variable chlorophyll fluorescence for spinach. (A) Trial 2, control

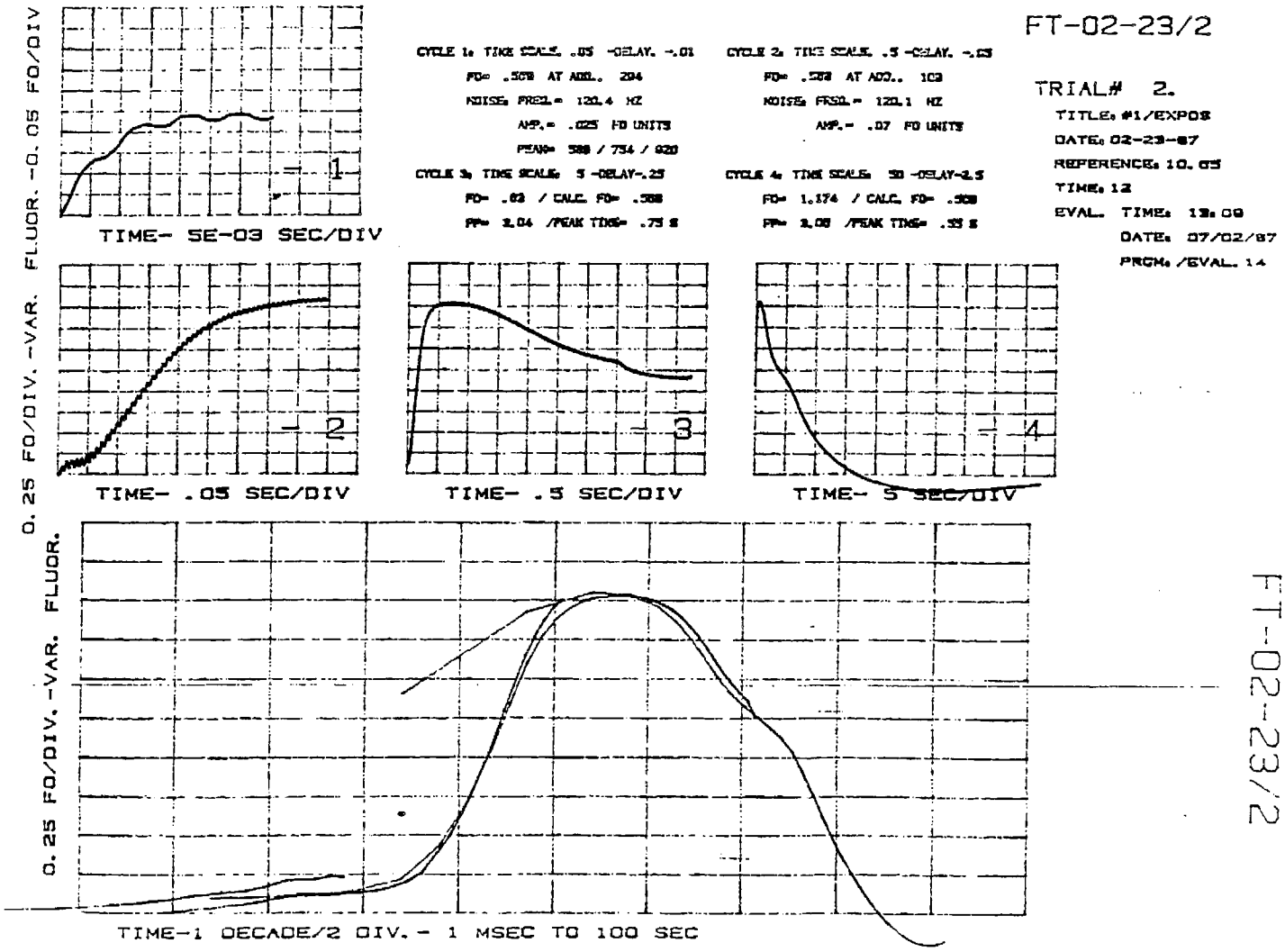

Figure 31. Variable chlorophyll fluorescence for spinach. (B) Trial 2, ozone

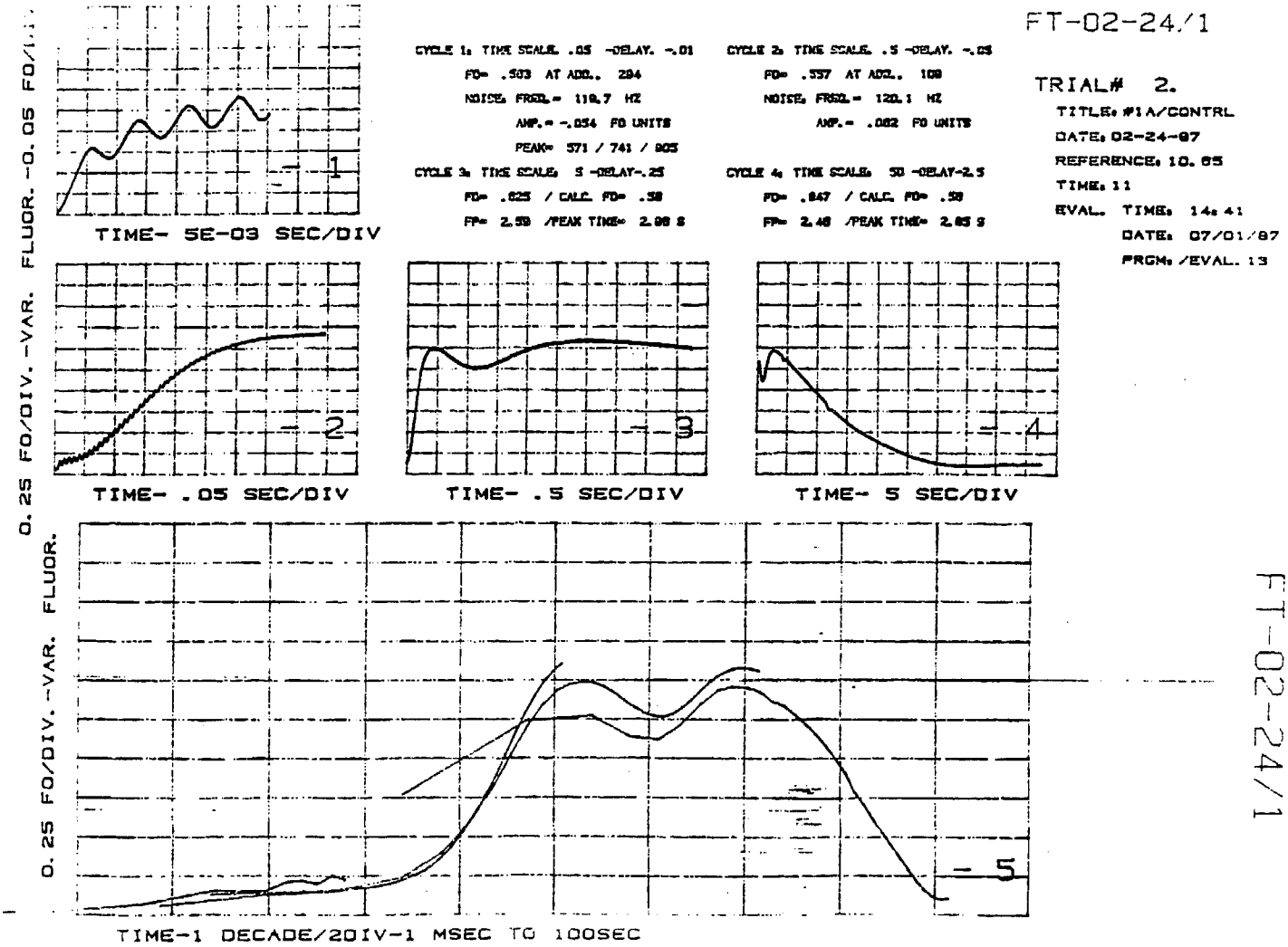

Figure 31. Variable chlorophyll flourescence for spinach. (C) Trial 1(A), control.

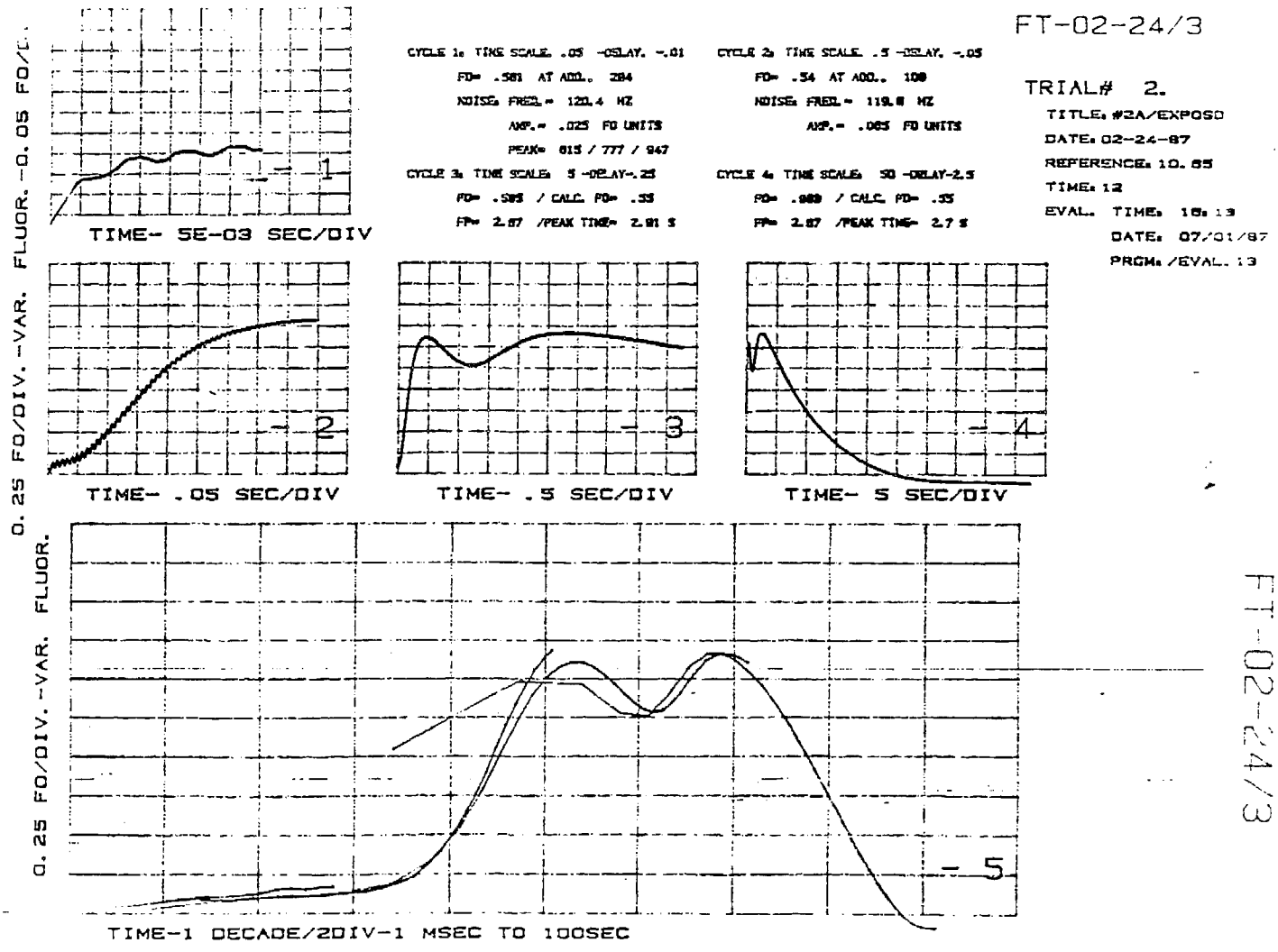

Figure 31. Variable chlorophyll flourescence for spinach. (D) Trial 2(A), ozone.

