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Chlorophyll Fluorescence as a Measure of Ozone Induced Stress

Jerry L. Faulkner
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To the Graduate Council:

I am submitting herewith a dissertation written by Jerry L. Faulkner entitled "Chlorophyll Fluorescence as a Measure of Ozone Induced Stress." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Botany.

Edward E. C. Clebsch, Major Professor

We have read this dissertation and recommend its acceptance:

David K. Smith, Otto J. Schwarz, Scott E. Schlarbaum

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Chlorophyll Fluorescence as a Measure of Ozone Induced Stress

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Jerry L. Faulkner

August, 1994

DEDICATION

Dedicated to

Travis Anthony Faulkner

1973 - 1990

ACKNOWLEDGMENTS

Although this dissertation bears only my name it is actually a reflection of the contributions of many people. Dr. Edward E. C. Clebsch has served as my major professor and his guidance and patience are greatly appreciated. The other members of my committee, Dr. Otto J. Schwarz, Dr. Scott E. Schlarbaum and Dr. David K. Smith have offered helpful comments and assistance. Dr. William L. Sanders provided generously of his time and expertise in assisting with the statistical design and analysis.

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Last but not least, I owe a huge debt of gratitude to my number one field assistant, my wife Wanda. She has sacrificed and stood by me throughout this dissertation. Her patience, encouragement and love have made it all possible.

ABSTRACT

Chlorophyll fluorescence is a nondestructive, intrinsic probe of photosynthesis. It has been used in a variety of physiological and environmental research. This study attempted to investigate the usefulness of chlorophyll fluorescence as a monitor of ozone induced stress in *Quercus rubra* L.

Chlorophyll fluorescence measurements were taken from mature trees and seedlings in open top chambers located at the Tennessee Valley Authority Air Pollution Research Facility at Norris, Tennessee. Chambers reflected one of three ozone concentration treatments: sub-ambient, ambient, or twice ambient. Spring leaf expansion was also measured as was spring and autumn chlorophyll content of leaves from mature trees.

Fluorescence measurements proved to reflect differences between treatments and tree size. The data indicates two response types. The first type produces an inducible response at ambient concentrations and homeostasis at twice ambient concentrations. The second type of response is homeostatic at ambient concentrations with apparent injury occurring in twice ambient treatments. These patterns indicate a multiphasic, multisite ozone effect. The fluorescence measurements indicated that seedlings were less sensitive to ozone than mature trees. No fluorescence parameters were sensitive to leaf expansion although some exhibited good correlation with seasonal changes in chlorophyll content. Treatment did not significantly affect chlorophyll content.

Chlorophyll fluorescence is a useful tool for analyzing the effect of ozone on the photosynthesis of *Quercus rubra.* The results of this study also give insight into the effects of ozone on photosynthesis and how plants respond.

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1. INTRODUCTION

Objective

A recent USDA survey of 66 air pollution researchers revealed that ozone is perceived as the most damaging single pollutant in American forest ecosystems (de Steiguer, Pye, and Love, 1990). Despite this perception, no adequate method exists for measuring ozone effects on mature forest trees in the field. Because chlorophyll fluorescence is a non-invasive, intrinsic method for measuring the state of the photosynthetic mechanism of plants it should be a valuable tool for measuring ozone stress in plants. The character of the fluorescence induction curve provides information about different parts of the photosynthetic process and consequently should contribute to the understanding of the action of ozone in plant tissue. The objective of this study is to examine the utility of chlorophyll fluorescence as an assay of ozone induced plant stress. To achieve this goal, a correlation between changing ozone concentrations and variations in chlorophyll fluorescence must be established.

Chlorophyll Fluorescence

It has been known since 1864 that certain pigments, especially those associated with photosynthesis, were capable of fluorescence at wavelengths slightly beyond the longest absorbed wavelength. G. G. Stokes first observed *in vivo* chlorophyll fluorescence and his work formed the basis for the modern science of photosynthetic fluorescence (Duysens, 1986). Research continued but was limited by the technology of the time. In

1874, Muller discovered that the fluorescence yield of dark adapted photosynthetic cells changes during the first few minutes of illumination (Duysens, 1986). The changing fluorescence yield is often referred to as the Katusky effect after the investigator who first made quantitative measurements of the yield (Katusky and Hirsch, 1931). The graphing of the changes is called an induction curve. Shortly after Katusky's work, chlorophyll fluorescence was recognized as an intrinsic probe of the photosynthetic process and much work has been done using a variety of methods.

The value of chlorophyll fluorescence as an assay technique is based on two concepts. First, it is accepted that any substance or action that interrupts or changes the photosynthetic process will result in a change in the yield of fluorescence. In a healthy, unstressed plant only a small portion of absorbed energy is re-emitted as fluorescence. Normally, absorbed light elevates electrons from the photosynthetic reaction centers to higher energy states where they reduce the first quinone electron acceptor, Q. Electrons are returned to ground state by an electron transport system that uses the energy to produce ATPs for powering the light independent reactions of photosynthesis. Any blockage or uncoupling of the transfer of energy to the reaction centers or to the light dependent photochemical machinery will result in excited electrons returning to ground state independent of the electron acceptor or electron transfer system. The result is altered fluorescence.

Secondly, the changes in fluorescence yield (the induction curve) following initial illumination are a result of the various elements of the photosynthetic machinery coming up to speed (Figure 1). These phases of the induction curve are labeled as OIDPSMT (Papageorgiou, 1975). While Photosystem I (PS I) does contribute to fluorescence it is commonly assumed that the majority of fluorescence originates from Photosystem II (PS II) (Duysens, 1986). Blockage or uncoupling of photosynthesis will cause changes in the induction curve that indicate the portion of the photosynthetic operation affected. The OIDP portion of the induction curve is often referred to as fast fluorescence. It is related to the primary photochemistry of PS II. The SMT complex is the slow phase of fluorescence and is an indication of the transfer of electrons beyond electron acceptor Q. The initial fluorescence value (F_O) is often referred to as the instantaneous fluorescence since it appears in less than 1 nanosecond. F_O has also been referred to as constant fluorescence because it is independent of photochemical events (Krause and Weis, 1984). Instantaneous fluorescence results from blockage of electron transfer within the light harvesting antennae or between the antennae and the reaction centers. It is inversely related to the number of PS II reaction centers that are open and undamaged and has also been interpreted as a measure of the oxidized pool of Q (Bolhar-Nordenkampf *et al.*, 1989). Comparisons by Schreiber *et al.* (1986) between *in vivo* fluorescence and fluorescence from ethanol chlorophyll extracts indicated that F_O is positively correlated to the amount of chlorophyll present. F_I and F_D are intermediates in the initial rise of fluorescence that are not well understood. Schreiber and Vidaver (1974) suggested that the I-D complex arises from the interplay of PS II and PS I. Karukstis (1992) indicated

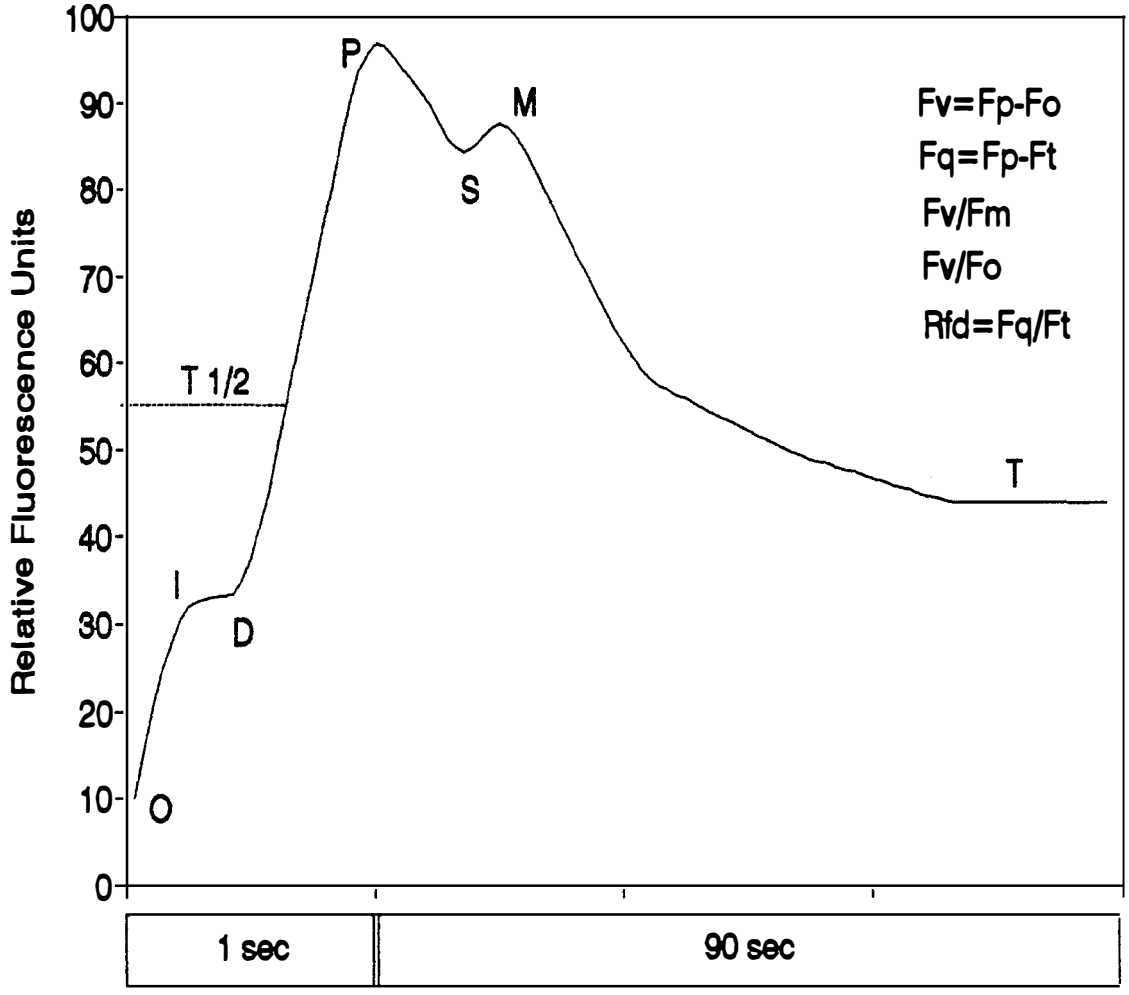

Figure 1. Fluorescence induction curve.