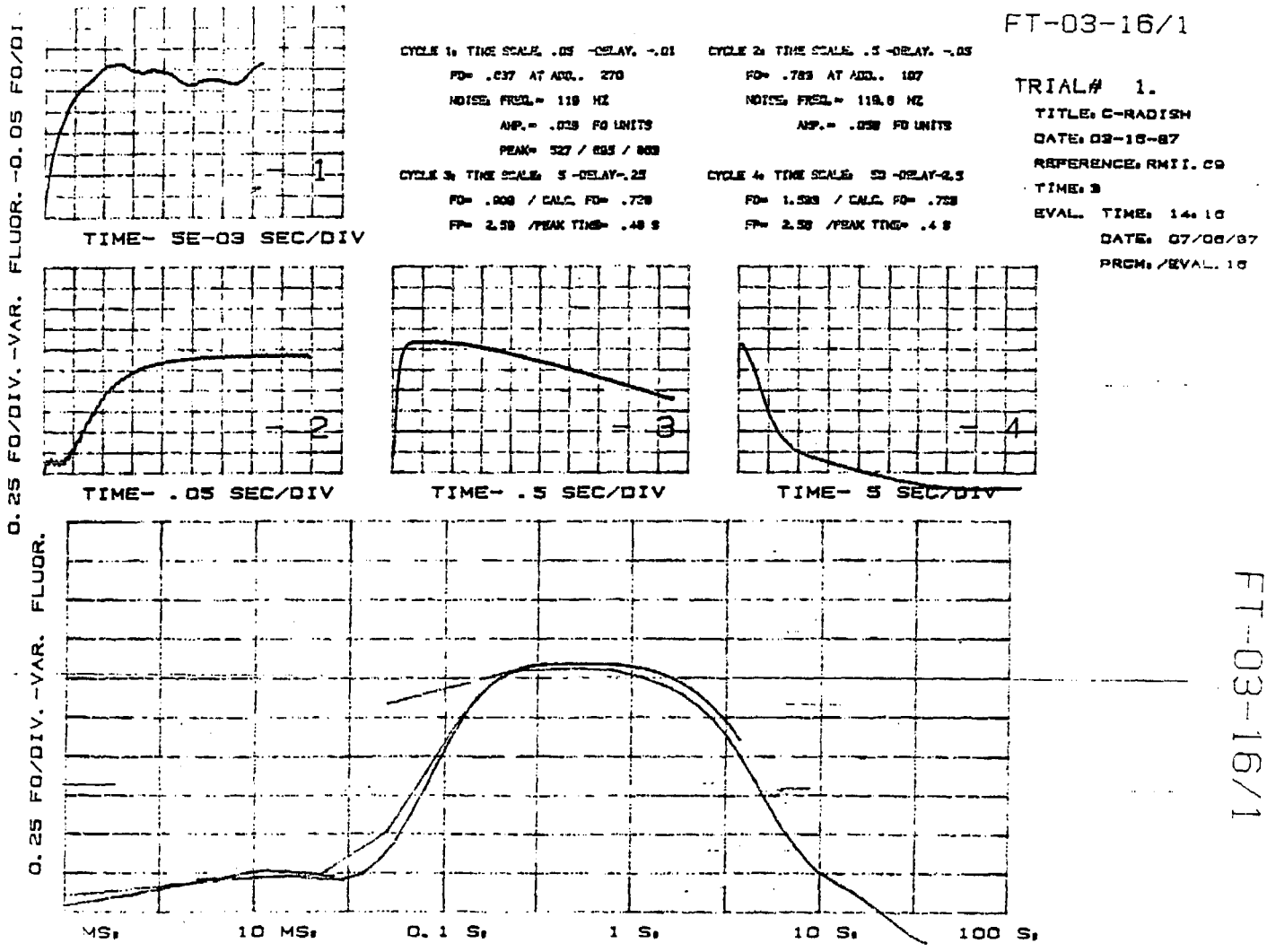

Figure 32. Variable chlorophyll fluorescence for radish. (A) Trial 1, control

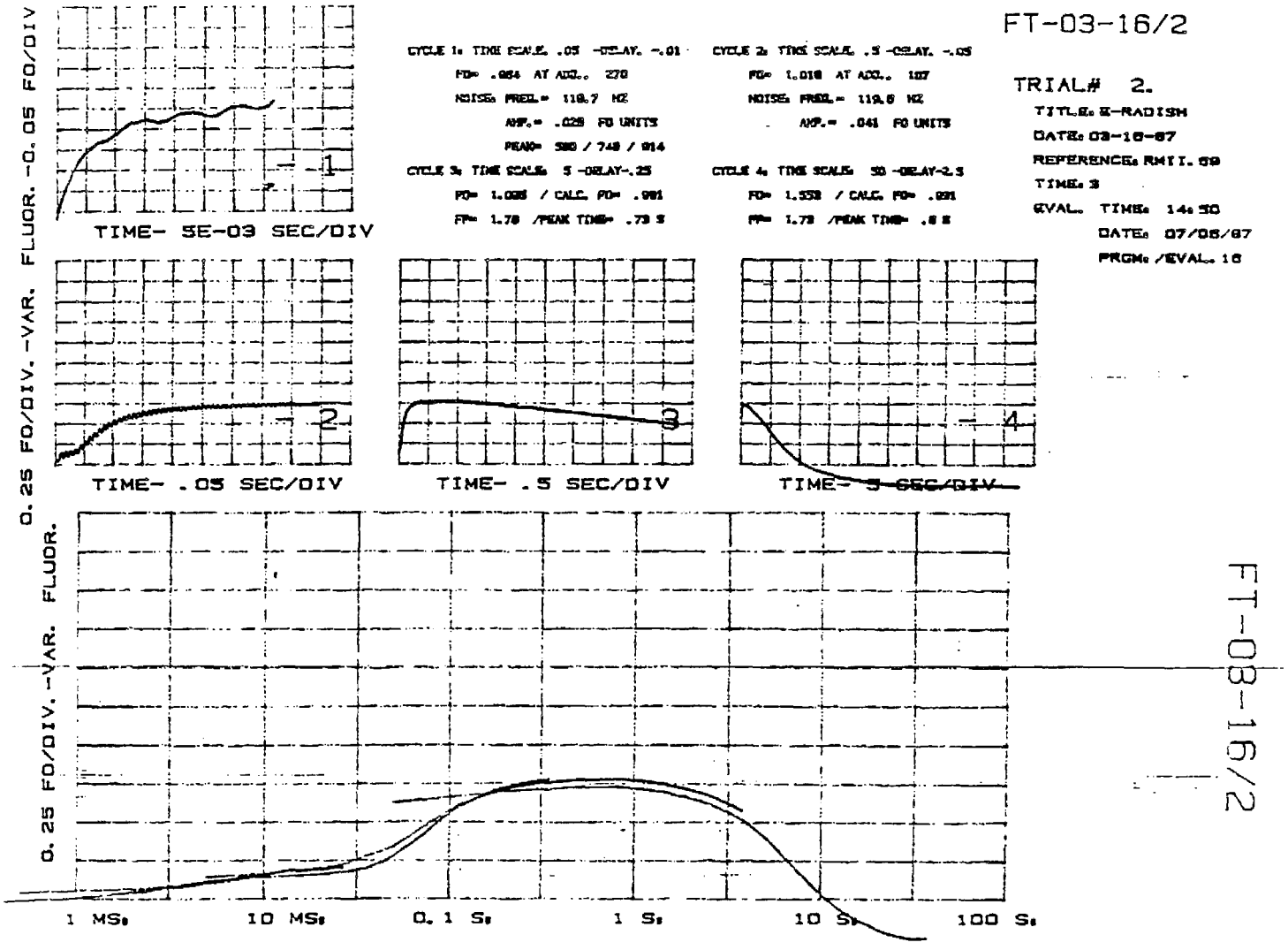

Figure 32. Variable chlorophyll fluorescence for radish. (B) Trial 2, exposed

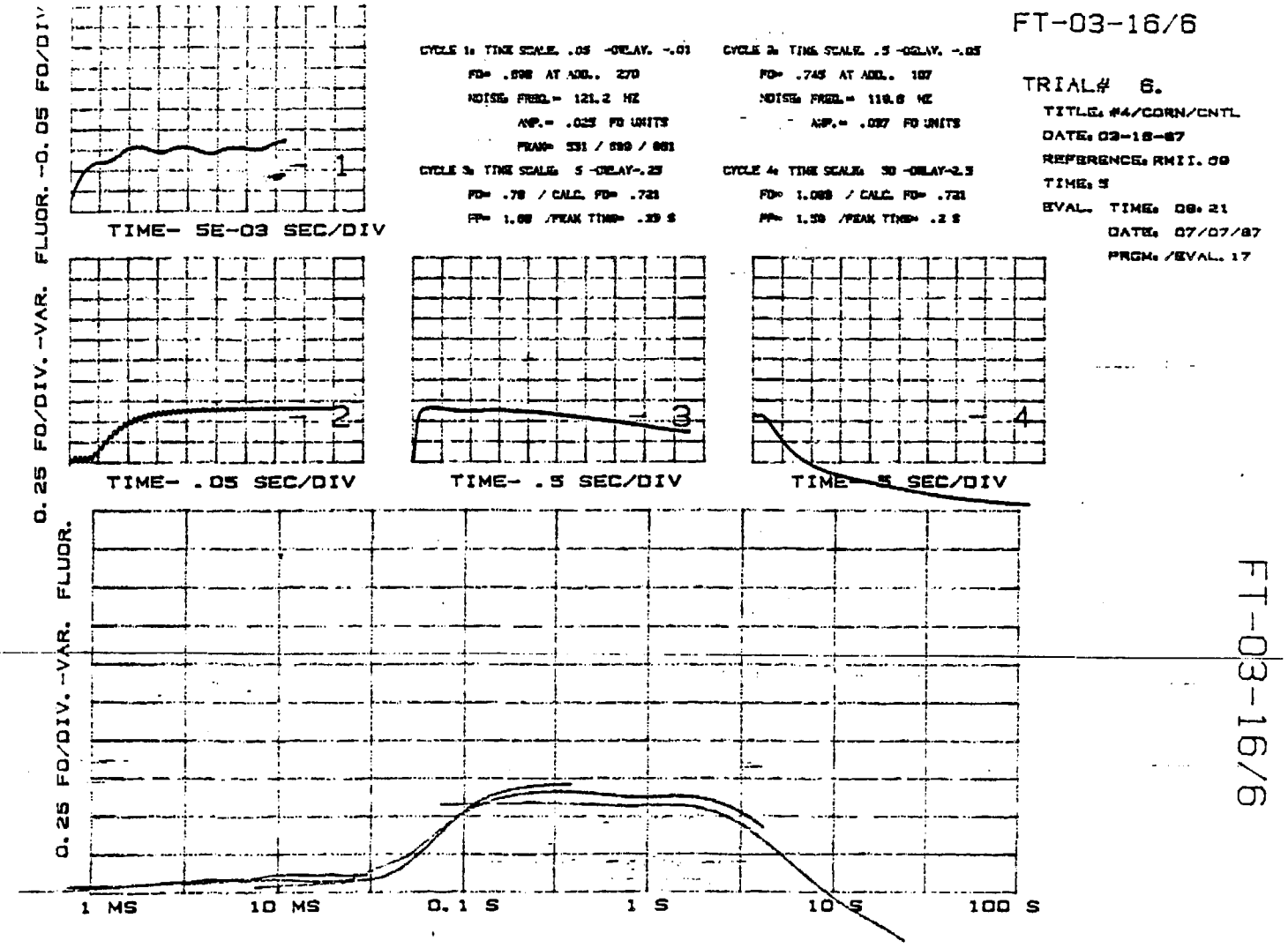

Figure 33. Variable chlorophyll fluorescence for corn. (A) Trial 6, control

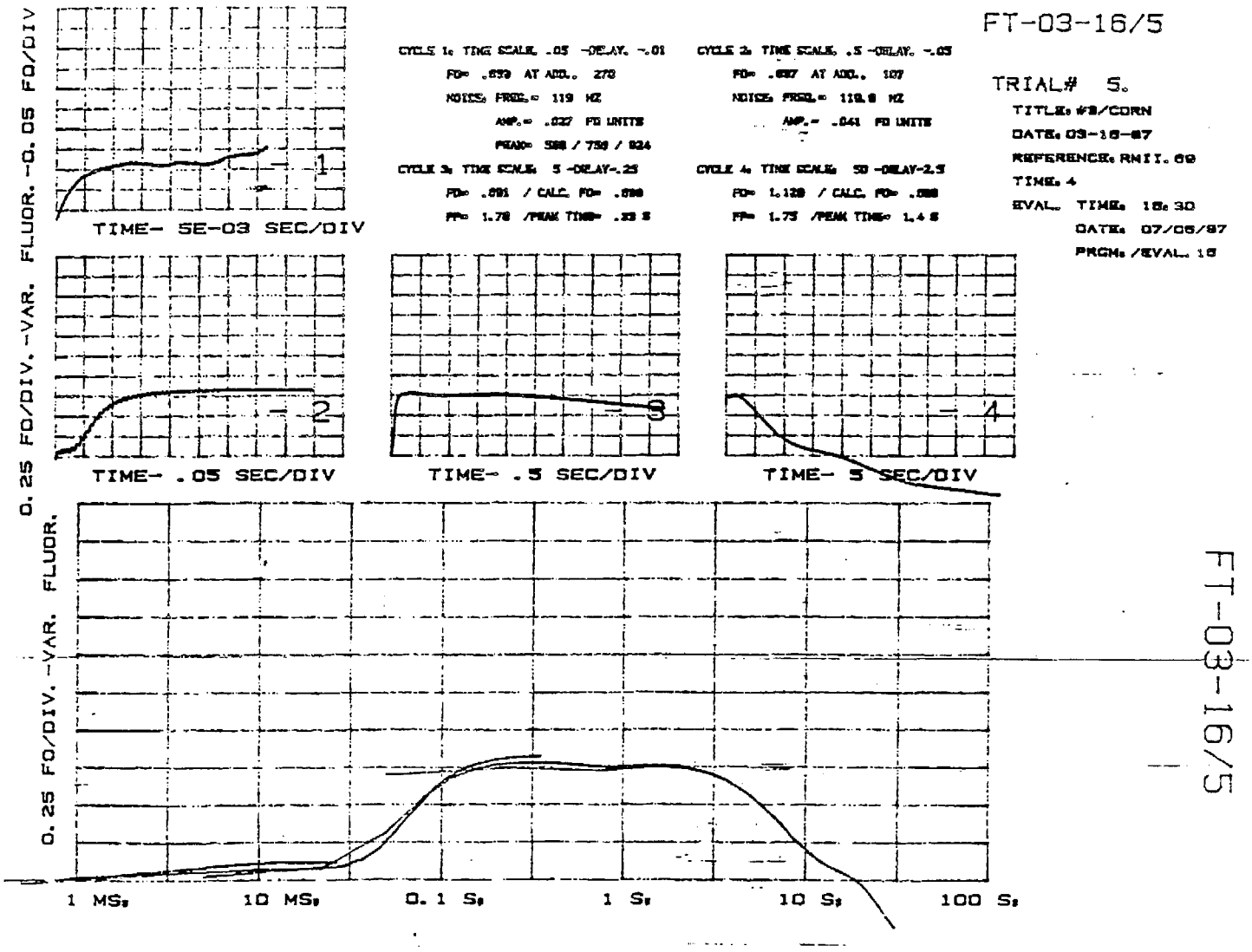

Figure 33. Variable chlorophyll fluorescence for corn. (B) Trial 5, ozone

determination of the chlorophyll fluorescence. The two curves for control and exposed plants can be precisely superimposed.

In conclusion, we have found that at this level of ozone, the effects upon chlorophyll fluorescence are small and subtle. For the most sensitive species examined, namely spinach and radish, we can determine that the peak (F_p) is slightly depressed and this makes the long-time scale shoulders of the secondary peaks more pronounced. But these effects are small and to show differences a large number of leaves must be sampled. There are, however, no changes that are obvious for the F_o patterns in these studies. In past studies, the F_o level has been identified with the amount of chlorophyll that exists within the leaf. In these studies, no visible injury in the leaf surfaces is observed and thus F_o does not change because there is no chlorosis within the leaf.

For the species that showed very little productivity loss and no visible injury (e.g., corn), we saw no changes in the chlorophyll fluorescence patterns, either at the gross kinetic level or for individual points of F_o or F_p.

The technique of utilizing the computer to calculate the variable flourescence and to make a logarithmic plot with respect to time of that fluorescence will allow a superimposition of these plots to find where smaller, more subtle affects, occur. At the present time, we believe that the majority of the effects on chlorophyll flourescence, due to injury occurring within the sensitive plants at this low level of ozone, is within the fluorescence pattern exhibited between one-half and two seconds. We see very little evidence for changes at fast time periods (within 10 milliseconds) and very few effects occurring after 10 seconds of illumination. These results should allow a focusing upon a specific time period which should be studied in more detail so that more samples can be routinely analyzed.

E. Applications of This Research

1. Relationship Between Ozone-Induced Changes in Physiology and Productivity

This study demonstrated that reductions in productivity due to O_3 exposure occur, but are difficult to associated with reductions in gas exchange rates. Only rarely were reductions in productivity (as measured by RGR) associated with the instantaneous measurements in gas exchange.

Furthermore, a periodic sampling (twice weekly) was necessary to detect the physiology-productivity associations.

The results of the present study provide evidence that twice weekly O_3 exposures can disrupt physiological responses in hydroponically-grown spinach. Stomatal conductance of water vapor responses were most sensitive to the impacts of O_3 as significant treatment effects were observed in all four experimental groups. Transpiration responses were also found to be sensitive (possibly as a consequence of decreased stomatal conductance), whereas photosynthesis was reduced in only one group. Significant reductions in whole-plant fresh weight were observed in all four groups, but the decreases were usually due to lower tissue water contents rather than decreased dry matter accumulation (except in Group 2). However, despite a lack of consistent growth reduction in spinach, ozone-induced alterations in physiology were detected, which suggests that the applied stress caused intermittent disruption, but did not cause permanent physiological dysfunction.

Despite the lack of clear causal relationship between photosynthesis and dry matter accumulation in this pilot study on spinach, several observations are noteworthy, which may be useful in future investigations: (1) visible injury to spinach leaves was observed only during the first week of ozone exposures, when the plants were very young; (2) ozone-induced alterations in physiology were greatest immediately following exposure to ozone; and (3) recovery to normal physiological function occurred within 48-72 hours post-fumigation. Based on these findings, more detailed examinations should be made on injury, physiology, and growth during the first week of ozone exposure. Identification of the parameters or characteristics that determine plant ozone-sensitivity must be made at this time if injury to leaves only occurs during this period of development. Along these lines, diurnal patterns of stomatal conductance to water vapor need to be measured to gain insights relative to the rate of recovery to normal physiological function.

In terms of the experimental design, several factors may have contributed to our inability to determine a clear causal relationship between the impacts of ozone on spinach photosynthesis and growth. Included among the considerations are: (1) The use of a relatively low and intermittently applied ozone dose (i.e., allowed the plants to recover from a moderate air pollution stress event); (2) variation in environmental con-

ditions between groups (i.e., day length, day and night temperature, light intensity); (3) number of replicate experiments (i.e., may have not conducted enough replicates to statistically discriminate differences of 10-15%); and (4) pot-size limitations (i.e., restriction of root growth and problems with nutrient deficiencies). If these factors reduced the physiological process rates in control plants, the deleterious impacts of ozone may not have been fully expressed, owing to potential interactive effects with the other stresses.

Relative to the interspecific study, radish was found to be a good model species for studying the effects of ozone. In this crop, decreased photosynthetic activity was correlated with reductions in whole-plant dry weight. Radish plants exhibited the highest physiological process rates of the crops examined in the interspecific study, which may be characteristic of ozone-sensitive plants, since ozone uptake rates may be greater than in less active plants.

This study clearly indicated that with ozone stressed plants can exhibit short-term stomatal closure responses which are not necessarily associated with reductions in photosynthetic rate or changes in chlorophyll fluorescence.

2. Use of Physiological Responses as Indicators of Ozone Stress in the Field

While this study does indicate that changes in plant physiology due to ozone can be detected, the study also indicated the limitations for direct use of gas exchange rates or chlorophyll fluorescence as a quick assessment tool for the field. First, the ozone responses could not always be detected on a week by week or experiment by experiment basis. There is considerable variability in physiological responses due to environmental conditions at the time of measurement which may mask any responses induced by ozone. For example, low light intensities on a number of measurement days reduced control stomatal conductance. Second, single day measurements would be relatively meaningless for correlation with yield in mechanistic studies, as yield reflects the sum total of response to stress over a long period of time, and not individual days. Thus, multiple measurements over an entire growth period would be necessary to correlate yield changes due to ambient ozone exposure. Because of the amount of the work and time required for these repeated physiological measurements, they would have little value for predicting

yield losses compared to the actual harvest data. However, multiple physiological measurements would help indicate the mechanisitic basis for any effects of ozone on yield.

Furthermore, use of photosynthetic rate by itself to indicate potential yield effects is questionable as many biochemical events occur between capture of CO_2 as measured by current instruments, and final accumulation of biomass. In fact, allocation of the fixed carbon from CO_2 to different plant organs may be more important in determining the final yield. A more appropriate physiological or biochemical assessment tool would be one which reflects the cumulative ozone exposure "burden" over time, even if the parameter was measured only once during a study. This would be analogous to measuring total sulfur concentration in leaves as an indicator of the sulfur dioxide exposure associated with yield decreases (Bytnerowicz et al. 1987).

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APPENDIX A

Comparison of Gas Exchange Measurements with Dual-Isotope Porometer and LI-COR Systems

Three trial exposures with pinto beans and one with spinach were made prior to the full scale study with spinach. From the pinto bean studies, it was evident that plants had more visible injury with "peak" than square wave exposure even though both patterns resulted in equivalent mean concentrations and doses. The trial spinach study indicated that the plants were severely injured with a single 0.30 ppm ozone peak exposure. Thus ozone concentrations were reduced for subsequent spinach exposures to 0.24 ppm for the peak and 0.12 ppm for the square wave exposure.

Pinto bean plants were rated for injury to illustrate the difference in plant response with the peak vs. square wave exposure pattern (Table A-1). Plants exposed with the peak pattern had much more injury than the square wave plants.

Ozone exposures were performed using 16 to 18 day old chamber-grown pinto beans on 8/5 and 8/12/86 to determine whether the square and peak exposure patterns provided ozone doses capable of causing visible and physiological disruption, and if those exposure patterns caused different levels of disruption. Mean irradiance, relative humidity and leaf temperature values during the physiological measurements are listed in Table A-2. On both dates, plants exposed to the peak pattern exhibited the most physiological disruption evident as lower rates of stomatal conductance of water vapor (Cs), net photosynthesis (Pn) or transpiration (Ts) (Table A-3). However, the amount of visible injury differed greatly between the two exposures in that the plants exposed on 8/12 exhibited very little (if any) necrosis 24 h post-fumigation. Lower irradiance levels during fumigation as well as differences in physiological age in the 8/12 plants (i.e. younger than the 8/5 plants) may have contributed to the lower amount of injury. The utility of the square wave and peak pattern exposures with respect to inducing injury was also tested with greenhouse-grown plants on 8/12. Plant responses were similar to those reported for the 8/5 fumigation.

Based on these single-fumigation trials, we conducted a 'double-fumigation' trial in which pinto beans were exposed to ozone on 8/19 and 8/22. Plant responses to ozone were similar to those recorded previously on 8/5 with respect physiological disruption (Table A-3). The results from 8/22 indicate that primary leaf physiological responses are lower after the second square wave fumigation, however this may be an age-

influenced phenomenon since the control plants also exhibited lower physiological activity. The apparent increase in Cs and Ts in the peak pattern exposed plants on the second day is due to sampling only those leaves which exhibited less than 50% necrosis.

On 8/26 visible injury responses of hydroponically-grown spinach to the square wave and peak pattern ozone exposures were examined in a trial run. Considerable injury was observed only in older leaves of peak pattern exposed plants. Since we planned to expose the plants to ozone eight times within a four-week period, lower ozone concentrations would need to be used since leaf injury was extensive after only one exposure. Hereafter, the square wave concentration was 0.12 ppm and the maximum ozone concentration in the peak pattern exposure was 0.24 ppm.

Table A-1. Leaf Injury to Pinto Bean Plants with Different Exposure Patterns Providing an Average Concentration of 0.30 $\mu l\ L^{-1}$ Ozone for Four Hours on Two Days[a]

Leaf	Control	Square Wave	Peak
		Percent Leaf Area Necrotic	
Unifoliate	0	37 ± 18	99 ± 2
Trifoliate	0	8 ± 4	88 ± 10

[a]Values are means ± SD for six replicate plants.

Table A-2. Environmental Conditions During the Pinto Bean Ozone Exposure Trials

Date	Irradiance ($\mu mol\ m^{-2}\ s^{-1}$)	Humidity (%)	Leaf Temperature (C)
8/5/86	947 ± 46	43 ± 8	32.4 ± 1.4
8/12/86	683 ± 116	42 ± 5	32.2 ± 0.9
8/19/86	870 ± 244	47 ± 6	33.2 ± 1.2
8/22/86	914 ± 145	44 ± 4	33.3 ± 0.8

Table A-3. Physiological Responses of Pinto Beans to Ozone in Square Wave or Peak Patter of Exposure[a]

Date	Treatment	Conductance ($cm\ s^{-1}$)	Photosynthesis ($mg\ CO_2\ m^{-2}\ s^{-1}$)	Transpiration ($mgH_2O\ m^{-2}\ s^{-1}$)
8/5/86	Control	2.64 ± 0.18	0.98 ± 0.16	222 ± 2
	Square W.	1.50 ± 0.26	0.67 ± 0.04	178 ± 6
	Peak	0.29 ± 0.07	0.24 ± 0.24	61 ± 13
8/12/86	Control	2.83 ± 0.52	0.72 ± 0.07	244 ± 21
	Square W.	1.04 ± 0.15	0.59 ± 0.07	156 ± 21
	Peak	0.79 ± 0.43	0.48 ± 0.09	127 ± 47
8/19/86	Control	2.34 ± 0.28	0.89 ± 0.09	233 ± 16
	Square W.	1.58 ± 0.31	0.65 ± 0.02	195 ± 19
	Peak	0.46 ± 0.15	0.25 ± 0.15	97 ± 20
8/22/86	Control	1.74 ± 0.27	0.72 ± 0.06	221 ± 23
	Square W.	1.25 ± 0.29	0.60 ± 0.10	188 ± 29
	Peak	0.75 ± 0.17	0.19 ± 0.17	141 ± 19

[a]Values are mean ± SD for three single plant replicates.

Results from a comparison between the LI-6000 and dual-isotope porometer before the Spinach study are shown in Figures A-1 through A-4. For stomatal conductance there was a high correlation between the dual isotope and LI-COR 6000 porometer results for both spinach (Figure A-1) and pinto beans (Figure A-2). However, conductance values were generally twice as high with the LI-COR 6000 compared to dual-isotope porometer. For photosynthesis there was no correlation between the dual-isotope porometer and LI-COR 6000 results for spinach (Figure A-3), but a high correlation for pinto beans (Figure A-4). For both species LI-6000 values were generally only slightly higher (25%) as with the dual-isotope porometer. However, for spinach there was too much scatter between replicates to detect a statistically significant regression equation.

The comparison between the two types of gas exchange porometers indicate that photosynthetic measurements are relatively compatible, provided that enough measurements are made. Stomatal conductance measurements are quite different with the two instruments. This has been suspected for quite some time and conductance data from the dual-isotope porometer has not been used. The LI-COR 6000 stomatal conductance can be considered to be more reliable as the values are comparable to those collected with the LI-COR 1600, a steady state porometer operating on a different air exchange principle.

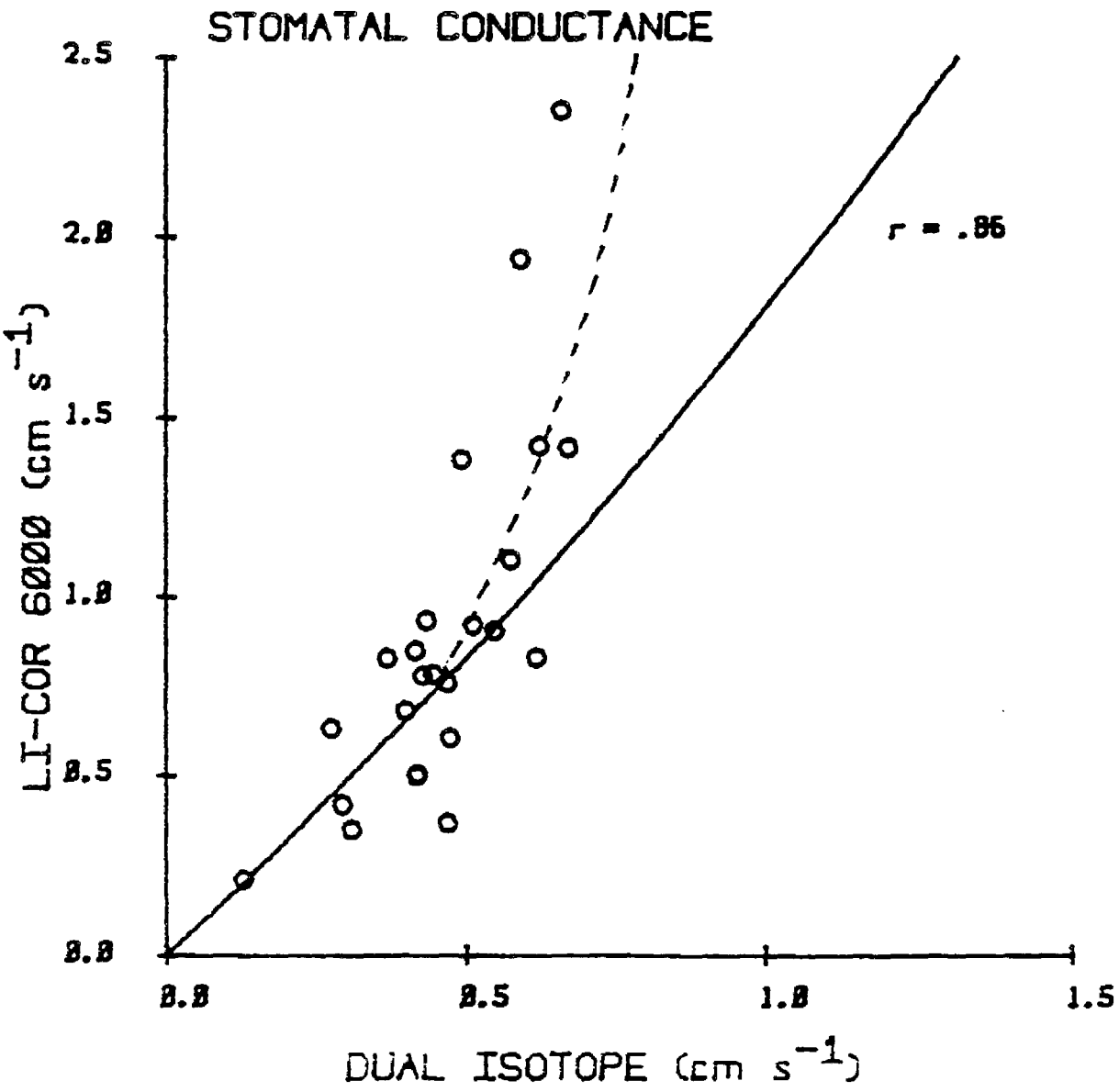

Figure A-1. Relationship for spinach stomatal conductance between dual-isotope and LI-COR 6000 porometers.