that the inflection is the result of electron transfer from the primary acceptor Q_A to a secondary acceptor Q_B. The induction curve continues to rise to a peak, F_P. Provided that the initial actinic light was saturating, F_P, also often given as F_{MAX}, occurs when all available Q acceptors are fully reduced (Renger and Schreiber, 1986). The decline from F_P to F_S reflects the flow of electron energy away from Q and the subsequent increase in the pool of the oxidized electron acceptors (Krause and Weis, 1984). The second rise in fluorescence from F_S to F_M indicates the initiation of carbon dioxide assimilation (Ireland *et al.*, 1984). F_T is referred to as the steady state and indicates that the photosynthetic machinery has reached equilibrium (Renger and Schreiber, 1986). Sivak and Walker (1985) have demonstrated that there is a reciprocal but phase shifted correlation between F_T and carbon assimilation. $T_{1/2}$ is one-half the time from F_O to F_P and indicates the efficiency of the light gathering antennas (Malkin and Fork, 1981). Various computed values have also been interpreted as indications of the condition of the photosynthetic apparatus. Variable fluorescence (F_V) is the difference between F_P and F_O. A more commonly used ratio is called the vitality index and is computed as F_V/F_P. This value is accepted as a good measure of the physiological state of the chloroplast (Miles, 1990). There is evidence that this index is highly correlated with the quantum yield of net photochemistry (Gentry *et al.*, 1989 and Seaton and Walker, 1990). Another ratio, F_V/F_O, has been suggested as a measure of the total electron flow in photosystem II (Havaux *et al.*, 1988). The difference between F_P and F_T is denoted as F_Q and represents the quenching capacity of the system. The quenching ratio (Rfd) is the quotient of F_Q/F_T (Krause and Weis, 1984) and is believed to be a good measure of photosynthetic activity

(D'Ambrosio *et al.*, 1992). This paper will consider F_O, F_P, F_V, F_V/F_P, F_T, F_Q, $T_{1/2}$, F_V/F_O, and R*fd*.

Stokes and Muller in the first observations of chlorophyll fluorescence used colored glass filters and the human eye as detectors. Actinic light was provided by the sun or by carbon arc lights (Renger and Schreiber, 1986). Fifty years later Katusky and Hirsch used crude photometers and hand plotting of data to describe the induction of fluorescence. The technology development associated with World War II brought the use of shuttered, high intensity lights and phosphoroscopic study into existence. These were shortly followed by photomultiplier tubes and eventually by light measuring devices that could be connected to oscilloscopes and chart recorders. The advent of solid state technology provided the possibility for compact fluorescence measuring devices such as those created by Schreiber *et al.* (1975) and introduced the potential for field measurement. Field use of these devices required portable oscilloscopes for recording the induction curve. Hard copies of curves could only be produced by photographing the oscilloscope screen or returning to the lab after each reading to produce a strip chart recording, consequently limiting field applications. Larcher and Cernusca (1985) attempted to produce efficient field use by interfacing a device patterned after Schreiber's with a pocket computer. Induction data recorded by the computer was stored on magnetic cassette tapes for later analysis. This arrangement was limited by the memory size of the pocket computer and the recording ability of the cassette tape. More modern devices incorporate pulse modulation of the light source or laser technology. Many of the pulse

amplitude modulation (PAM) instruments are capable of interfacing with micro-computers although those currently portable enough for field use lack suitable data storage. Presently there are at least three portable devices available that combine chlorophyll fluorescence monitoring with a built in data logger and microprocessor. The stored information can be down loaded to a personal computer or printed directly by printer or plotter.

While the majority of research using chlorophyll fluorescence has centered on photosynthetic physiology, a recent realization has emerged that fluorescence monitoring is an excellent assay of plant stress. Many environmental factors that affect the photochemical process result in detectable changes in the induction curve (Table 1). Changes in fluorescence may be observed prior to or in the absence of visible damage, making it an even more valuable assay technique.

Previous research to assess ozone damage using chlorophyll fluorescence has produced mixed results. Schreiber *et al.* (1978) found that there was good agreement between fluorescence and visual assays of damage to *Phaseolus vulgaris.* Chlorophyll fluorescence induction curves demonstrated significant changes up to 20 hours before visual symptoms appeared. Testing conducted by Heath (1980) showed that *Chlorella* species exhibited almost complete electron flow inhibition as measured by chlorophyll fluorescence at ozone concentrations of 60 p.p.m. . Ennis *et al.* (1990) found no significant changes in fluorescence induction in *Picea rubens* due to ozone fumigation.

Table 1. Plant stress studies utilizing chlorophyll fluorescence.

Plant	Stress	Reference
Pseudotsuga menziesii	Cold & dormancy	Hawkins & Lister, 1985
Nicotiana plumbaginifolia	Nitrate deficiency	Saux *et al.*, 1987
Salix sp.	Drought	Ogren & Oquist, 1985
Afroalpine sp.	Chill	Bodner & Beck, 1987
Pinus sylvestris	Cold	Oquist & Wass, 1988
Passiflora sp.	Chill	McRae *et al.*, 1986
Trifolium ambiguum	Drought	Larcher & Cernusca, 1985
Tsuga heterophylla	Chill	Melcarek, *et al.*, 1977
Phaseolus vulgaris	Ozone	Schreiber *et al.*, 1978
Chlorella sp.	Ozone	Heath, 1980
Helianthus sp.	Drought	Conroy *et al.*, 1988
Pinus radiata	Phosphorus deficiency	Conroy *et al.*, 1986
Spinacia oleracea	SO_2, O_3, & NO_2	Schmidt *et al.*, 1990
26 different species	Heat	Bilger *et al.*, 1984

Tests by Ruth (1990) on an unidentified spruce species showed a decrease in the quotient of F_Q/F_T at extremely high levels of ozone (1000 parts per billion).

Tropospheric Ozone

Tropospheric ozone is a secondary pollutant formed chiefly by the action of ultraviolet light on NO_2. It has been implicated in forest decline in North America and Europe. Ozone damage is usually diagnosed visibly by chlorosis of the foliage. The appearance may vary extensively from a mottling previously referred to as "weather flecking" (Taylor, 1984) to a uniform fading of the leaf. There is mounting evidence that the major effects of ozone are subliminal and may or may not result in visible damage.

Ozone can affect plants in a variety of ways. Surface contact between leaves and ozone results in erosion or breakdown of the cuticle (Treshow and Anderson, 1989). Treshow and Anderson (1989) also reported that ozone influences stomatal conductance by leaching potassium from guard cells although Tingey and Taylor (1982) suggested that this is a minor effect and should not be an *a priori* assumption when investigating ozone foliar damage.

The more serious damage by ozone occurs internally both in the gaseous and liquid phase. Ozone is highly reactive, dissolves easily in the water coating intercellular surfaces, and decomposes readily to form ions and free radicals. Ozone interferes with internal physiological processes (Treshow and Anderson, 1989; Tseng *et al.*, 1988 and

Wallin *et al.*, 1990) resulting in reduced vigor and productivity. Most important among these processes is photosynthesis and it is believed the effect is of sufficient magnitude to be detectable by chlorophyll fluorescence monitoring (Heath, 1989 and Schreiber *et al.*, 1978).

There are several theories about the way ozone damages photosynthesis. One method is through the action of free radicals formed when ozone decomposes. Treshow and Anderson (1989) suggested that the sulfhydryl groups of enzymes are susceptible to free radical degradation. Mehlhorn and Wellburn (1987) and Mehlhorn *et al.* (1991) also support degradation of enzymes but theorize that it is the result of peroxide formation from ozone and stress ethylene reactions. The most prominent current theory is that ozone affects photosynthesis by damaging cell membranes either by direct oxidation or by the formation of multiple free radicals. While the plasmalemma, endoplasmic reticulum, and other organelle membranes are affected, the earliest site is apparently the membranes of the chloroplasts (Treshow and Anderson, 1989). This is supported by microscopic evidence as reported by Tingey and Taylor (1982). The mechanism of membrane damage involves oxidation of membrane proteins and ozonolysis of unsaturated fatty acids (Treshow and Anderson, 1989). Disruptions of these membranes and the proteins that comprise the photosynthetic "machinery" result in disruption of energy flow and should produce increased chlorophyll fluorescence.

Ozone effects are mitigated by a variety of factors. As is true in any chemical reaction, temperature will affect the rate of reaction. Ozone damage will be more severe at higher temperatures. Response also depends on duration and magnitude of exposure, period between exposures, and other environmental conditions at the time of exposure (Treshow and Anderson, 1989). Melhorn *et al.* (1991) suggested that ozone is more toxic in brief high doses and that plants experiencing chronic exposure may develop a tolerance. Evidence exists that ozone damage is related to the cumulative dose.

Hypothesis

In order to achieve the objective of examining the utility of chlorophyll fluorescence as an indicator of ozone induced stress, several questions will be addressed by this study. They are:

1. Do different ozone treatment regimes produce statistically significant differences in chlorophyll fluorescence measurements?
2. Is chlorophyll fluorescence measurement sensitive enough to detect the effects of diurnal changes in ozone concentrations?
3. Since temperature also affects chlorophyll fluorescence, can the effects of ozone be discriminated from temperature effects?
4. What is the nature of the relationship between chlorophyll content and chlorophyll fluorescence?
5. Are chlorophyll fluorescence measurements sensitive to internal changes during leaf expansion and senescence and can chlorophyll

fluorescence measurement detect any effects of ozone during these critical periods?

While not directly related to the objective of this study, the results should provide insight into the following questions:

1. Are there detectably different patterns of chlorophyll fluorescence between adult trees and seedlings?

2. What is the effect of different ozone treatments on chlorophyll content?

3. What is the effect of different ozone treatments on the expansion and senescence of leaves?

2. METHODS AND MATERIALS

TVA Fumigation Facility

This research was conducted at the Tennessee Valley Authority ozone fumigation facility located at the Norris Dam Reservation near Norris, Tennessee. The facility consists of eighteen open top fumigation chambers located in a Northern red oak (*Quercus rubra* L.) seed orchard. Nine of the chambers enclose single, sexually mature red oaks. The remaining nine chambers contain potted seedlings. The large chambers are 4.6 m diameter x 9.2 m tall. Each large chamber consists of seven sections including two air supply plenums and a fustrum (Edwards *et al.*, 1994). The small chambers are 3.0 m diameter x 2.4 m tall with one plenum.

The chambers are divided equally into three treatment regimes. Three large chambers and three small chambers receive the same treatment. The three treatments are subambient concentrations of ozone, ambient ozone, and twice ambient ozone. Subambient concentrations are achieved by filtering the air through activated charcoal filters. Ozone is created from oxygen and supplied to the chambers through underground Teflon tubing. Ozone addition was discontinued for the 1993 growing season on September 27. Concentrations are controlled and recorded by computer.

The mature northern red oaks were planted in the mid-1960's. Different clones of phenotypically selected trees were grafted onto a common root stock and spaced 6.5

m x 6.5 m. The nine trees selected for the study originally had a diameter range of 20-25 cm. and were 6-8 m tall. (Samuelson and Edwards, 1993). The current size of the trees is below site index and may reflect graft incompatability which is common in oaks.

The three year old seedlings were grown from acorns collected in the seed orchard. They are presently contained in 24 l. plastic pots in soil collected from the A horizon adjacent to the site (Samuelson and Edwards, 1993). The pots are placed in a styrofoam base in order to mediate soil temperature fluctuations. Each chamber contains a random selection of seedlings.

Both large trees and seedlings were watered with nozzles located at the top of the chambers to restore field capacity when the soil water potential fell below -0.2 to -0.5 MPa (Edwards *et al.*, 1994). Pesticides were applied as needed. No fertilizer or herbicide was used.

Chlorophyll Fluorescence Measurement

Chlorophyll fluorescence measurements were made using the CF-1000 Chlorophyll Fluorescence Measurement System (P. K. Morgan Instruments, Inc., Andover, MA). In each large chamber, three first flush leaves near the tip of a branch at mid-chamber height on the north side of the chamber were selected and tagged with numbered plastic rings. The north side of the chamber was chosen to minimize diurnal effects and to avoid the

possible effects of sampling for other studies on the south side. One leaf from each of three seedlings in the small chambers was similarly marked.

Chlorophyll fluorescence measurements were taken from each marked leaf during the 1993 growing season. Measurements were made weekly from May 9 through June 19 and every other week through September 11. Seedlings in small chambers were only sampled once more on September 18 due to the enclosure of seedlings in wire cages to facilitate leaf biomass collection. Weekly sampling of large chambers resumed until October 28. Each leaf was dark adapted 30 ± 0.5 minutes using the Morgan adaptation clips. Measurements were taken using an actinic light of 600 μ mol m^{-2} sec^{-1} for 90 seconds. These parameters were determined from preliminary tests during 1992. Light level was determined from a comparison of the resulting $T_{1/2}$ values for different dark adaptation times. Figure 2 shows that an asymptote appears at approximately 600 μ mol m^{-2} sec^{-1}. Observations of F_O, F_V, F_P, and F_V/F_P were more erratic at higher light concentrations and often super-saturated the leaf to the point of obscuring the P-S-M complex. Thirty minutes was selected as the appropriate dark time because longer times were impractical and also did not dramatically increase F_V and F_P (Figure 3). The duration of the exposure and reading was selected to be as brief as possible but still permit stabilization of quenching. Figure 4 illustrates that 90 seconds is appropriate. Fluorescence kinetics values F_O, F_V, F_P, F_V/F_P, $T_{1/2}$, F_Q and F_T and time of day and date were measured and calculated by the instrument and downloaded to a personal computer at the end of each day's sampling. F_V/F_O and R*fd* were calculated using functions of the

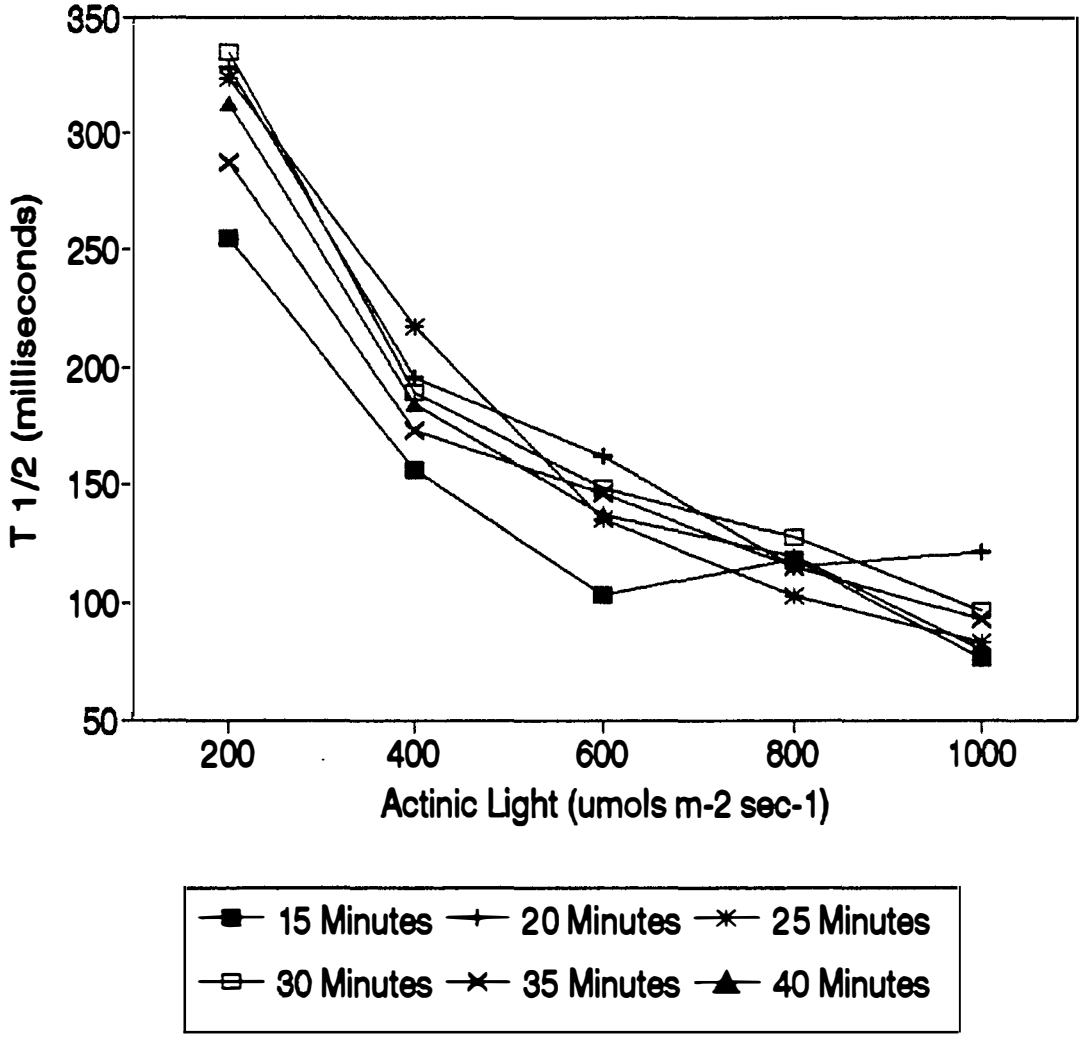

Figure 2. Mean $T_{1/2}$ values for different dark adaptation times and different light levels. N = 3.