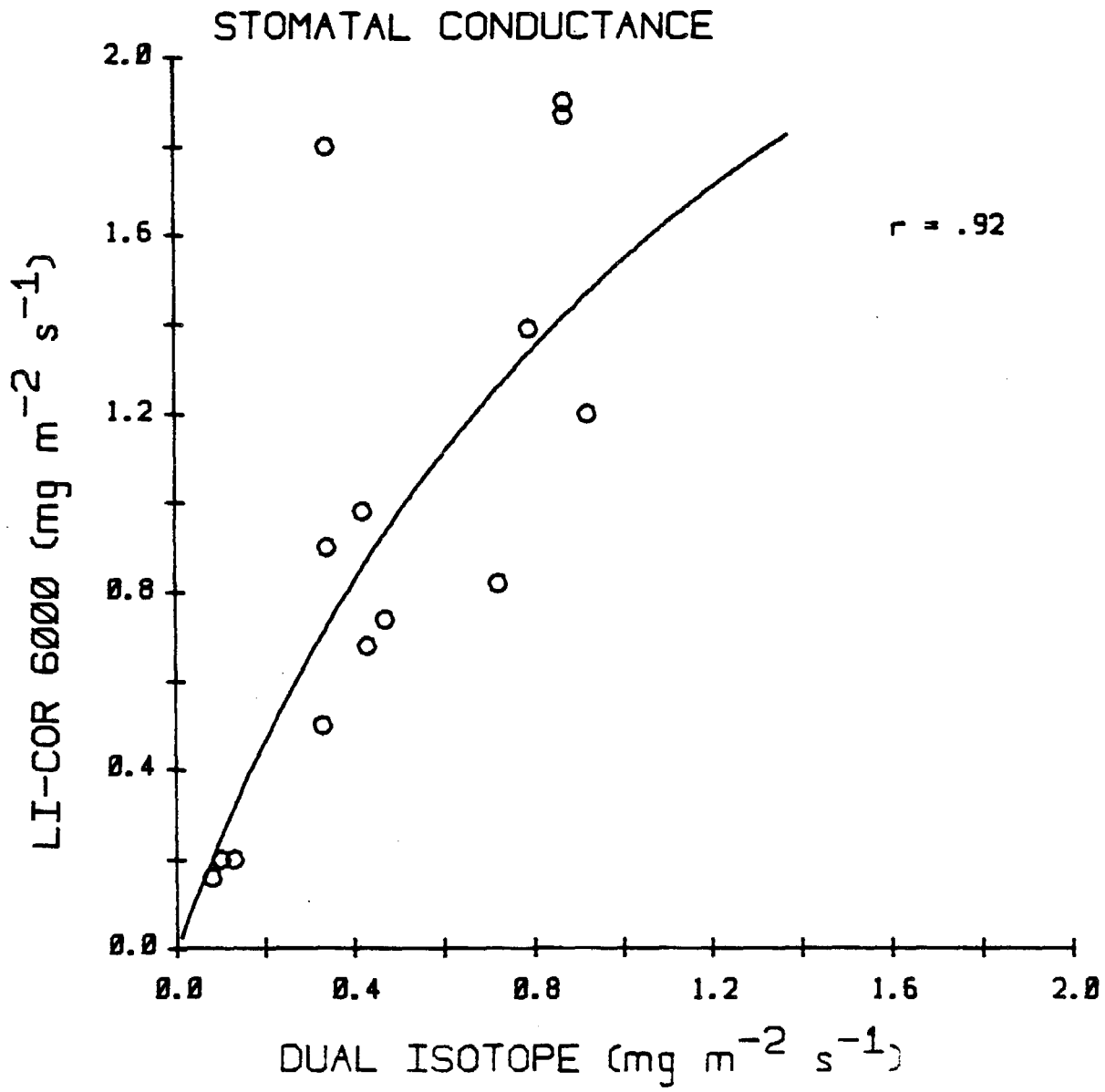

Figure A-2. Relationship for pinto bean stomatal conductance between dual-isotope and LI-COR 6000 porometers.

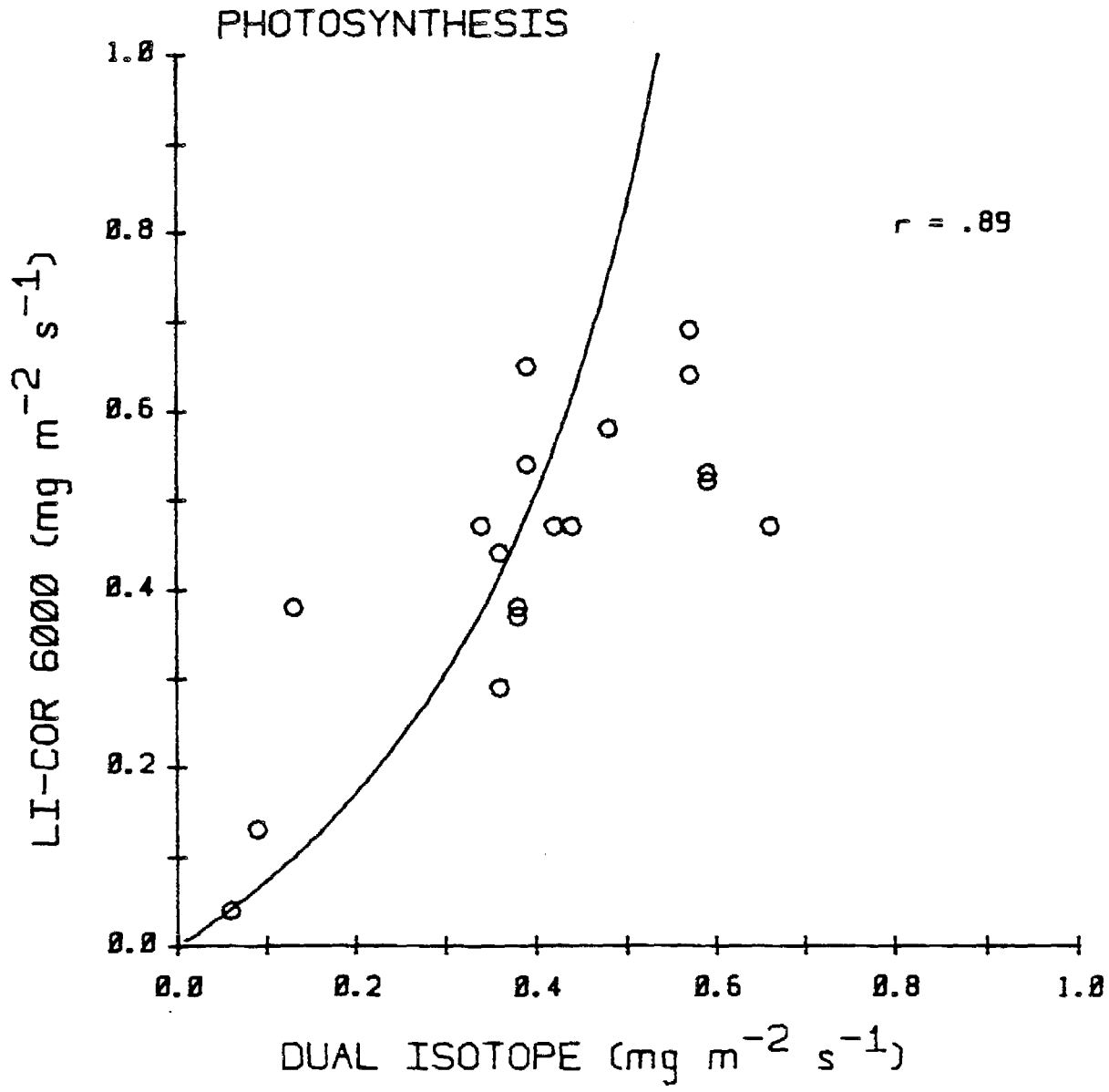

Figure A-3. Relationship for pinto bean photosynthesis between dual-isotope and LI-COR 6000 porometers.

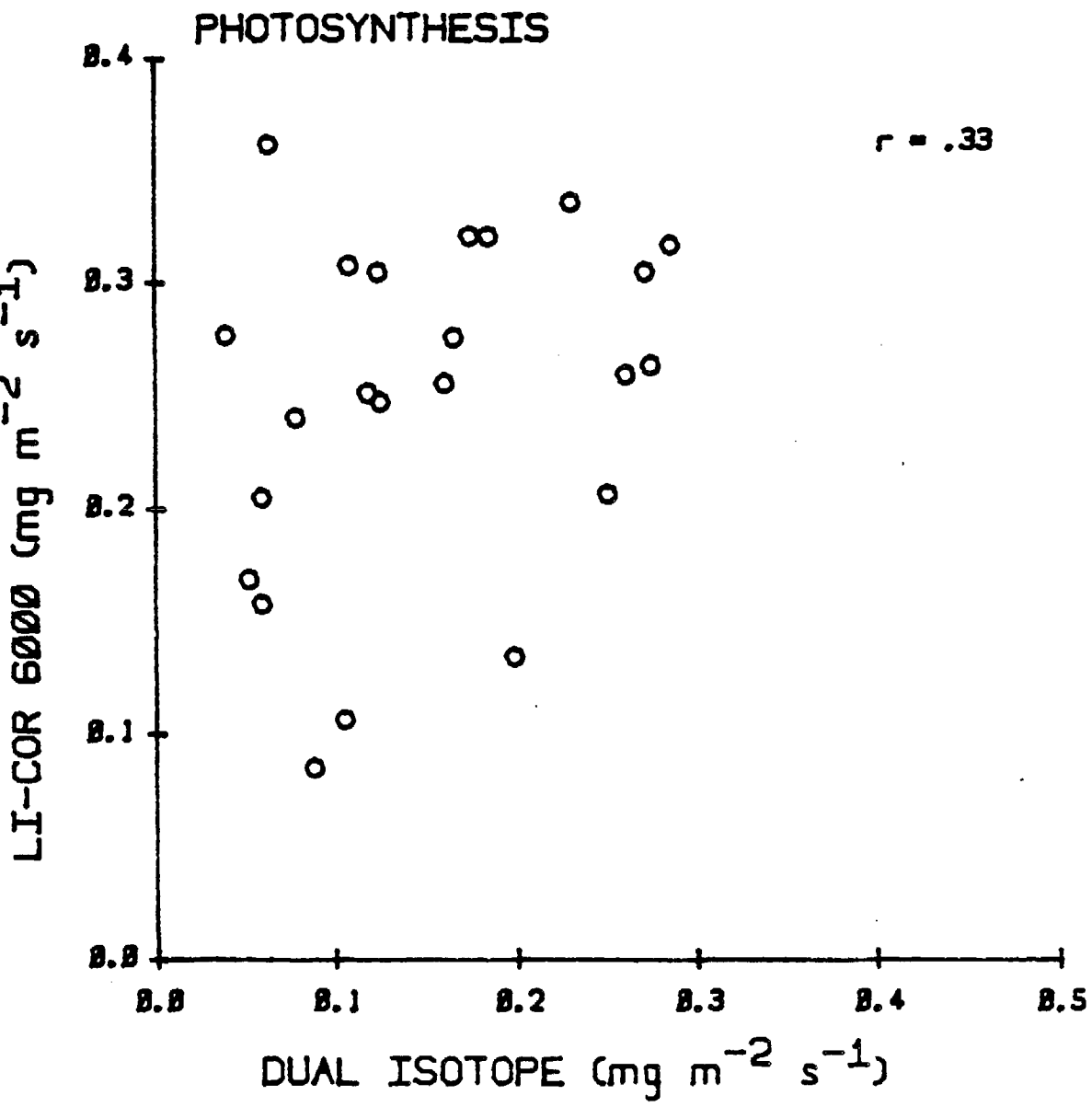

Figure A-4. Relationship for spinach photosynthesis between dual-isotope and LI-COR 6000 porometers.

APPENDIX B

Raw Data for Physiology and Productivity Measurements

Table B-1. Environmental Conditions During First Spinach Group[a]

Date	Irradiance ($\mu mol\ m^{-2}\ s^{-1}$)	Humidity (%)	Cuvette Temperature (C)
9/2/86	343 ± 21	50 ± 4	28.1 ± 0.3
9/5/86	347 ± 23	52 ± 3	31.4 ± 0.2
9/8/86	342 ± 6	61 ± 5	28.9 ± 0.2
9/9/86	354 ± 28	59 ± 4	27.8 ± 0.3
9/12/86	364 ± 19	57 ± 4	26.9 ± 0.2
9/15/86	350 ± 14	54 ± 2	25.1 ± 0.3
9/16/86	365 ± 11	49 ± 5	26.5 ± 0.2
9/19/86	352 ± 23	53 ± 5	25.3 ± 0.1
9/22/86	313 ± 95	47 ± 3	25.1 ± 0.3
9/23/86	338 ± 49	46 ± 8	22.7 ± 0.4
9/26/86	409 ± 75	46 ± 8	24.6 ± 0.8
9/29/86	395 ± 8	47 ± 6	26.5 ± 0.5
Average[b]	356 ± 8	52 ± 5	26.6 ± 2.3

[a]Values are mean ± SD for 6-24 single plant replicates. Data taken with a LI-COR 6000 porometer, except for days with temperature data missing when all data were taken with a LI-COR 1600 porometer.
[b]Average ± SD is for daily means.

Table B-2. Environmental Conditions During Second Spinach Group[a]

Date	Irradiance ($\mu mol\ m^{-2}\ s^{-1}$)	Humidity (%)	Cuvette Temperature (C)
10/21/86	111 ± 31	32 ± 2	21.9 ± 0.3
10/24/86	162 ± 47	33 ± 1	24.0 ± 0.4
10/27/86	202 ± 40	25 ± 2	25.9 ± 1.6
10/28/86	122 ± 14	33 ± 1	21.9 ± 0.4
10/31/86	193 ± 22	47 ± 9	23.4 ± 0.6
11/4/86	188 ± 18	39 ± 7	24.6 ± 1.0
11/7/86	157 ± 24	46 ± 6	23.3 ± 0.6
11/11/86	156 ± 19	41 ± 6	23.0 ± 0.6
11/14/86	134 ± 37	41 ± 6	22.7 ± 0.8
Average[b]	158 ± 32	37 ± 7	23.4 ± 1.3

[a]Values are mean ± SD for 12 single plant replicates. Data taken with a LI-COR 6000 porometer.
[b]Average ± SD is for daily means.

Table B-3. Environmental Conditions During Third Spinach Group[a]

Date	Irradiance ($\mu mol\ m^{-2}\ s^{-1}$)	Humidity (%)	Cuvette Temperature (C)
11/25/86	153 ± 13	18 ± 3	25.3 ± 1.9
11/28/86	160 ± 11	22 ± 2	26.9 ± 1.8
12/01/86	180 ± 51	20 ± 1	23.2 ± 0.1
12/02/86	148 ± 10	31 ± 6	---
12/05/86	124 ± 9	40 ± 6	---
12/09/86	167 ± 5	44 ± 6	---
12/12/86	157 ± 25	36 ± 6	---
12/15/86	307 ± 142	20 ± 3	19.5 ± 2.5
12/16/86	145 ± 5	38 ± 5	---
12/19/86	206 ± 18	47 ± 4	---
12/22/86	489 ± 233	21 ± 1	19.4 ± 1.4
Average[b]	203 ± 107	31 ± 11	22.9 ± 3.4

[a]Values are mean ± SD for 12 single plant replicates. Data taken with a LI-COR 6000 porometer, except for dates without cuvette data, when the LI-COR 1600 was used.
[b]Average ± SD is for daily means.

Table B-4. Environmental Conditions During First Interspecific Group[a]

Date	Irradiance ($\mu mol\ m^{-2}\ s^{-1}$)	Humidity (%)	Cuvette Temperature (C)
2/3/87	315 ± 142	24 ± 2	22.8 ± 0.7
2/6/87	991 ± 409	19 ± 1	22.8 ± 0.3
2/10/87	544 ± 189	32 ± 10	25.1 ± 0.9
2/13/87	67 ± 17	30 ± 10	20.1 ± 0.4
2/17/87	317 ± 213	22 ± 9	21.5 ± 0.9
2/19/87	418 ± 181	26 ± 12	19.2 ± 1.6
2/24/87	61 ± 19	18 ± 9	14.3 ± 0.9
2/26/86	551 ± 213	27 ± 9	23.3 ± 1.0
Average[b]	408 ± 301	25 ± 5	21.0 ± 3.3

[a]Values are mean ± SD for 32 single plant replicates. Data taken with a LI-COR 6000 porometer except for 2/3-2/6/87 when a LI-COR 1600 was used.
[b]Average ± SD is for daily means.