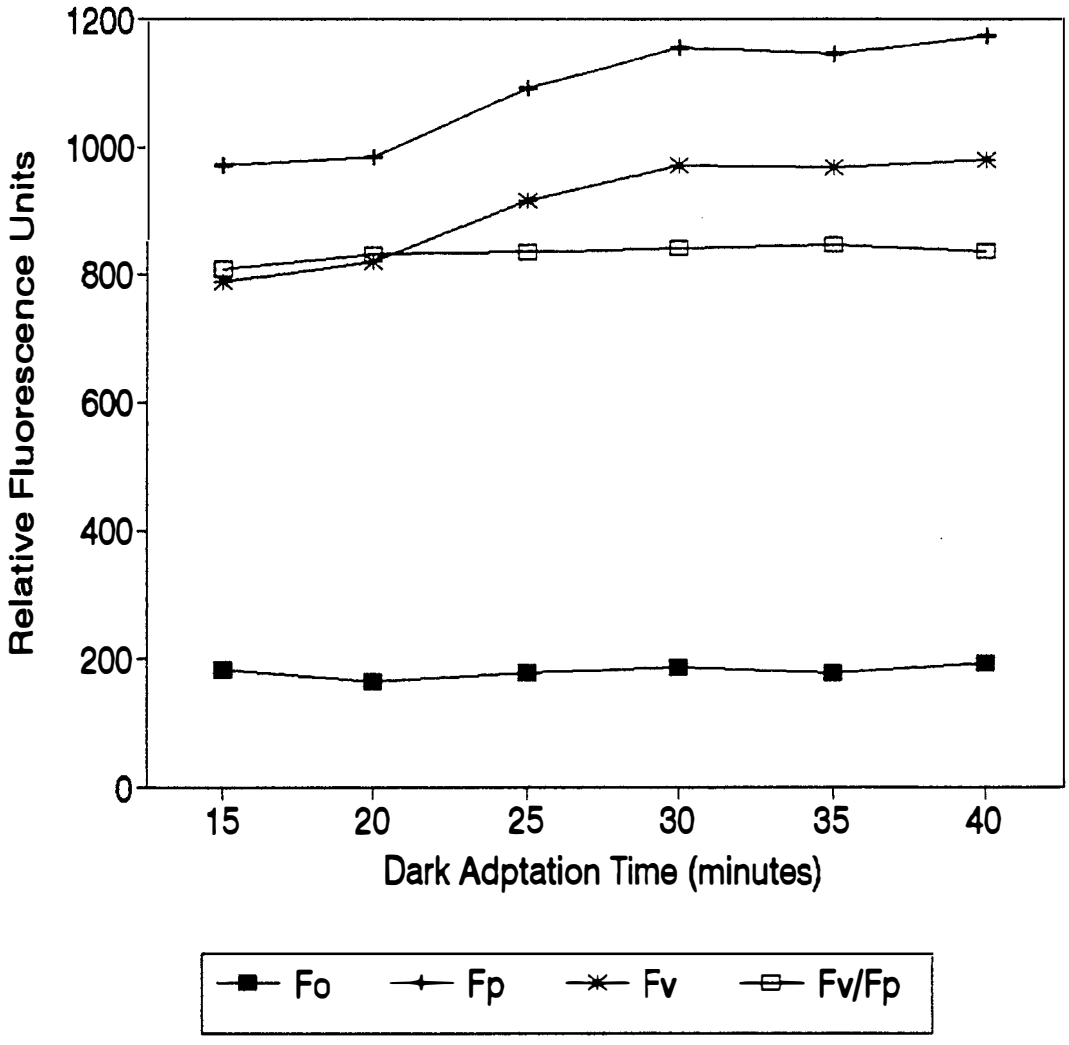

Figure 3. Mean fluorescence values for different dark adaptations at 600 μ mol m^{-2} sec^{-1}. N = 3.

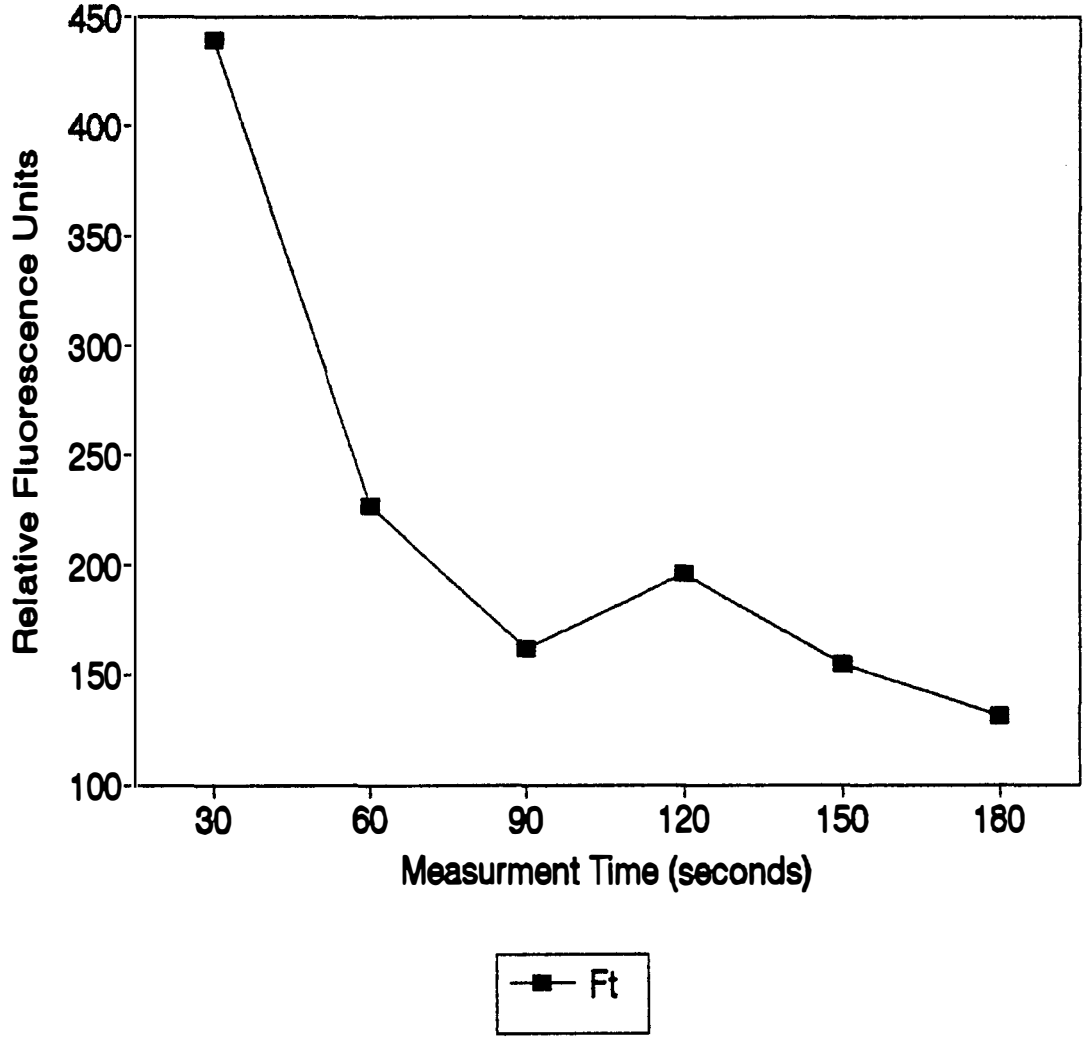

Figure 4. Mean F_T at different measurement times. N = 3.

statistical analysis programs. Ozone concentrations and temperatures at the time of the measurement were obtained from the facility's control and monitoring computer.

Leaf Expansion and Chlorophyll Content

From May 9 through June 19 the length of each marked leaf was measured and recorded to determine leaf expansion. During this same period and again during the autumn weekly sampling, three leaves were excised from each of the large trees each week. Leaves were chosen that approximated the size and orientation of the marked leaves. Collected leaves were placed in plastic, zipper type freezer bags and placed on ice immediately following harvest. At the end of each day's sampling leaves were wrapped in aluminum foil and placed in a freezer at -81° C. until extracted.

Extraction was conducted in a dimly lit room. Each leaf was cut into pieces and a 0.5 g portion of the leaf ground in a mortar and pestle with 10 ml of acetone and a small quantity of sand. The mixture was vacuum filtered through a glass fiber filter. The mortar, pestle, and residue were washed with an additional 10 ml of acetone. A one ml aliquot of the filtrate was diluted with 10 additional milliliters of acetone. The final dilution was refrigerated until spectrophotometric readings could be taken. Photometric measurements were made on a Shimadzu UV-160 spectrophotometer. Absorption values were recorded on each leaf extract at wavelengths of 663, 652, and 645 nm. Chlorophyll *a* and *b* concentrations were computed using the formulas proposed by Bruinsma (1963).

The formulas are:

$$C_a = 12.7\ A_{663} - 2.7\ A_{645}$$

$$C_b = 22.9\ A_{645} - 4.7\ A_{663}$$

where C_a and C_b are concentration of chlorophyll *a* and *b* respectively, A_{663} equals the absorbance reading at 663 nm., and A_{645} equals the absorbance reading at 645 nm. The calculated concentrations were summed and compared to the result of:

$$C_{a+b} = 27.8\ A_{652}$$

where A_{652} equals the absorbance at 652 nm. Small differences between these two indicate the absence of degradation products.

Statistical Design and Analysis

For purposes of this study the chambers were enumerated as 1 through 9 for large chambers and 11 through 19 for small chambers. Chambers 1,4,7,11,14, and 17 received subambient concentrations. Chambers 2,5,8,12,15, and 18 were ambient and 3,6,9,13,16, and 19 received twice ambient ozone. Chambers were divided into six blocks of three chambers each (one of each treatment) and sampled in an alternating scheme to minimize the effects of time of day and temperature.

Analysis was conducted using SAS (SAS Institute Inc., Cary, NC.) and GLMM (Department of Experimental Statistics, Louisiana State University Agriculture Center, Baton Rouge, LA.) on a personal computer. Mixed model analysis was performed to account for variance within and among chambers. The experimental unit was the

chamber and treatment and chamber size were the main effects tested. The design incorporated a rotating block design to diminish the confounding effect of time of day. Correlations between variables and fluorescence parameters were tested within the six combinations of treatment and chamber size.

3. RESULTS AND DISCUSSION

Chlorophyll Fluorescence

The chlorophyll fluorescence parameters did not exhibit the expected linear relationship with increasing ozone treatment. Heck *et al.* (1969) reported that plants do not respond linearly to ozone stress. The various measures demonstrated either highest or lowest values associated with the ambient treatment (Figure 5). For example, initial fluorescence (F_O) peaked at 266.9 relative fluorescence units for the ambient treatment while the subambient and twice ambient treatments had lower values. Differences in F_O between treatments were not significant. Peak fluorescence values (F_P) were significantly different between treatments. Again the ambient treatment resulted in the highest value with the measurement from subambient chambers being lower and the twice ambient chambers producing the lowest values. F_V (variable fluorescence), the steady state fluorescence values (F_T), and F_Q exhibited the same pattern and were also significantly different. $T_{1/2}$ produced an inverse pattern with the lowest values for measurements from the ambient chambers but was not statistically significant. Generalized least squares means for all fluorescence parameters are contained in Table 2.

The non-linear, peak and valley nature of the fluorescence measurements would seem to indicate that some type of resistance or repair mechanism is being triggered when a threshold level of ozone perturbation is surpassed. Schut (1985) achieved a workable model of ozone effects by incorporating a threshold and photosynthate controlled repair

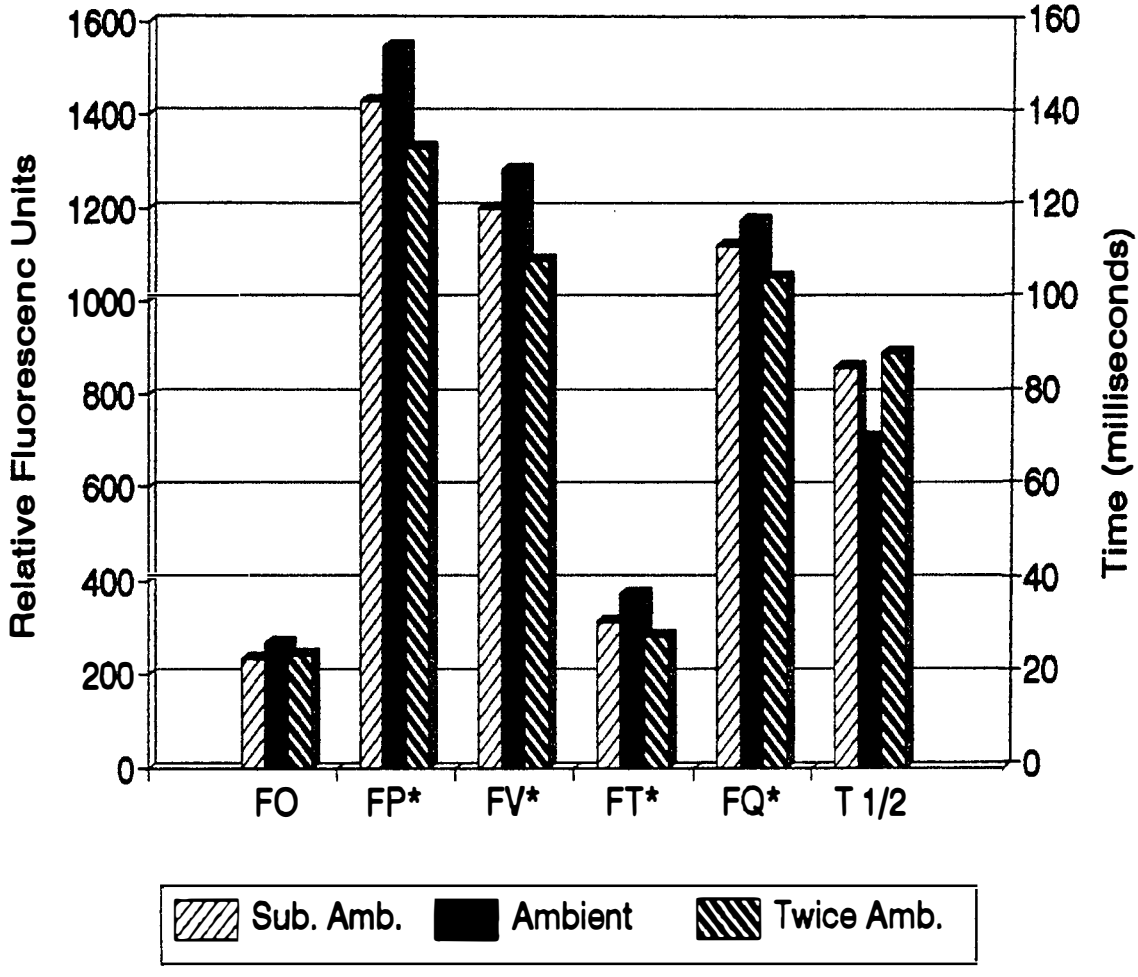

Figure 5. Generalized least squares means of fluorescence parameters for each treatment. * = Significant at $\alpha = 0.10$.

Table 2. Generalized least squares means of fluorescence parameters for each treatment.

Fluorescence Parameters	Treatments Subambient	Ambient	Twice ambient
Fo	230.7	266.9	242.8
Fp*	1429.9	1548.3	1330.2
Fv*	1198.9	1280.5	1087.4
T 1/2	85.6	70.7	88.7
Ft*	311.7	373.7	280.2
Fq*	1117.9	1174.1	1049.9
Fv/Fp	0.833	0.818	0.820
Fv/Fo	5.946	5.475	5.480
Rfd	4.132	3.717	4.041

* Differences between means are significant at $\alpha = 0.10$.

mechanism. Tingey and Taylor (1982) suggested that the physiological and biochemical responses of plants to ozone progresses from perturbation to a state of homeostasis followed by injury. Lichtenthaler (1988) proposed that stress can be divided into three phases. The first phase, the response phase, results in a deviation from the functional norms. Next, during the restitution phase, adaptation and reparation processes may be able to compensate for the perturbation. The final or end phase occurs when stress intensity is too high and injury appears. Both of these paradigms are applicable as a stressor increases in intensity or duration. The pattern of fluorescence readings from this experiment seems to support this concept.

Interpretation of the results in the light of this theory is best accomplished by dividing the fluorescence parameters into two groups: those expected to show a linear decrease and those expected to show a linear increase. F_V and F_P usually decrease in response to stress. F_O, F_T, and $T_{1/2}$ usually increase.

It has been reported that ozone treatment reduced F_V in *Triticum aestivum* (Barnes *et al.*, 1990), *Picea abies* (Barnes and Davison, 1988), and *Pisum sativum* (Barnes *et al.*, 1988) and reduced both F_P and F_V in *Vicia faba* (Guidi *et al.*, 1993) *Phaseolus vulgaris* (Schreiber *et al.*, 1978) and *Triticum aestivium* (Grimm and Fuhrer, 1992). In this experiment these fluorescence parameters were greater for ambient treatment than for subambient but then decreased to levels below subambient in chambers receiving twice ambient amounts of ozone. Heath *et al.* (1982) reported a similar phenomenon for

Chlorella sorokiniana. During the first minutes of ozone exposure, the algae demonstrated a rise in F_v followed by a decrease during continued ozone introduction. *Pisum sativum, Phaseolus vulgaris,* and two varieties of *Nicotiana tabacum* demonstrated reduction of stress ethylene production after 21 days of continuous ozone fumigations (Mehlhorn *et al.*, 1991). This supports the idea of a homeostatic or restitution phase for moderately stressed plants. Although Bennett *et al.* (1974) reported apparent stimulation of plant growth by air pollutants it would be prudent to stop short of referring to ambient ozone concentrations as eustress.

The data from the F_0 measurements would seem to suggest that the threshold for the restitution phase is between the levels of ambient and twice ambient exposure. The peak from the ambient treatments represents a deviation from the functional norm. Usually perturbation results in an increase in F_0 as the number of open reaction centers declines due to injury. Barnes *et al.* (1988) reported that ozone fumigation of *Pisum sativum* at 75 ppb resulted in an increase in F_0 that then reversed by day 4 of exposure. A similar example exists in *Phaseolus vulgaris* (snapbeans) although at higher ozone levels. Lee (1991) reported increased values for F_0 in snapbeans fumigated at an ozone level of 250 ppb. Schreiber *et al.* (1978) reported relatively unchanged levels of initial fluorescence from *Phaseolus vulgaris* exposed to ozone levels as high as 500 ppb. F_0 measurements from *Chlorella sorokiniana* demonstrate a similar pattern of initial increase followed by a decrease as ozone exposure continued (Heath *et al.*, 1982). Steady state fluorescence, F_T, was also greater in ambient chambers and lowest in twice ambient

treatments. F_T increases as the result of ozone stress are reported in *Phaseolus vulgaris* (Schreiber *et al.*, 1978 and Lee, 1991) and *Vicia faba* (Guidi *et al.*, 1993). The data plus these examples would seem to indicate that in Northern red oak, levels of ozone approaching the ambient levels in this experiment can have an effect but once ozone concentration exceeds this threshold value a defense or repair mechanism ameliorates the stress.