Table B-5. Environmental Conditions During Second Interspecific Group[a]

Date	Irradiance ($\mu mol\ m^{-2}\ s^{-1}$)	Humidity (%)	Cuvette Temperature (C)
3/3/87[b]	1163 ± 149	30 ± 8	23.5 ± 2.5
3/6/87[b]	201 ± 37	22 ± 1	23.0 ± 0.6
3/10/87	349 ± 79	43 ± 8	19.8 ± 0.5
3/13/87	677 ± 250	35 ± 8	25.2 ± 1.5
3/17/87	773 ± 226	34 ± 7	26.3 ± 0.8
3/20/87	798 ± 220	32 ± 9	20.5 ± 2.5
3/24/87	519 ± 523	28 ± 12	22.9 ± 1.8
3/27/87	827 ± 227	33 ± 7	26.4 ± 0.6
Average[c]	663 ± 302	30 ± 8	23.5 ± 2.5

[a]Values are mean ± SD for 12 single plant replicates. Data taken with a LI-COR 6000 porometer except for 3/24-27/87 when a LI-COR 1600 was used.
[b]For humidity, data taken with 1600 is usually 20% lower than data taken with 6000.
[c]Average ± SD is for daily means.

Table B-6. Physiological Measurements for First Spinach Group (2 reps)[a]

Date	Treatment	Conductance ($cm\ s^{-1}$)	Photosynthesis ($mg\ CO_2\ m^{-2}\ s^{-1}$)	Transpiration ($mg\ H_2O\ m^{-2}\ s^{-1}$)
9/2/86	Control	1.56 ± 0.49	0.46 ± 0.03	129 ± 22
	Square W.	1.31 ± 0.11	0.48 ± 0.00	116 ± 2
	Peak	0.77 ± 0.14	0.46 ± 0.05	85 ± 9
9/5/86	Control	1.76 ± 0.71	0.42 ± 0.08	143 ± 28
	Square W.	1.48 ± 0.04	0.47 ± 0.05	142 ± 12
	Peak	0.84 ± 0.14	0.30 ± 0.04	101 ± 12
9/8/86	Control	2.14 ± 1.07	0.39 ± 0.12	131 ± 37
	Square W.	2.83 ± 0.99	0.45 ± 0.00	144 ± 14
	Peak	1.51 ± 0.80	0.26 ± 0.18	116 ± 31
9/9/86	Control	2.64 ± 0.08	0.58 ± 0.01	141 ± 6
	Square W.	2.20 ± 0.08	0.50 ± 0.16	125 ± 11
	Peak	2.00 ± 0.05	0.40 ± 0.04	120 ± 24
9/12/86	Control	2.25 ± 0.01	0.52 ± 0.10	134 ± 2
	Square W.	1.21 ± 0.32	0.35 ± 0.05	91 ± 14
	Peak	1.98 ± 0.95	0.47 ± 0.04	96 ± 5
9/15/86	Control	1.76 ± 0.51	0.47 ± 0.01	114 ± 8
	Square W.	1.21 ± 0.05	0.38 ± 0.01	84 ± 3
	Peak	1.16 ± 0.03	0.34 ± 0.20	51 ± 5
9/16/86	Control	1.17 ± 0.06	0.37 ± 0.05	101 ± 6
	Square W.	1.32 ± 0.43	0.41 ± 0.10	72 ± 35
	Peak	0.44 ± 0.10	0.34 ± 0.04	31 ± 5
9/19/86	Control	1.40 ± 0.48	0.42 ± 0.03	102 ± 25
	Square W.	1.28 ± 0.40	0.36 ± 0.18	98 ± 17
	Peak	0.64 ± 0.35	0.34 ± 0.03	56 ± 14
9/22/86	Control	0.89 ± 0.51	0.27 ± 0.05	70 ± 31
	Square W.	1.34 ± 0.45	0.48 ± 0.09	96 ± 2
	Peak	0.69 ± 0.090	0.31 ± 0.08	72 ± 2
9/23/86	Control	0.98 ± 0.05	0.40 ± 0.15	76 ± 2
	Square W.	0.57 ± 0.38	0.27 ± 0.05	49 ± 25
	Peak	0.70 ± 0.64	0.26 ± 0.04	53 ± 32
9/26/86	Control	0.87 ± 0.45	0.43 ± 0.19	84 ± 35
	Square W.	0.72 ± 0.52	0.44 ± 0.08	81 ± 47
	Peak	0.88 ± 0.40	0.37 ± 0.14	89 ± 30

(continued)

Table B-6 (continued) - 2

Date	Treatment	Conductance ($cm\ s^{-1}$)	Photosynthesis ($mg\ CO_2\ m^{-2}\ s^{-1}$)	Transpiration ($mg\ H_2O\ m^{-2}\ s^{-1}$)
9/29/86	Control	0.73 ± 0.25	0.45 ± 0.08	77 ± 19
	Square W.	0.54 ± 0.31	0.29 ± 0.18	70 ± 37
	Peak	1.32 ± 0.06	0.50 ± 0.17	116 ± 3
Average[b]	Control	1.50 ± 0.73	0.43 ± 0.10	107 ± 32
	Square W.	1.30 ± 0.74	0.41 ± 0.10	98 ± 33
	Peak	1.11 ± 0.03	0.35 ± 0.11	90 ± 31

[a]Values are means ± SD for two plants per treatment.
[b]Average for only 9/5-9/29/86. The data for 9/2/86 were not included as they were not used for across dates statistical analysis.

Table B-7. Physiological Measurements for First Spinach Group (Days More than Two Replicates)[a]

Date	Treatment	Conductance (cm s^{-1})	Photosynthesis (mg CO_2 m^{-2} s^{-1})	Transpiration (mg H_2O m^{-2} s^{-1})
9/2/86	Control	1.54 ± 0.32	0.41 ± 0.06	126 ± 13
	Square W.	1.27 ± 0.19	0.41 ± 0.05	113 ± 7
	Peak	0.68 ± 0.16	0.35 ± 0.09	78 ± 13
9/5/86	Control	1.78 ± 0.32	0.41 ± 0.08	151 ± 14
	Square W.	1.43 ± 0.24	0.39 ± 0.10	137 ± 14
	Peak	1.09 ± 0.36	0.30 ± 0.05	116 ± 19
9/9/86	Control	2.38 ± 0.47	0.52 ± 0.07	133 ± 13
	Square W.	1.53 ± 0.54	0.41 ± 0.07	107 ± 23
	Peak	1.47 ± 0.04	0.42 ± 0.09	102 ± 23
9/12/86	Control	2.10 ± 0.47	0.49 ± 0.10	125 ± 13
	Square W.	1.53 ± 0.54	0.41 ± 0.07	107 ± 23
	Peak	1.47 ± 0.64	0.42 ± 0.09	102 ± 23
9/16/86	Control	1.04 ± 0.37	0.36 ± 0.04	92 ± 23
	Square W.	1.08 ± 0.51	0.39 ± 0.06	92 ± 32
	Peak	0.72 ± 0.35	0.32 ± 0.05	72 ± 26
9/19/86	Control	1.48 ± 0.29	0.41 ± 0.03	104 ± 15
	Square W.	1.14 ± 0.31	0.40 ± 0.13	93 ± 12
	Peak	1.06 ± 0.50	0.38 ± 0.06	81 ± 31

[a]Values are means ± SD for 10 (9/2), eight (9/5), six (9/9, 9/12), or four (9/16, 9/19) plants per treatment.

Table B-8. Physiological Measurements During Second Spinach Group[a]

Date	Treatment	Conductance ($cm\ s^{-1}$)	Photosynthesis ($mg\ CO_2\ m^{-2}\ s^{-1}$)	Transpiration ($mg\ H_2O\ m^{-2}\ s^{-1}$)
10/21/86	Control	1.24 ± 0.12	---	12 ± 1
	Square W.	0.70 ± 0.08	---	8 ± 1
	Peak	0.53 ± 0.03	---	6 ± 0
10/24/86	Control	1.43 ± 0.32	---	15 ± 1
	Square W.	0.94 ± 0.41	---	12 ± 5
	Peak	0.59 ± 0.05	---	7 ± 4
10/27/86	Control	2.10 ± 0.69	---	23 ± 8
	Square W.	1.86 ± 0.24	---	22 ± 3
	Peak	1.41 ± 0.41	---	18 ± 4
10/28/86	Control	1.28 ± 0.21	---	13 ± 1
	Square W.	0.70 ± 0.26	---	8 ± 3
	Peak	0.73 ± 0.16	---	8 ± 2
10/31/86	Control	1.27 ± 0.26	0.23 ± 0.10	86 ± 7
	Square W.	0.87 ± 0.15	0.26 ± 0.07	70 ± 5
	Peak	0.42 ± 0.14	0.21 ± 0.07	45 ± 8
11/4/86	Control	0.75 ± 0.09	0.19 ± 0.08	73 ± 10
	Square W.	0.30 ± 0.22	0.16 ± 0.05	35 ± 25
	Peak	0.23 ± 0.07	0.14 ± 0.04	30 ± 8
11/7/86	Control	0.79 ± 0.15	0.13 ± 0.07	63 ± 6
	Square W.	0.39 ± 0.24	0.13 ± 0.04	36 ± 21
	Peak	0.20 ± 0.06	0.16 ± 0.11	21 ± 7
11/11/86	Control	0.66 ± 0.15	0.15 ± 0.06	59 ± 9
	Square W.	0.39 ± 0.14	0.12 ± 0.05	40 ± 13
	Peak	0.20 ± 0.14	0.10 ± 0.03	24 ± 16
11/14/86	Control	0.55 ± 0.24	0.27 ± 0.15	49 ± 17
	Square W.	0.27 ± 0.15	0.15 ± 0.12	27 ± 15
	Peak	0.19 ± 0.17	0.13 ± 0.07	19 ± 16
Average	Control	0.80 ± 0.31	0.18 ± 0.08	66 ± 16
	Square W.	0.45 ± 0.28	0.16 ± 0.10	42 ± 21
	Peak	0.25 ± 0.14	0.15 ± 0.07	28 ± 14

[a]Values are means ± SD for four single plant replicates. For days where photosynthesis data were available, data were taken with the LI-COR 6000; the LI-COR 1600 was used on the other dates.

Table B-9. Physiological Measurements During Third Spinach Group[a]

Date	Treatment	Conductance ($cm\ s^{-1}$)	Photosynthesis ($mg\ CO_2\ m^{-2}\ s^{-1}$)	Transpiration ($mg\ H_2O\ m^{-2}\ s^{-1}$)
11/25/86	Control	0.35 ± 0.14	---	52 ± 20
	Square W.	0.37 ± 0.34	---	54 ± 24
	Peak	0.39 ± 0.14	---	56 ± 25
11/28/86	Control	0.67 ± 0.07	---	92 ± 15
	Square W.	0.37 ± 0.15	---	57 ± 21
	Peak	0.27 ± 0.10	---	47 ± 15
12/1/86	Control	1.10 ± 0.19	---	125 ± 20
	Square W.	1.14 ± 0.13	---	129 ± 9
	Peak	1.15 ± 0.24	---	129 ± 16
12/2/86	Control	0.64 ± 0.31	0.10 ± 0.07	81 ± 29
	Square W.	0.41 ± 0.18	0.10 ± 0.05	59 ± 19
	Peak	0.18 ± 0.05	0.07 ± 0.07	29 ± 7
12/5/86	Control	0.42 ± 0.05	0.14 ± 0.03	40 ± 7
	Square W.	0.25 ± 0.15	0.15 ± 0.10	26 ± 15
	Peak	0.13 ± 0.05	0.10 ± 0.04	14 ± 7
12/9/86	Control	0.40 ± 0.04	0.18 ± 0.04	32 ± 4
	Square W.	0.20 ± 0.08	0.18 ± 0.06	19 ± 6
	Peak	0.11 ± 0.06	0.16 ± 0.05	11 ± 6
12/12/86	Control	0.39 ± 0.05	0.17 ± 0.07	39 ± 2
	Square W.	0.17 ± 0.07	0.14 ± 0.04	18 ± 6
	Peak	0.14 ± 0.10	0.08 ± 0.06	15 ± 10
12/15/86	Control	1.00 ± 0.23	---	107 ± 12
	Square W.	0.95 ± 0.28	---	101 ± 12
	Peak	0.91 ± 0.32	---	101 ± 21
12/16/86	Control	0.49 ± 0.11	0.19 ± 0.04	50 ± 11
	Square W.	0.19 ± 0.07	0.18 ± 0.05	21 ± 8
	Peak	0.10 ± 0.08	0.16 ± 0.08	11 ± 9
12/19/86	Control	0.55 ± 0.09	0.31 ± 0.03	44 ± 11
	Square W.	0.25 ± 0.06	0.23 ± 0.05	23 ± 4
	Peak	0.15 ± 0.04	0.25 ± 0.05	13 ± 3
12/22/86	Control	0.79 ± 0.13	---	96 ± 2
	Square W.	0.89 ± 0.07	---	102 ± 5
	Peak	0.73 ± 0.09	---	86 ± 4

(continued)

Table B-9 (continued) - 2

Date	Treatment	Conductance (cm s^{-1})	Photosynthesis (mg CO_2 m^{-2} s^{-1})	Transpiration (mg H_2O m^{-2} s^{-1})
Average[b]	Control	0.48 ± 0.16	0.18 ± 0.08	48 ± 20
	Square W.	0.20 ± 0.13	0.16 ± 0.07	28 ± 17
	Peak	0.14 ± 0.07	0.14 ± 0.09	15 ± 9

[a]Values are means ± SD for four plants per treatment.
[b]Average for only 12/2-12/12, 12/16-19/86. The other data are included as they were not used for across dates statistical analysis.

Table B-10. Physiological Measurements During Fourth Spinach Group[a]

Date	Treatment	Conductance ($cm\ s^{-1}$)	Photosynthesis ($mg\ CO_2\ m^{-2}\ s^{-1}$)	Transpiration ($mg\ H_2O\ m^{-2}\ s^{-1}$)
2/10/87	Control	0.93 ± 0.17	0.44 ± 0.02	86 ± 10
	Peak	0.39 ± 0.11	0.50 ± 0.07	56 ± 13
2/13/87	Control	0.56 ± 0.07	0.10 ± 0.07	38 ± 5
	Peak	0.35 ± 0.08	0.13 ± 0.02	37 ± 3
2/17/87	Control	0.38 ± 0.24	0.31 ± 0.07	40 ± 19
	Peak	0.22 ± 0.00	0.23 ± 0.04	30 ± 4
2/20/87	Control	0.59 ± 0.31	0.47 ± 0.09	49 ± 14
	Peak	0.39 ± 0.23	0.45 ± 0.13	45 ± 15
2/24/87	Control	0.30 ± 0.07	0.08 ± 0.12	20 ± 4
	Peak	0.26 ± 0.24	0.09 ± 0.07	20 ± 15
2/27/87	Control	0.37 ± 0.13	0.43 ± 0.10	50 ± 12
	Peak	0.49 ± 0.14	0.38 ± 0.13	60 ± 10
Average	Control	0.52 ± 0.27	0.30 ± 0.18	46 ± 23
	Peak	0.35 ± 0.17	0.30 ± 0.18	40 ± 18

[a]Values are means ± D for four single plant replicates.