The half rise time ($T_{1/2}$) requires separate consideration because of the pattern of response exhibited in this experiment (Figure 5). $T_{1/2}$ usually increases as a result of stress. Barnes *et al.* (1988), Barnes and Davison (1988), and Lee (1991) reported ozone induced increases in *Pisum sativum*, *Picea abies*, and *Phaseolus vulgaris* respectively. Although not statistically significant, $T_{1/2}$ was lower for ambient than for the subambient treatment but highest for the twice ambient treatment. Because $T_{1/2}$ is inversely related to the rate of rise from F_O to F_P, this would indicate a response similar to that of F_P and F_V where a homeostatic or restitution phase occurred at ambient concentrations while distress occurred at twice ambient concentrations. Reiling and Davison (1992) reported initially decreasing then increasing patterns in the responses of *Cerastium fontanum* and *Plantago major* over a period of 5 days of ozone fumigation.

If the above assumptions are correct it would suggest that ozone produces a biphasic response in plants and that the intra-chloroplast sites of ozone perturbation and injury are different at different concentrations. Pell (1987) proposed that ozone effects

on plants demonstrated more than one mechanism of action. Lee (1991) suggested a dual action of ozone stress as a result of similar data from *Phaseolus vulgaris*. There are two response types apparent from these results. In the first type (type A), perturbation occurs at ambient concentrations while homeostasis and restitution occur at twice ambient concentrations. The second type (type B) demonstrates homeostasis and restitution at ambient concentrations with injury becoming apparent at twice ambient levels. Because different portions of the fluorescence induction curve are indicative of events at distinctive locations in the photosynthetic machinery, it is possible to make some inferences about a possible multiphasic, multisite effect of ozone.

F_O fluorescence levels are presumed to be an indication of the number and integrity of the photosystem II reaction centers and the efficiency of electron transfer from light harvesting antenna pigments to the reaction centers. Perturbation and injury increase F_O. In this experiment mean F_O values were highest from measurements taken from ambient treated trees. Values from twice ambient treatments were only slightly higher than subambient readings. This response is indicative of type A.

F_T also demonstrates a type A response. F_T values are expected to increase due to stress. Ambient levels of F_T were highest with twice ambient levels actually slightly lower than subambient levels. As a steady state fluorescence value, F_T gives insight into the condition of electron transfer beyond Q, including the stromal light independent reactions of photosynthesis.

The half rise time $T_{1/2}$ was also expected to rise in response to ozone stress. It is a measure of the rate of electron transfer from the reaction centers to the primary electron acceptor, Q. $T_{1/2}$ is inversely related to the transfer rate. From this data it appears that the transfer rate was higher among trees exposed to ambient concentrations than in subambient chambers although the twice ambient treatment exhibited a rate less than the subambients. This is response type B in which homeostasis occurs at ambient concentrations and injury at twice ambient levels.

Peak fluorescence occurs when all reaction centers are closed and transfer of electrons to the primary acceptor, Q, occurs. As cited above, ozone stress usually reduces F_P. The decrease in F_P is due to the reduction of the back pressure of the proton gradient across the thylakoid membrane (Walker, 1987). The reduction of pressure is due to holes in the thylakoid membrane produced by ozone. F_P levels were highest in the ambient chambers and least for twice ambient treatments. This is response type B also.

Since these two response types are occurring simultaneously, one portion of the photosynthetic machinery may be operating at homeostasis while another is injured or impaired. The cumulative effect is to reduce photosynthesis in a linear fashion such that ambient treated trees have a reduced net photosynthesis and twice ambient trees have the lowest net photosynthesis.

Fluorescence parameters F_V and F_O support these conclusions. Since these two are the result of differences between measured parameters they chiefly function to indicate that the significant variation between differences in treatment response was not the result of a raised or reduced base. For example, the statistically significant difference in F_V between treatments shows that the difference in F_P is not merely an artifact of differences in F_O. F_V/F_P was not statistically significant ($P = 0.35$) and did not exhibit a response pattern similar to the other parameters. The F_V/F_P values of 0.82 to 0.83 are within the normal range for C_3 plants (Bjorkman and Demmig, 1987). Samuelson and Edwards (1993) reported identical results from a preliminary study. In *Picea abies*, reductions in net photosynthesis were not accompanied by changes in F_V/F_P (Havaranek *et al.*, 1989). F_V/F_O and R*fd* were also not significant. F_V/F_O did produce lower values for ambient and twice ambient treatments (Figure 6). Ozone treatment of approximately 100 ppb. produced no significant change in F_V/F_O in *Picea rubens* (Ennis, *et al.*, 1990). R*fd* demonstrates a reduced value for ambient treatments with twice ambient and subambient producing higher results respectively. Although Ruth (1990) reported a decrease in R*fd* in spruce fumigated at ozone levels exceeding 300 ppb., Strasser *et al.* (1987) showed no change in R*fd* in beech and poplar treated with ozone concentrations less than 180 ppb. These ratio measures of plant vitality may have limited application in plants that exhibit the proposed multiphasic, multisite response.

The mechanisms by which the homeostatic or restitution phases are achieved can not be determined from the present information. A variety of anti-oxidant defense

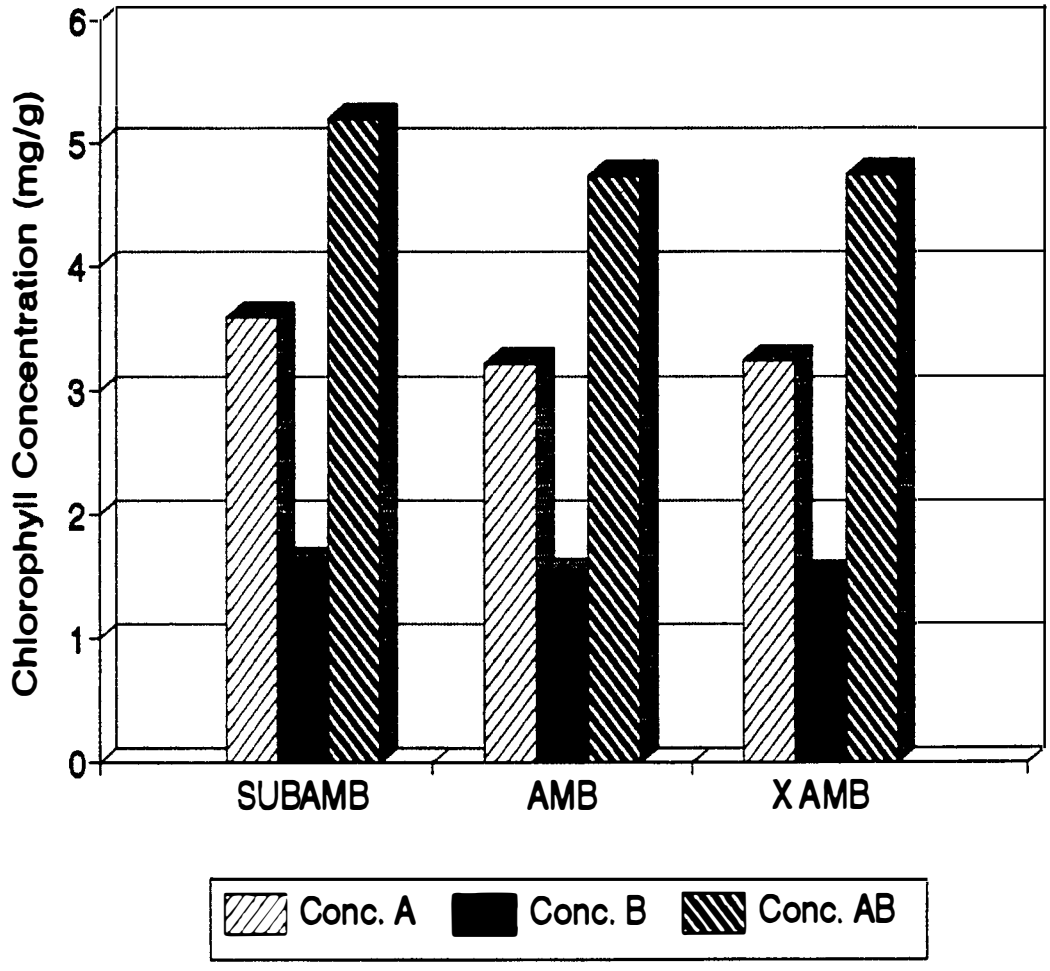

Figure 6. Generalized least squares means of fluorescence ratios for each treatment.

mechanisms have been proposed including ascorbate, super-oxide dismutase (SOD), and glutathione. Because Mehlhorn *et al.* (1991) have tied ozone damage to ethylene production, they propose that reduction of effects may be due to controlled production of ethylene. Each of these singularly or in combination could be ameliorating the effects of ozone at different concentrations at various locations in the chloroplast. Bennett *et al.* (1984) have shown that there are several types of SOD localized within the same cell. Consequently it seems reasonable that different isozymes may exist within the same organelle and that each may have distinctive activation concentrations and pathways.

Most fluorescence parameters were significantly different between chambers (and consequently trees) of different sizes. Table 3 shows that F_O, F_V, F_V/F_P, $T_{1/2}$, F_T, F_V/F_O, and R*fd* were significantly different. F_P and F_Q were nearly significant with P = 0.13 and 0.19 respectively. These differences reflect the physiological immaturity of the seedlings rather than an increased response to stress. Larger F_O and $T_{1/2}$ values for smaller chambers indicate that the transfer of electrons between light harvesting pigments and the reaction centers and from reaction centers to the primary electron acceptor are lower in small trees. Lower F_P and F_v values demonstrate transfer deficiencies beyond the primary electron acceptor. This data is supported by net photosynthesis measurements and will be discussed in a subsequent section of this paper.

Table 3. Generalized least squares means of fluorescence parameters for each chamber size.

Fluorescence Parameters	Size	
	Large	Small
Fo*	194.0	299.6
Fp	1485.9	1386.3
Fv*	1291.1	1086.8
T 1/2*	70.5	92.8
Ft*	400.8	242.9
Fq	1084.5	1143.4
Fv/Fp*	0.869	0.779
Fv/Fo*	7.050	4.218
Rfd*	3.120	4.806

* Differences between means are significant at $\alpha = 0.10$.

The interaction of treatment and chamber size also produced significant differences in fluorescence measurements. As shown in Table 4, only F_O, F_V/F_P, F_V/F_O, and R*fd* did not demonstrate significant differences in analysis of treatment x chamber size effects. F_O was nearly significant with P = 0.15. Comparison of these means indicates that seedlings were less responsive to treatment differences than were large trees. Seedlings exhibited a more linear response to increased fumigation treatments but the linearity was not significant when analysis is performed on only small trees. This response is also supported by photosynthetic measurements and has serious implications for extrapolation of seedling effects to large tree predictions.

Differences in fluorescence parameters between leaves on the same tree were minimal. Differences between trees and seedlings within the same treatment were moderate and reflected the dissimilarity in physiological age and the genetic variation in the plants. A random effects statement was included in the computer analysis to compensate for intra- and inter-chamber differences.

Diurnal and Temperature Effects

Limited testing prior to this experiment indicated that there was a confounding effect of time of day, probably as a result of temperature, ozone concentration, and illumination changes during the course of the day. Statistical analysis showed that hour of day was a significant effect on all fluorescence parameters.

Table 4. Generalized least squares means of fluorescence parameters for treatment x size.

Fluorescence Parameter	Size	Treatment Subambient	Ambient	2X Ambient
Fo	Large	184.3	233.8	164.1
	Small	277.2	299.9	321.5
Fp*	Large	1551.8	1722.7	1183.1
	Small	1307.7	1373.8	1477.4
Fv*	Large	1367.5	1487.1	1018.1
	Small	1030.5	1073.8	1156.1
T 1/2*	Large	70.6	49.0	91.9
	Small	100.6	92.3	85.5
Ft*	Large	403.1	498.0	301.2
	Small	220.3	249.4	259.2
Fq*	Large	1148.5	1224.0	881.2
	Small	1087.5	1124.3	1218.6
Fv/Fp	Large	0.883	0.863	0.860
	Small	0.783	0.773	0.779
Fv/Fo	Large	7.739	6.748	6.662
	Small	4.153	4.203	4.298
Rfd	Large	3.226	2.842	3.293
	Small	5.038	4.591	4.789

* Differences between means are significant at $\alpha = 0.10$.

Fluorescence parameters are sensitive to temperature because of the effect of temperature on chemical reaction rate and because of the possibility of thermal quenching as an alternative means of reducing excess electron energy. Analysis of temperature and fluorescence parameter correlations within chambers of the same size and treatment revealed that only F_T exhibited a consistent, statistically significant correlation to temperature. Other parameters were either not significant or did not produce consistentlypositive or negative correlations. Across all combinations of treatment and chamber size, F_T produced a mean correlation coefficient of -0.435. This is a logical pattern since F_T is a measure of the steady state photosynthesis. Increasing temperatures (below an inhibition threshold) would increase the rate and consequently the efficiency of photosynthetic chemical reactions resulting in reduced fluorescence.

Because ozone is formed by photochemical reactions, ozone concentrations tend to increase as the day progresses. Correlations were analyzed to determine the instantaneous effect of ozone concentration on fluorescence parameters. Again only F_T demonstrated a consistent, statistically significant pattern of response to changing ozone concentrations. The mean correlation coefficient was -0.274. This is the inverse of what is expected as higher ozone levels would be expected to inhibit photosynthesis and produce more fluorescence. One possible explanation for this seeming contradiction is that dose (exposure x time) is more important than immediate effects in reducing photosynthetic rate (Reich, 1987).

The design of this experiment enabled discernment of treatment effects apart from these confounding diurnal influences. Treatment x time of day analysis produced no significant differences in any of the fluorescence parameters. The blocking of chambers, alternation of first chamber sampled, and the compression of sampling times within blocks made possible the resolution of treatment differences.

Chlorophyll Content

Chlorophyll content was not significantly different between treatments. Figure 7 illustrates that there was a slight but insignificant decline in concentrations from subambient to ambient to twice ambient. This is contrary to evidence from sugar maple which demonstrated declines in concentrations of chlorophyll with increasing ozone fumigation (Reich *et al.*, 1986). The work on sugar maple was done on seedlings. Since this experiment took leaves for chlorophyll testing from only mature trees, the difference in tree size may account for the discrepancy. Where ozone treatment has reduced chlorophyll content (Tenga and Ormrod, 1990 and Wallin *et al.*, 1990) there have also been visible signs of damage (chlorosis, necrosis, etc.) indicating advanced injury.

The chlorophyll content differences between days was statistically significant for both chlorophyll *a* and *b* and their total. This follows the expected pattern of seasonal change as leaves mature and then become senescent. Figure 8 shows the generalized least squares means concentration for the sample days by treatment. There was also a significant treatment x day effect for chlorophyll *a* and the $a + b$ total. Variation

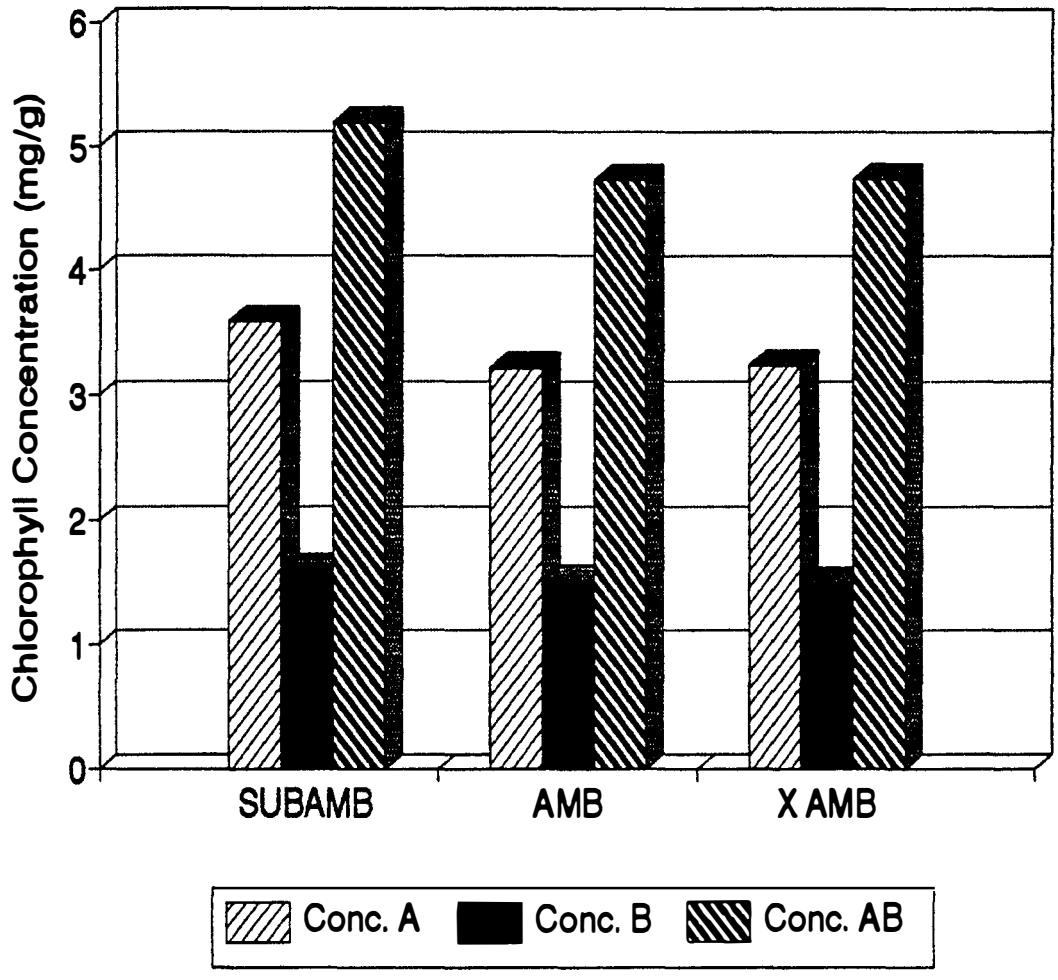

Figure 7. Generalized least squares means of chlorophyll concentrations.