Table B-11. Physiological Measurements During First Interspecific Group[a]

Date	Treatment	Conductance ($cm\ s^{-1}$)	Photosynthesis ($mg\ CO_2\ m^{-2}\ s^{-1}$)	Transpiration ($mg\ H_2O\ m^{-2}\ s^{-1}$)
			Corn	
2/10/87	Control	0.14 ± 0.10	0.26 ± 0.10	19 ± 9
	Peak	0.09 ± 0.03	0.28 ± 0.01	15 ± 4
2/13/87	Control	0.07 ± 0.02	0.08 ± 0.09	7 ± 2
	Peak	0.07 ± 0.01	0.06 ± 0.02	10 ± 3
2/17/87	Control	0.08 ± 0.04	0.23 ± 0.07	12 ± 8
	Peak	0.05 ± 0.01	0.16 ± 0.03	6 ± 1
2/20/87	Control	0.06 ± 0.03	0.21 ± 0.07	7 ± 4
	Peak	0.06 ± 0.02	0.16 ± 0.04	9 ± 3
2/24/87	Control	0.07 ± 0.07	0.08 ± 0.05	6 ± 6
	Peak	0.01 ± 0.01	0.06 ± 0.02	1 ± 0
2/27/87	Control	0.19 ± 0.05	0.28 ± 0.08	24 ± 5
	Peak	0.08 ± 0.02	0.20 ± 0.05	12 ± 2
Average	Control	0.10 ± 0.06	0.19 ± 0.09	13 ± 7
	Peak	0.06 ± 0.03	0.15 ± 0.08	9 ± 5
			Squash	
2/10/87	Control	0.34 ± 0.10	0.29 ± 0.03	44 ± 10
	Peak	0.30 ± 0.10	0.35 ± 0.08	45 ± 12
2/13/87	Control	0.16 ± 0.04	0.05 ± 0.02	15 ± 2
	Peak	0.13 ± 0.03	0.05 ± 0.01	15 ± 3
2/17/87	Control	0.09 ± 0.03	0.10 ± 0.04	12 ± 4
	Peak	0.07 ± 0.06	0.16 ± 0.14	11 ± 9
2/20/87	Control	0.14 ± 0.06	0.15 ± 0.03	16 ± 6
	Peak	0.16 ± 0.04	0.19 ± 0.07	18 ± 5
2/24/87	Control	0.04 ± 0.04	0.03 ± 0.03	4 ± 4
	Peak	0.01 ± 0.01	0.02 ± 0.02	1 ± 1
2/27/87	Control	0.19 ± 0.05	0.24 ± 0.06	27 ± 7
	Peak	0.14 ± 0.04	0.18 ± 0.05	22 ± 5
Average	Control	0.16 ± 0.10	0.14 ± 0.16	20 ± 14
	Peak	0.13 ± 0.10	0.16 ± 0.12	19 ± 5

(continued)

Table B-11 (continued) - 2

Date	Treatment	Conductance (cm s^{-1})	Photosynthesis (mg CO_2 m^{-2} s^{-1})	Transpiration (mg H_2O m^{-2} s^{-1})
			Radish	
2/10/87	Control	0.87 ± 0.25	0.32 ± 0.07	68 ± 8
	Peak	0.36 ± 0.12	0.16 ± 0.07	53 ± 16
2/13/87	Control	0.31 ± 0.05	0.10 ± 0.08	27 ± 2
	Peak	0.45 ± 0.14	0.07 ± 0.09	45 ± 12
2/17/87	Control	0.56 ± 0.16	0.27 ± 0.05	48 ± 10
	Peak	0.39 ± 0.14	0.10 ± 0.03	40 ± 13
2/20/87	Control	0.57 ± 0.15	0.47 ± 0.11	47 ± 8
	Peak	0.52 ± 0.02	0.29 ± 0.02	49 ± 6
2/24/87	Control	0.19 ± 0.14	0.08 ± 0.02	14 ± 8
	Peak	0.27 ± 0.04	0.03 ± 0.03	20 ± 3
2/27/87	Control	0.63 ± 0.34	0.33 ± 0.03	56 ± 20
	Peak	0.45 ± 0.06	0.17 ± 0.06	57 ± 6
Average	Control	0.52 ± 0.24	0.26 ± 0.15	43 ± 20
	Peak	0.41 ± 0.09	0.14 ± 0.09	44 ± 13
			Lettuce	
2/17/87	Control	0.19 ± 0.05	0.09 ± 0.03	23 ± 6
	Peak	0.12 ± 0.02	0.11 ± 0.01	16 ± 3
2/20/87	Control	0.24 ± 0.06	0.12 ± 0.10	22 ± 4
	Peak	0.24 ± 0.06	0.13 ± 0.05	23 ± 5
2/24/87	Control	0.10 ± 0.05	0.11 ± 0.06	10 ± 4
	Peak	0.06 ± 0.05	0.07 ± 0.03	6 ± 4
2/27/87	Control	0.25 ± 0.12	0.19 ± 0.04	33 ± 12
	Peak	0.32 ± 0.05	0.28 ± 0.05	44 ± 4
Average	Control	0.20 ± 0.07	0.13 ± 0.04	22 ± 9
	Peak	0.19 ± 0.13	0.15 ± 0.09	22 ± 16

[a]Values are means ± SD for four single plant replicates. For days where photosynthesis data were available, data were taken with the LI-COR 6000; the LI-COR 1600 was used on the other dates.

Table B-12. Physiological Measurements During Second Interspecific Group[a]

Date	Treatment	Conductance ($cm\ s^{-1}$)	Photosynthesis ($mg\ CO_2\ m^{-2}\ s^{-1}$)	Transpiration ($mg\ H_2O\ m^{-2}\ s^{-1}$)
			Corn	
3/10/87	Control	0.23 ± 0.02	0.38 ± 0.04	20 ± 1
	Peak	0.15 ± 0.04	0.36 ± 0.10	16 ± 3
3/13/87	Control	0.19 ± 0.03	0.53 ± 0.08	33 ± 5
	Peak	0.14 ± 0.04	0.36 ± 0.12	25 ± 5
3/17/87	Control	0.14 ± 0.03	0.31 ± 0.07	21 ± 4
	Peak	0.16 ± 0.02	0.40 ± 0.05	27 ± 3
3/20/87	Control	0.14 ± 0.04	0.25 ± 0.06	16 ± 3
	Peak	0.12 ± 0.02	0.27 ± 0.04	15 ± 2
3/24/87	Control	0.09 ± 0.02	0.04 ± 0.02	14 ± 2
	Peak	0.08 ± 0.01	0.09 ± 0.02	12 ± 1
3/27/87	Control	0.13 ± 0.01	0.34 ± 0.05	22 ± 2
	Peak	0.14 ± 0.02	0.36 ± 0.02	24 ± 2
Average	Control	0.15 ± 0.05	0.31 ± 0.16	21 ± 7
	Peak	0.13 ± 0.03	0.31 ± 0.11	20 ± 6
			Squash	
3/10/87	Control	0.42 ± 0.19	0.29 ± 0.09	33 ± 11
	Peak	0.40 ± 0.06	0.39 ± 0.06	35 ± 5
3/13/87	Control	0.22 ± 0.05	0.27 ± 0.12	37 ± 9
	Peak	0.25 ± 0.02	0.34 ± 0.05	40 ± 3
3/17/87	Control	0.17 ± 0.06	0.23 ± 0.05	29 ± 8
	Peak	0.19 ± 0.02	0.28 ± 0.02	38 ± 4
3/20/87	Control	0.15 ± 0.05	0.20 ± 0.04	18 ± 6
	Peak	0.19 ± 0.05	0.24 ± 0.04	24 ± 5
3/24/87	Control	0.14 ± 0.06	0.23 ± 0.10	25 ± 12
	Peak	0.17 ± 0.05	0.28 ± 0.08	32 ± 9
3/27/87	Control	0.27 ± 0.05	0.34 ± 0.07	47 ± 8
	Peak	0.20 ± 0.05	0.28 ± 0.08	38 ± 7
Average	Control	0.23 ± 0.11	0.26 ± 0.12	32 ± 10
	Peak	0.23 ± 0.09	0.30 ± 0.05	35 ± 6

(continued)

Table B-12 (continued) - 2

Date	Treatment	Conductance (cm s^{-1})	Photosynthesis (mg CO_2 m^{-2} s^{-1})	Transpiration (mg H_2O m^{-2} s^{-1})
			Radish	
3/10/87	Control	1.14 ± 0.27	0.46 ± 0.12	60 ± 9
	Peak	0.48 ± 0.18	0.30 ± 0.01	38 ± 8
3/13/87	Control	0.96 ± 0.27	0.44 ± 0.15	77 ± 11
	Peak	0.62 ± 0.34	0.34 ± 0.04	71 ± 24
3/17/87	Control	0.55 ± 0.14	0.46 ± 0.37	65 ± 11
	Peak	0.69 ± 0.13	0.48 ± 0.06	86 ± 10
3/20/87	Control	0.58 ± 0.16	0.60 ± 0.10	58 ± 10
	Peak	0.86 ± 0.20	0.52 ± 0.14	90 ± 18
3/24/87	Control	0.77 ± 0.18	0.70 ± 0.18	81 ± 11
	Peak	1.00 ± 0.40	0.68 ± 0.17	86 ± 20
3/27/87	Control	0.83 ± 0.37	0.62 ± 0.12	101 ± 29
	Peak	0.77 ± 0.36	0.52 ± 0.11	89 ± 26
Average	Control	0.81 ± 0.23	0.55 ± 0.11	74 ± 17
	Peak	0.74 ± 0.18	0.47 ± 0.14	76 ± 20
			Lettuce	
3/10/87	Control	0.34 ± 0.13	0.23 ± 0.01	34 ± 10
	Peak	0.44 ± 0.14	0.26 ± 0.05	36 ± 9
3/13/87	Control	0.26 ± 0.11	0.21 ± 0.08	36 ± 12
	Peak	0.30 ± 0.10	0.23 ± 0.08	35 ± 10
3/13/87	Control	0.27 ± 0.05	0.24 ± 0.06	40 ± 6
	Peak	0.29 ± 0.07	0.26 ± 0.09	46 ± 9
3/17/87	Control	0.18 ± 0.12	0.21 ± 0.08	22 ± 14
	Peak	0.27 ± 0.03	0.34 ± 0.05	29 ± 4
3/20/87	Control	0.08 ± 0.03	0.08 ± 0.02	11 ± 4
	Peak	0.14 ± 0.06	0.08 ± 0.01	18 ± 6
3/27/87	Control	0.27 ± 0.11	0.27 ± 0.02	40 ± 13
	Peak	0.27 ± 0.06	0.29 ± 0.09	41 ± 8
Average	Control	0.23 ± 0.09	0.21 ± 0.07	31 ± 12
	Peak	0.29 ± 0.10	0.24 ± 0.09	34 ± 10

[a]Values are means ± SD for four single plant replicates. For days where photosynthesis data were available, data were taken with the LI-COR 6000; the LI-COR 1600 was used on the other dates.

Table B-13. Dry Weight Measurements for the First Spinach Group[a]

Date	Treatment	Leaf (g)	Stem (g)	Root (g)
9/8/86	Control	1.52 ± 0.46	0.46 ± 0.10	0.38 ± 0.38
	Square	1.11 ± 0.33	0.34 ± 0.08	0.32 ± 0.12
	Peak	0.94 ± 0.50	0.31 ± 0.09	0.32 ± 0.00
9/15/86	Control	2.43 ± 0.75	0.65 ± 0.10	0.77 ± 0.43
	Square	2.82 ± 0.71	0.84 ± 0.09	0.79 ± 0.26
	Peak	3.07	1.09	0.94
9/22/86	Control	5.12 ± 2.82	1.41 ± 0.88	0.82 ± 0.45
	Square	2.85 ± 0.58	0.73 ± 0.20	0.75 ± 0.28
	Peak	2.13 ± 0.47	0.62 ± 0.40	0.65 ± 0.50
9/29/86	Control	5.63 ± 0.38	1.56 ± 0.16	1.46 ± 0.09
	Square	2.80 ± 1.69	1.00 ± 0.40	1.42 ± 0.60
	Peak	4.53	1.41	1.54

Date	Treatment	Shoot (g)	Total (g)	Shoot/Root Ratio
9/8/86	Control	1.90 ± 0.60	2.48 ± 0.94	4.97 ± 2.68
	Square	1.46 ± 0.40	1.76 ± 0.52	4.68 ± 0.51
	Peak	1.25 ± 0.59	1.57 ± 0.59	3.89 ± 1.83
9/15/86	Control	3.08 ± 0.85	3.65 ± 1.28	6.88 ± 3.75
	Square	3.66 ± 0.79	4.45 ± 1.05	4.72 ± 0.52
	Peak	4.16	5.10	4.43
9/22/86	Control	6.53 ± 3.70	7.60 ± 4.14	5.86 ± 1.01
	Square	3.58 ± 0.78	4.33 ± 1.05	4.95 ± 0.79
	Peak	2.75 ± 0.87	3.40 ± 1.36	5.23 ± 2.69
9/29/86	Control	7.19 ± 0.54	8.61 ± 0.62	5.06 ± 0.08
	Square	4.86 ± 2.09	6.27 ± 2.69	3.43 ± 0.02
	Peak	5.94	7.48	3.86

[a]Values are means ± SD for two single plant replicates except for one replicate for peak on 9/8 and 9/22/86.

Table B-14. Fresh Weight Measurements for the First Spinach Group[a]

Date	Treatment	Leaf (g)	Stem (g)	Root (g)
9/8/86	Control	21.56 ± 7.25	8.33 ± 2.52	8.36 ± 4.40
	Square	14.36 ± 4.84	5.39 ± 1.58	7.43 ± 1.27
	Peak	10.22 ± 5.55	4.64 ± 1.22	5.63 ± 0.94
9/15/86	Control	30.23 ± 8.50	9.61 ± 0.94	10.57 ± 8.74
	Square	31.52 ± 7.74	13.09 ± 1.11	14.00 ± 3.68
	Peak	27.00	13.65	16.54
9/22/86	Control	60.83 ± 24.5	25.02 ± 13.3	27.03 ± 9.38
	Square	33.86 ± 3.51	12.80 ± 2.29	13.83 ± 0.86
	Peak	24.60 ± 4.64	9.72 ± 5.34	14.02 ± 7.53
9/29/86	Control	60.70 ± 9.40	24.42 ± 4.31	21.31 ± 2.79
	Square	41.84 ± 12.9	14.85 ± 2.16	23.29 ± 3.45
	Peak	45.64	19.65	26.67

Date	Treatment	Shoot (g)	Total (g)	Shoot/Root Ratio
9/8/86	Control	29.89 ± 9.77	38.25 ± 14.2	3.79 ± 0.83
	Square	19.75 ± 6.41	27.17 ± 7.68	2.62 ± 0.42
	Peak	14.85 ± 6.77	20.48 ± 7.71	2.58 ± 0.77
9/15/86	Control	39.84 ± 9.44	40.41 ± 18.2	5.17 ± 3.38
	Square	44.61 ± 8.85	58.60 ± 11.5	3.22 ± 0.21
	Peak	40.65	57.19	2.46
9/22/86	Control	85.84 ± 37.7	112.87 ± 47.1	3.12 ± 0.31
	Square	46.66 ± 8.85	60.48 ± 4.95	3.39 ± 0.63
	Peak	34.32 ± 9.98	48.33 ± 17.5	2.64 ± 0.71
9/29/86	Control	91.11 ± 13.7	112.41 ± 16.5	4.27 ± 0.08
	Square	56.69 ± 15.1	79.98 ± 18.5	2.41 ± 0.29
	Peak	65.29	91.96	2.45

[a]Values are means ± SD for two single plant replicates except for one replicate for peak on 9/8 and 9/22/86.

Table B-15. Dry Weight Measurements for the Second Spinach Group[a]

Date	Treatment	Leaf (g)	Stem (g)	Root (g)
10/27/86	Control	0.38 ± 0.08	0.07 ± 0.01	0.16 ± 0.03
	Square	0.25 ± 0.09	0.05 ± 0.02	0.12 ± 0.06
	Peak	0.21 ± 0.12	0.06 ± 0.02	0.09 ± 0.04
11/3/86	Control	1.07 ± 0.24	0.24 ± 0.07	0.25 ± 0.07
	Square	0.76 ± 0.44	0.17 ± 0.60	0.15 ± 0.09
	Peak	0.68 ± 0.21	0.17 ± 0.05	0.19 ± 0.06
11/10/86	Control	2.77 ± 0.84	0.71 ± 0.28	0.56 ± 0.22
	Square	1.95 ± 0.29	0.60 ± 0.10	0.44 ± 0.14
	Peak	2.27 ± 0.48	0.63 ± 0.14	0.50 ± 0.16
11/17/86	Control	6.12 ± 0.28	1.79 ± 0.71	0.96 ± 0.49
	Square	4.81 ± 1.03	1.05 ± 0.43	0.61 ± 0.11
	Peak	3.06 ± 1.25	0.86 ± 0.44	0.55 ± 0.29

Date	Treatment	Shoot (g)	Total (g)	Shoot/Root Ratio
10/27/86	Control	0.45 ± 0.09	0.60 ± 0.12	2.88 ± 0.12
	Square	0.30 ± 0.11	0.42 ± 0.15	2.70 ± 1.00
	Peak	0.27 ± 0.13	0.36 ± 0.15	3.17 ± 1.34
11/3/86	Control	1.31 ± 0.31	1.56 ± 0.38	5.31 ± 0.32
	Square	0.92 ± 0.54	1.07 ± 0.62	6.33 ± 1.35
	Peak	0.85 ± 0.26	1.04 ± 0.31	4.44 ± 0.63
11/10/86	Control	3.49 ± 1.11	4.04 ± 1.32	6.48 ± 0.93
	Square	2.56 ± 0.19	2.99 ± 0.22	6.54 ± 2.83
	Peak	2.85 ± 0.66	3.35 ± 0.70	6.07 ± 1.82
11/17/86	Control	7.90 ± 2.91	8.86 ± 3.33	8.67 ± 2.19
	Square	5.85 ± 0.81	6.46 ± 0.71	10.00 ± 3.20
	Peak	3.92 ± 1.69	4.47 ± 1.95	7.66 ± 1.75

[a]Values are means ± SD for four single plant replicates.

Table B-16. Fresh Weight Measurements for the Second Spinach Group[a]

Date	Treatment	Leaf (g)	Stem (g)	Root (g)
10/27/86	Control	3.82 ± 0.79	0.95 ± 0.23	1.95 ± 0.41
	Square	2.51 ± 1.02	0.60 ± 0.27	1.24 ± 0.62
	Peak	2.02 ± 1.09	0.59 ± 0.35	1.09 ± 0.59
11/3/86	Control	14.24 ± 3.21	4.42 ± 1.45	4.93 ± 1.77
	Square	8.93 ± 6.40	2.89 ± 2.11	3.09 ± 2.06
	Peak	7.68 ± 2.85	2.81 ± 1.11	2.85 ± 0.96
11/10/86	Control	39.44 ± 11.9	14.69 ± 6.09	14.52 ± 4.68
	Square	26.41 ± 4.23	10.73 ± 3.12	10.69 ± 3.47
	Peak	27.32 ± 7.11	12.29 ± 3.31	11.50 ± 1.97
11/17/86	Control	101.56 ± 39.6	52.57 ± 22.4	29.91 ± 13.8
	Square	60.84 ± 9.16	29.18 ± 11.1	20.31 ± 4.96
	Peak	43.48 ± 19.0	21.56 ± 10.8	13.81 ± 6.49

Date	Treatment	Shoot (g)	Total (g)	Shoot/Root
10/27/86	Control	4.77 ± 1.00	6.72 ± 1.41	2.45 ± 0.11
	Square	3.11 ± 1.28	4.35 ± 1.83	2.63 ± 0.58
	Peak	2.61 ± 1.43	3.69 ± 1.96	2.65 ± 0.94
11/3/86	Control	18.66 ± 4.61	23.58 ± 6.25	3.91 ± 0.58
	Square	11.82 ± 8.48	14.91 ± 10.5	3.54 ± 0.88
	Peak	10.49 ± 3.93	13.31 ± 4.86	3.67 ± 0.35
11/10/86	Control	54.13 ± 17.6	68.65 ± 22.2	3.72 ± 0.33
	Square	37.14 ± 7.73	47.82 ± 10.7	3.67 ± 0.88
	Peak	39.61 ± 10.3	51.12 ± 11.6	3.45 ± 0.73
11/17/86	Control	154.13 ± 59.6	184.03 ± 73.1	5.29 ± 0.48
	Square	90.02 ± 20.0	110.33 ± 22.8	4.54 ± 0.93
	Peak	65.04 ± 29.8	78.85 ± 35.6	4.95 ± 1.22

[a]Values are means ± SD for four single plant replicates.

Table B-17. Dry Weight Measurements for the Third Spinach Group[a]

Date	Treat-ment	Leaf (g)	Stem (g)	Root (g)
12/1/86	Control	0.89 ± 0.20	0.19 ± 0.04	0.28 ± 0.06
	Square	0.93 ± 0.21	0.21 ± 0.04	0.37 ± 0.07
	Peak	0.56 ± 0.06	0.13 ± 0.02	0.22 ± 0.04
12/8/86	Control	1.92 ± 0.16	0.45 ± 0.04	0.41 ± 0.08
	Square	1.27 ± 0.23	0.30 ± 0.07	0.43 ± 0.16
	Peak	1.17 ± 0.15	0.26 ± 0.03	0.27 ± 0.09
12/15/86	Control	4.09 ± 0.38	0.72 ± 0.09	0.91 ± 0.15
	Square	3.81 ± 0.92	0.84 ± 0.23	0.98 ± 0.29
	Peak	3.51 ± 0.82	0.83 ± 0.28	0.94 ± 0.08
12/22/86	Control	5.34 ± 1.39	1.26 ± 0.38	1.86 ± 0.34
	Square	5.77 ± 1.64	1.49 ± 0.42	1.69 ± 0.46
	Peak	4.67 ± 1.35	1.11 ± 0.27	1.42 ± 0.27
		Shoot (g)	Total (g)	Shoot/Root
12/1/86	Control	1.07 ± 0.24	1.35 ± 0.23	3.87 ± 0.06
	Square	1.14 ± 0.25	1.51 ± 0.30	3.12 ± 0.43
	Peak	0.69 ± 0.08	0.90 ± 0.10	3.26 ± 0.65
12/8/86	Control	2.36 ± 0.19	2.77 ± 0.26	5.82 ± 0.75
	Square	1.57 ± 0.30	1.99 ± 0.45	3.91 ± 0.94
	Peak	1.43 ± 0.17	1.70 ± 0.26	5.70 ± 1.50
12/15/86	Control	4.81 ± 0.41	5.72 ± 0.50	5.35 ± 0.77
	Square	4.65 ± 1.13	5.63 ± 1.38	4.81 ± 0.74
	Peak	4.34 ± 1.09	5.28 ± 1.04	4.71 ± 1.55
12/22/86	Control	6.61 ± 1.76	8.47 ± 2.09	3.52 ± 0.35
	Square	7.26 ± 2.03	8.95 ± 2.48	4.29 ± 0.26
	Peak	5.78 ± 1.59	7.20 ± 1.81	4.04 ± 0.83

[a]Values are means ± SD for four single plant replicates.

Table B-18. Fresh Weight Measurements for the Third Spinach Group[a]

Date	Treat-ment	Leaf (g)	Stem (g)	Root (g)
12/1/86	Control	9.35 ± 2.40	2.54 ± 0.68	4.28 ± 0.60
	Square	10.14 ± 2.23	2.87 ± 0.64	4.92 ± 0.52
	Peak	5.44 ± 0.73	1.72 ± 0.18	2.86 ± 0.49
12/8/86	Control	20.89 ± 1.62	5.92 ± 0.32	7.52 ± 3.36
	Square	13.79 ± 2.98	4.05 ± 1.18	7.48 ± 1.38
	Peak	11.64 ± 1.70	3.22 ± 0.38	4.63 ± 1.83
12/15/86	Control	42.62 ± 4.33	10.02 ± 1.96	19.43 ± 3.92
	Square	38.16 ± 9.13	11.68 ± 3.93	19.90 ± 4.49
	Peak	35.52 ± 10.2	11.72 ± 4.79	19.79 ± 2.56
12/22/86	Control	58.13 ± 14.5	15.65 ± 5.15	28.04 ± 6.34
	Square	54.75 ± 16.5	18.63 ± 7.06	26.87 ± 8.96
	Peak	40.25 ± 12.4	13.57 ± 4.51	18.66 ± 5.80

		Shoot (g)	Total (g)	Shoot/Root
12/1/86	Control	11.88 ± 3.06	16.16 ± 3.65	2.75 ± 0.31
	Square	13.01 ± 2.84	17.92 ± 3.83	2.77 ± 0.84
	Peak	7.16 ± 0.81	10.03 ± 1.16	2.54 ± 0.41
12/8/86	Control	26.81 ± 1.74	34.33 ± 4.92	4.11 ± 1.71
	Square	17.84 ± 4.03	25.33 ± 5.35	2.38 ± 0.18
	Peak	14.86 ± 1.96	19.49 ± 3.66	3.51 ± 1.04
12/15/86	Control	52.64 ± 5.44	72.07 ± 8.88	2.76 ± 0.38
	Square	49.83 ± 12.8	69.73 ± 17.0	2.50 ± 0.28
	Peak	47.24 ± 15.0	67.03 ± 16.7	2.36 ± 0.54
12/22/86	Control	70.78 ± 19.6	98.82 ± 24.9	2.53 ± 0.37
	Square	73.38 ± 22.9	100.24 ± 30.0	2.77 ± 0.44
	Peak	53.81 ± 16.4	72.47 ± 22.1	2.88 ± 0.19

[a]Values are means ± SD for four single plant replicates.

Table B-19. Fresh Weight Measurements for the First Interspecific Group[a]

Date	Treatment	Root (g)	Shoot (g)	Total (g)	Shoot/Root
			Corn		
2/9/87	Control	5.03 ± 2.76	3.11 ± 1.54	8.14 ± 4.37	0.63 ± 0.20
	Peak	5.49 ± 3.18	2.96 ± 1.28	8.45 ± 4.54	0.56 ± 0.11
2/16/87	Control	6.50 ± 2.73	4.63 ± 1.40	11.13 ± 4.27	0.74 ± 0.16
	Peak	4.05 ± 1.26	2.49 ± 1.14	6.53 ± 2.54	0.59 ± 0.15
2/23/87	Control	7.49 ± 2.44	3.31 ± 1.27	10.79 ± 3.89	0.43 ± 0.05
	Peak	7.47 ± 4.39	4.75 ± 2.58	12.22 ± 7.31	0.64 ± 0.09
3/2/87	Control	9.04 ± 3.96	5.55 ± 3.22	14.59 ± 7.65	0.55 ± 0.25
	Peak	9.52 ± 2.24	4.77 ± 2.47	14.29 ± 5.05	0.48 ± 0.16
			Squash		
2/9/87	Control	1.33 ± 0.64	6.76 ± 0.80	8.09 ± 1.28	6.81 ± 5.24
	Peak	1.78 ± 0.61	6.14 ± 0.32	7.92 ± 0.68	3.93 ± 1.90
2/16/87	Control	2.47 ± 0.16	8.86 ± 1.15	11.33 ± 1.37	3.60 ± 0.52
	Peak	2.47 ± 0.16	9.34 ± 1.06	11.80 ± 1.57	4.06 ± 0.10
2/23/87	Control	3.09 ± 0.77	11.85 ± 2.09	14.30 ± 2.31	3.71 ± 0.61
	Peak	2.43 ± 0.49	11.82 ± 0.98	13.94 ± 1.20	4.83 ± 1.14
3/2/87	Control	3.51 ± 0.94	17.13 ± 1.90	20.44 ± 2.71	5.09 ± 1.14
	Peak	3.42 ± 1.03	17.29 ± 2.75	20.54 ± 3.70	5.22 ± 0.96
			Radish		
2/9/87	Control	2.71 ± 1.66	2.15 ± 0.53	4.85 ± 1.35	1.27 ± 1.30
	Peak	1.86 ± 0.84	1.51 ± 0.54	3.36 ± 1.37	0.83 ± 0.27
2/16/87	Control	6.11 ± 0.88	2.14 ± 0.53	8.25 ± 1.11	0.35 ± 0.09
	Peak	2.86 ± 1.17	1.48 ± 0.68	4.33 ± 1.87	0.52 ± 0.19
2/23/87	Control	8.74 ± 1.45	3.41 ± 2.09	11.50 ± 2.04	0.32 ± 0.18
	Peak	4.85 ± 1.89	2.45 ± 0.57	6.79 ± 2.78	0.39 ± 0.18
3/2/87	Control	10.70 ± 0.64	2.75 ± 0.79	13.25 ± 0.79	0.24 ± 0.05
	Peak	5.43 ± 1.03	1.87 ± 0.78	6.93 ± 0.93	0.29 ± 0.10

(continued)

Table B-19 (continued) - 2

Date	Treat-ment	Root (g)	Shoot (g)	Total (g)	Shoot/Root
			Lettuce		
2/9/87	Control	0.64 ± 0.17	1.59 ± 0.29	2.23 ± 0.47	2.53 ± 0.42
	Peak	0.53 ± 0.14	1.06 ± 0.15	1.59 ± 0.31	2.06 ± 0.35
2/16/87	Control	0.44 ± 0.12	2.79 ± 0.31	3.23 ± 0.42	6.61 ± 1.51
	Peak	0.34 ± 0.18	2.19 ± 1.21	2.53 ± 1.57	6.26 ± 1.86
2/23/87	Control	2.83 ± 1.09	5.04 ± 1.91	7.86 ± 3.26	1.78 ± 0.21
	Peak	1.77 ± 0.11	3.53 ± 0.18	5.30 ± 0.21	2.00 ± 0.20
3/2/87	Control	3.91 ± 0.49	6.84 ± 0.51	10.75 ± 0.61	1.89 ± 0.76
	Peak	3.36 ± 1.05	6.05 ± 1.61	9.39 ± 2.78	1.83 ± 0.31

[a]Values are means ± SD for four single plant replicates.

Table B-20. Fresh Weight Measurements for the Second Interspecific Group[a]

Date	Treatment	Root (g)	Shoot (g)	Total (g)	Shoot/Root
			Corn		
3/9/87	Control	8.07 ± 1.70	6.95 ± 1.33	15.03 ± 3.22	0.86 ± 0.04
	Peak	6.64 ± 1.15	6.30 ± 0.89	12.94 ± 2.11	0.95 ± 0.08
3/16/87	Control	10.24 ± 2.51	9.59 ± 1.56	19.83 ± 4.25	0.95 ± 0.11
	Peak	9.79 ± 2.17	11.20 ± 1.30	20.99 ± 3.60	1.16 ± 0.12
3/23/87	Control	14.28 ± 3.69	15.66 ± 1.05	27.44 ± 8.40	0.92 ± 0.36
	Peak	16.55 ± 1.30	16.43 ± 0.66	33.47 ± 2.25	0.97 ± 0.11
3/30/87	Control	17.86 ± 1.66	19.73 ± 1.07	37.39 ± 1.82	1.11 ± 0.13
	Peak	19.69 ± 3.60	20.97 ± 1.01	40.67 ± 4.31	1.09 ± 0.16
			Squash		
3/9/87	Control	3.39 ± 0.20	11.10 ± 0.53	14.49 ± 0.81	3.28 ± 0.06
	Peak	3.28 ± 0.32	10.12 ± 0.85	13.39 ± 1.23	3.10 ± 0.23
3/16/87	Control	4.35 ± 0.62	17.41 ± 1.51	21.88 ± 2.35	4.07 ± 0.42
	Peak	4.40 ± 1.15	15.41 ± 1.18	19.81 ± 2.39	3.63 ± 0.75
3/23/87	Control	4.95 ± 0.77	21.78 ± 0.85	26.72 ± 2.09	4.49 ± 0.66
	Peak	5.48 ± 0.76	21.32 ± 2.40	26.80 ± 3.21	3.98 ± 1.03
3/30/87	Control	3.37 ± 0.52	30.96 ± 1.34	34.33 ± 1.87	9.35 ± 1.37
	Peak	3.40 ± 0.50	28.37 ± 1.59	31.77 ± 2.18	8.46 ± 1.21
			Radish		
3/9/87	Control	8.13 ± 2.66	3.19 ± 0.25	11.32 ± 2.79	0.42 ± 0.14
	Peak	6.02 ± 1.16	3.08 ± 0.42	9.10 ± 1.62	0.52 ± 0.38
3/16/87	Control	16.83 ± 3.85	4.13 ± 1.05	20.95 ± 3.90	0.25 ± 0.09
	Peak	9.66 ± 2.99	3.70 ± 0.34	13.36 ± 2.75	0.42 ± 0.18
3/23/87	Control	22.91 ± 4.78	4.80 ± 0.64	27.71 ± 5.05	0.21 ± 0.05
	Peak	17.32 ± 3.71	4.79 ± 0.99	22.11 ± 4.21	0.28 ± 0.04
3/30/87	Control	24.20 ± 7.46	5.25 ± 0.69	29.44 ± 6.92	0.24 ± 0.10
	Peak	23.23 ± 5.46	5.20 ± 0.67	28.43 ± 5.97	0.23 ± 0.05

(continued)

Table B-20 (continued) - 2

Date	Treatment	Root (g)	Shoot (g)	Total (g)	Shoot/Root
			Lettuce		
3/9/87	Control	1.08 ± 0.22	3.36 ± 0.79	4.45 ± 0.98	3.19 ± 1.03
	Peak	0.99 ± 0.34	3.19 ± 0.73	4.19 ± 1.13	3.32 ± 0.80
3/16/87	Control	2.21 ± 1.07	6.72 ± 1.83	8.98 ± 3.10	3.43 ± 1.18
	Peak	2.87 ± 0.30	7.76 ± 0.73	10.63 ± 1.12	2.70 ± 0.14
3/23/87	Control	4.66 ± 0.65	11.58 ± 1.73	16.24 ± 2.44	2.49 ± 0.40
	Peak	5.11 ± 1.05	12.43 ± 1.39	17.54 ± 1.66	2.51 ± 0.62
3/30/87	Control	7.20 ± 0.86	17.11 ± 2.57	24.24 ± 2.19	2.42 ± 0.63
	Peak	6.50 ± 2.60	14.87 ± 2.60	21.36 ± 4.50	2.71 ± 1.42

[a]Values are means ± SD for four single plant replicates.

Table B-21. Dry Weight Measurements for the First Interspecific Group[a]

Date	Treatment	Root (g)	Shoot (g)	Total (g)	Shoot/Root
			Corn		
2/9/87	Control	0.38 ± 0.18	0.37 ± 0.19	0.74 ± 0.35	1.00 ± 0.38
	Peak	0.58 ± 0.42	0.35 ± 0.19	0.93 ± 0.59	0.66 ± 0.22
2/16/87	Control	0.58 ± 0.21	0.58 ± 0.16	1.16 ± 0.37	1.03 ± 0.12
	Peak	0.42 ± 0.15	0.38 ± 0.13	0.81 ± 0.28	0.91 ± 0.13
2/23/87	Control	0.72 ± 0.21	0.50 ± 0.21	1.21 ± 0.42	0.68 ± 0.10
	Peak	0.71 ± 0.37	0.64 ± 0.36	1.35 ± 0.72	0.90 ± 0.20
3/2/87	Control	0.74 ± 0.30	0.72 ± 0.36	1.45 ± 0.67	0.95 ± 0.11
	Peak	0.66 ± 0.09	0.58 ± 0.30	1.24 ± 0.38	0.86 ± 0.33
			Squash		
2/9/87	Control	0.05 ± 0.03	0.52 ± 0.01	0.58 ± 0.14	13.68 ± 11.3
	Peak	0.08 ± 0.03	0.55 ± 0.05	0.62 ± 0.04	9.33 ± 6.90
2/16/87	Control	0.39 ± 0.18	1.00 ± 0.14	1.39 ± 0.32	2.83 ± 0.87
	Peak	0.26 ± 0.07	1.00 ± 0.15	1.25 ± 0.20	4.03 ± 0.75
2/23/87	Control	0.42 ± 0.11	1.41 ± 0.21	1.83 ± 0.31	3.51 ± 0.54
	Peak	0.28 ± 0.10	1.40 ± 0.19	1.68 ± 0.19	5.76 ± 2.67
3/2/87	Control	0.34 ± 0.04	1.82 ± 0.26	2.16 ± 0.29	5.39 ± 0.43
	Peak	0.28 ± 0.08	1.94 ± 0.26	2.23 ± 0.31	7.08 ± 1.21
			Radish		
2/9/87	Control	0.16 ± 0.08	0.16 ± 0.04	0.32 ± 0.07	1.45 ± 1.38
	Peak	0.09 ± 0.03	0.14 ± 0.02	0.24 ± 0.05	1.62 ± 0.35
2/16/87	Control	0.41 ± 0.05	0.21 ± 0.04	0.62 ± 0.08	0.50 ± 0.05
	Peak	0.20 ± 0.07	0.16 ± 0.07	0.36 ± 0.13	0.82 ± 0.32
2/23/87	Control	0.67 ± 0.15	0.28 ± 0.04	0.94 ± 0.19	0.42 ± 0.04
	Peak	0.39 ± 0.17	0.25 ± 0.12	0.64 ± 0.28	0.63 ± 0.11
3/2/87	Control	0.74 ± 0.01	0.30 ± 0.06	1.04 ± 0.06	0.40 ± 0.08
	Peak	0.39 ± 0.07	0.22 ± 0.06	0.61 ± 0.05	0.60 ± 0.21

(continued)

Table B-21 (continued) - 2

Date	Treatment	Root (g)	Shoot (g)	Total (g)	Shoot/Root
			Lettuce		
2/9/87	Control	0.03 ± 0.01	0.07 ± 0.03	0.10 ± 0.02	2.83 ± 1.59
	Peak	0.03 ± 0.00	0.04 ± 0.01	0.07 ± 0.01	1.64 ± 0.82
2/16/87	Control	0.03 ± 0.01	0.17 ± 0.03	0.19 ± 0.03	6.13 ± 1.66
	Peak	0.03 ± 0.01	0.14 ± 0.07	0.29 ± 0.31	2.94 ± 1.66
2/23/87	Control	0.20 ± 0.10	0.46 ± 0.21	0.66 ± 0.31	2.42 ± 0.51
	Peak	0.13 ± 0.02	0.32 ± 0.02	0.44 ± 0.04	2.51 ± 0.30
3/2/87	Control	0.35 ± 0.08	0.70 ± 0.12	1.04 ± 0.13	2.15 ± 0.85
	Peak	0.23 ± 0.10	0.60 ± 0.21	0.83 ± 0.30	2.79 ± 0.78

[a]Values are means ± SD for four single plant replicates.

Table B-22. Dry Weight Measurements for the Second Interspecific Group[a]

Date	Treatment	Root (g)	Shoot (g)	Total (g)	Shoot/Root
			Corn		
3/9/87	Control	0.60 ± 0.15	0.85 ± 0.22	1.44 ± 0.37	1.42 ± 0.08
	Peak	0.49 ± 0.12	0.63 ± 0.10	1.12 ± 0.21	1.31 ± 0.20
3/16/87	Control	0.86 ± 0.23	1.25 ± 0.22	2.11 ± 0.44	1.50 ± 0.23
	Peak	1.03 ± 0.52	1.60 ± 0.24	2.63 ± 0.59	1.78 ± 0.62
3/23/87	Control	1.25 ± 0.27	2.23 ± 0.15	3.48 ± 0.41	1.83 ± 0.30
	Peak	1.56 ± 0.19	2.33 ± 0.12	3.88 ± 0.20	1.51 ± 0.22
3/30/87	Control	1.73 ± 0.19	3.46 ± 0.43	5.19 ± 0.45	2.02 ± 0.36
	Peak	1.79 ± 0.26	3.74 ± 0.15	5.53 ± 0.39	2.12 ± 0.22
			Squash		
3/9/87	Control	0.18 ± 0.01	1.04 ± 0.05	1.21 ± 0.05	5.94 ± 0.61
	Peak	0.18 ± 0.02	0.96 ± 0.10	1.14 ± 0.12	5.23 ± 0.16
3/16/87	Control	0.31 ± 0.04	2.05 ± 0.19	2.36 ± 0.23	6.64 ± 0.46
	Peak	0.26 ± 0.07	1.86 ± 0.14	2.12 ± 0.17	7.62 ± 2.02
3/23/87	Control	0.43 ± 0.05	3.28 ± 1.13	3.71 ± 1.09	7.85 ± 3.72
	Peak	0.42 ± 0.05	3.41 ± 0.75	3.83 ± 0.73	8.27 ± 2.38
3/30/87	Control	0.25 ± 0.05	3.01 ± 0.16	3.26 ± 0.15	12.66 ± 3.03
	Peak	0.24 ± 0.03	2.96 ± 0.23	3.20 ± 0.24	12.40 ± 1.18
			Radish		
3/9/87	Control	0.45 ± 0.11	0.27 ± 0.03	0.72 ± 0.11	0.63 ± 0.20
	Peak	0.34 ± 0.06	0.29 ± 0.06	0.63 ± 0.10	0.86 ± 0.16
3/16/87	Control	1.11 ± 0.13	0.45 ± 0.12	1.56 ± 0.24	0.40 ± 0.07
	Peak	0.63 ± 0.16	0.47 ± 0.02	1.10 ± 0.15	0.80 ± 0.27
3/23/87	Control	1.56 ± 0.23	0.50 ± 0.06	2.05 ± 0.27	0.32 ± 0.04
	Peak	1.08 ± 0.23	0.62 ± 0.05	1.71 ± 0.25	0.59 ± 0.12
3/30/87	Control	1.77 ± 0.44	0.50 ± 0.09	2.27 ± 0.37	0.31 ± 0.11
	Peak	1.53 ± 0.40	0.54 ± 0.11	2.07 ± 0.48	0.37 ± 0.09

(continued)

Table B-22 (continued) - 2

Date	Treatment	Root (g)	Shoot (g)	Total (g)	Shoot/Root
			Lettuce		
3/9/87	Control	0.05 ± 0.01	0.18 ± 0.05	0.23 ± 0.04	4.31 ± 1.60
	Peak	0.03 ± 0.01	0.19 ± 0.05	0.22 ± 0.06	6.30 ± 1.18
3/16/87	Control	0.14 ± 0.08	0.57 ± 0.21	0.70 ± 0.29	4.60 ± 1.09
	Peak	0.20 ± 0.03	0.71 ± 0.10	0.91 ± 0.12	3.50 ± 0.31
3/23/87	Control	0.65 ± 0.57	1.23 ± 0.24	1.87 ± 0.68	2.71 ± 1.23
	Peak	0.43 ± 0.10	1.28 ± 0.16	1.70 ± 0.20	3.09 ± 0.66
3/30/87	Control	0.68 ± 0.11	1.69 ± 1.03	2.37 ± 1.02	2.57 ± 1.85
	Peak	0.63 ± 0.19	2.09 ± 0.31	2.72 ± 0.49	3.47 ± 0.76

[a]Values are means ± SD for four single plant replicates.

Table B-23. Fresh Weight Measurements for the Fourth Spinach Group[a]

Date	Treatment	Root (g)	Shoot (g)	Total (g)	Shoot/Root
2/9/87	Control	4.60 ± 0.45	8.32 ± 1.25	12.92 ± 1.66	1.80 ± 0.14
	Peak	4.23 ± 0.64	7.34 ± 0.16	11.57 ± 6.77	1.77 ± 0.26
2/16/87	Control	10.19 ± 0.88	21.95 ± 5.96	32.13 ± 6.57	2.14 ± 0.46
	Peak	6.71 ± 0.83	15.18 ± 1.59	21.89 ± 2.10	2.28 ± 0.25
2/23/87	Control	43.59 ± 10.5	75.28 ± 24.6	118.87 ± 34.0	1.72 ± 0.33
	Peak	33.38 ± 4.58	53.16 ± 3.05	86.54 ± 5.25	1.63 ± 0.23
3/2/87	Control	75.08 ± 25.4	97.56 ± 12.8	172.64 ± 48.1	1.33 ± 0.12
	Peak	68.85 ± 7.87	86.17 ± 15.9	155.02 ± 23.4	1.24 ± 0.12

[a]Values are means ± SD for four single plant replicates.

Table B-24. Dry Weight Measurements for the Fourth Spinach Group[a]

Date	Treat-ment	Root (g)	Shoot (g)	Total (g)	Shoot/Root
2/9/87	Control	0.26 ± 0.06	0.81 ± 0.13	1.07 ± 0.15	3.16 ± 0.60
	Peak	0.26 ± 0.06	0.71 ± 0.05	0.96 ± 0.09	2.88 ± 0.70
2/16/87	Control	0.63 ± 0.16	1.95 ± 0.38	2.58 ± 0.35	3.27 ± 1.18
	Peak	0.50 ± 0.10	1.47 ± 0.18	2.00 ± 0.25	3.03 ± 0.45
2/23/87	Control	2.32 ± 0.45	6.42 ± 1.58	8.74 ± 1.99	2.77 ± 0.36
	Peak	1.63 ± 0.16	5.00 ± 0.24	6.63 ± 0.38	3.09 ± 0.23
3/2/87	Control	3.55 ± 0.73	10.79 ± 2.33	13.63 ± 3.06	2.84 ± 0.10
	Peak	3.47 ± 0.35	9.12 ± 1.53	12.59 ± 1.81	2.62 ± 0.29

[a]Values are means ± SD of four single plant replicates.

APPENDIX C

Experimental Protocol for Fluorescence Measurements with Leaf Discs