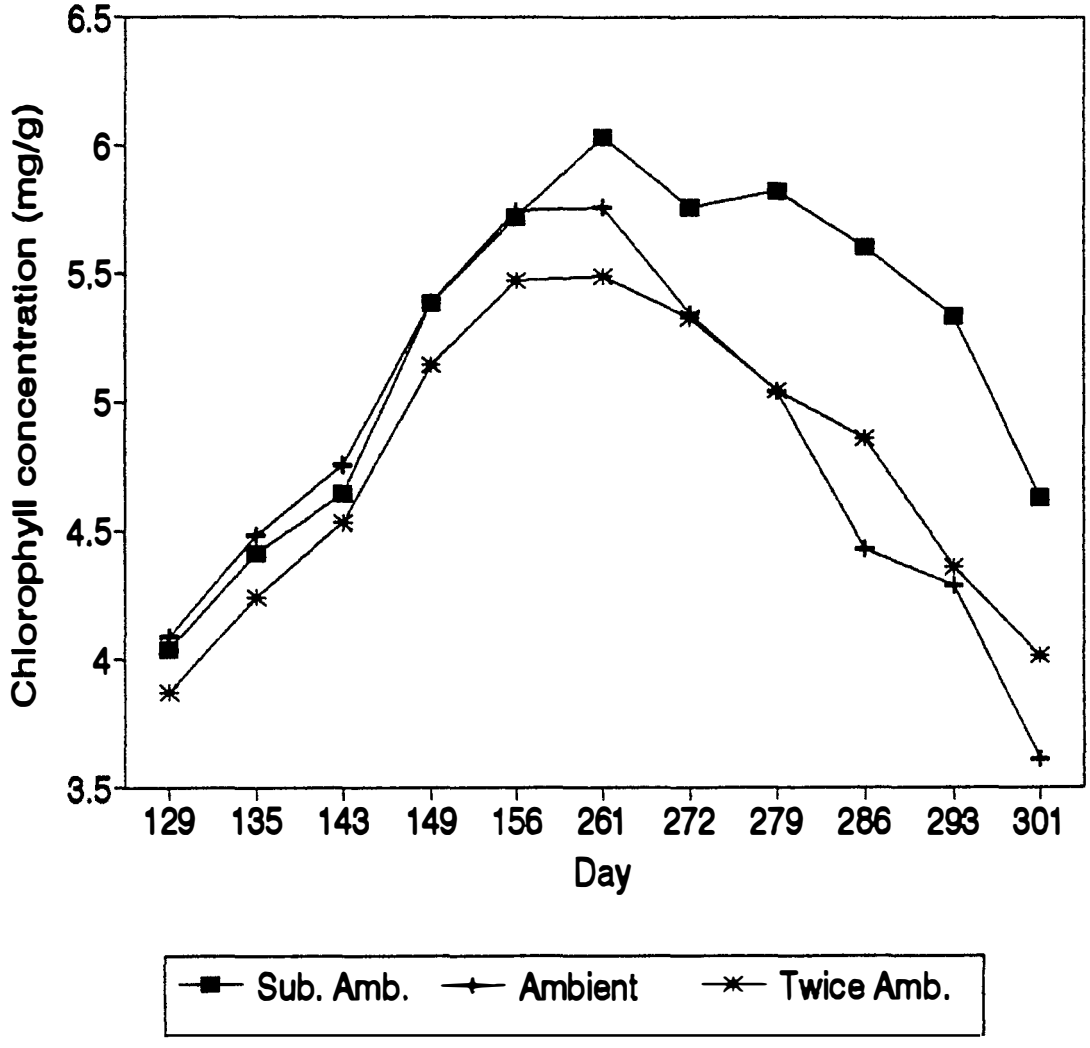

Figure 8. Generalized least squares means of chlorophyll concentration a + b by day for each treatment. Data smoothed using moving average function of Quattro Pro.

was moderate but consistent throughout with standard errors averaging 1.32. Chlorophyll concentrations for leaves from the twice ambient treatment are consistently lower than concentrations from ambient or subambient chambers until well into the autumn. After day 261 (September 18), chlorophyll in twice ambient and ambient leaves declines at a more rapid rate. This supports the idea that ozone stressed plants senesce more rapidly than unstressed (Davis and Skelly, 1992)

F_V/F_P and F_V/F_O are the only fluorescence parameters that correlate with chlorophyll concentration. Figure 9 shows the correlation between F_V/F_O and chlorophyll *ab* content. Within all three treatments, F_V/F_P and F_V/F_O are strongly, positively correlated with concentrations of *a*, *b*, and *ab* total. The average correlation coefficient of F_V/F_P with chlorophyll *a* concentration is 0.644. With chlorophyll *b* concentrations the mean coefficient of F_V/F_P is 0.505 and with *ab* total is 0.618. The mean correlation coefficients of F_V/F_O with chlorophylls *a*, *b*, and *ab* total are 0.571, 0.445 and 0.547 respectively. All are highly statistically significant. This data raises question about the validity of F_V/F_P and F_V/F_O as good measures of photosynthetic efficiency. As stated earlier, F_V/F_P and F_V/F_O do not differ significantly between treatments as did other fluorescence parameters. This would seem to suggest that other fluorescence parameters, i.e., F_O, F_P, F_T, and $T_{1/2}$, may be better indicators of stress to photosynthetic activity that is occurring independent of chlorophyll concentration changes.

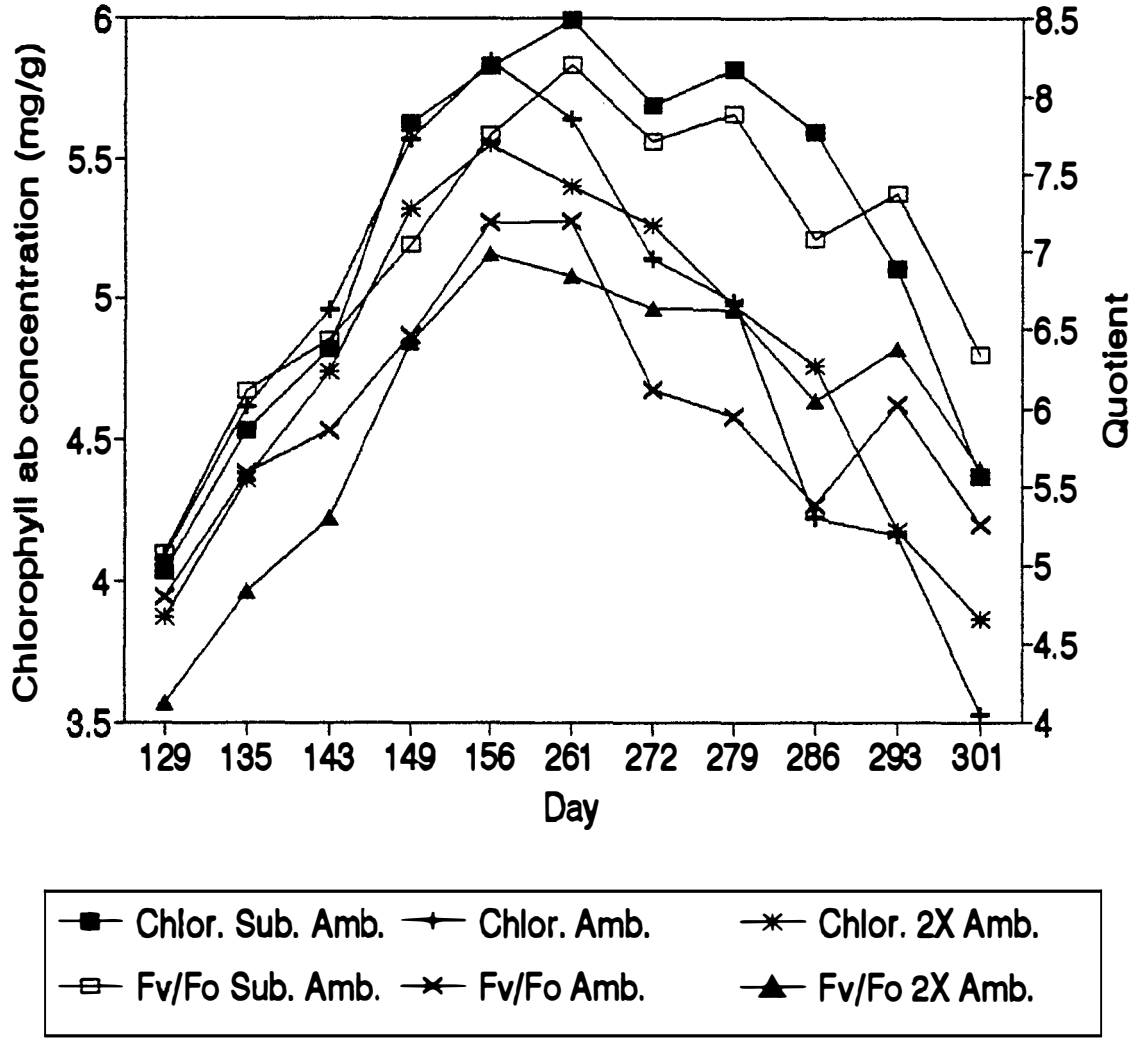

Figure 9. Mean F_V/F_O and chlorophyll *a* + *b* content. Data smoothed using moving average function of Quattro Pro.

No other fluorescence parameters demonstrated a consistent, significant correlation with chlorophyll content. F_P, F_T, and R*fd* did exhibit autumn senescence patterns similar to those reported by Lichtenthaler (1987). F_P and F_T increased and R*fd* decreased as the autumnal chlorophyll breakdown proceeded (Figure 10). D'Ambrosio *et al.* (1992) stated that R*fd* is independent of chlorophyll concentration. If this is true, the autumn decline of R*fd* may be an indicator of declining photosynthetic efficiency.

Seasonal Changes

Leaves were more than 90 per cent expanded when measurements began. Mean expansion lengths are contained in the Appendix. The rate of leaf expansion was not significantly different between treatments and no treatment x day effects were found. None of the fluorescence parameters were consistently and significantly correlated with leaf expansion. There was some difference between expansion in large versus small chambers (trees vs. seedlings). The expansion of seedling leaves occurred approximately one week later than those of large trees.

Chlorophyll content measures demonstrated a typical pattern of increase during spring expansion and a decrease during autumn senescence (Figure 8). As mentioned earlier twice ambient treated leaves had a more rapid decrease in chlorophyll content indicating more rapid senescence. Ozone exposure can accelerate leaf aging (Reich and Amundson, 1985).

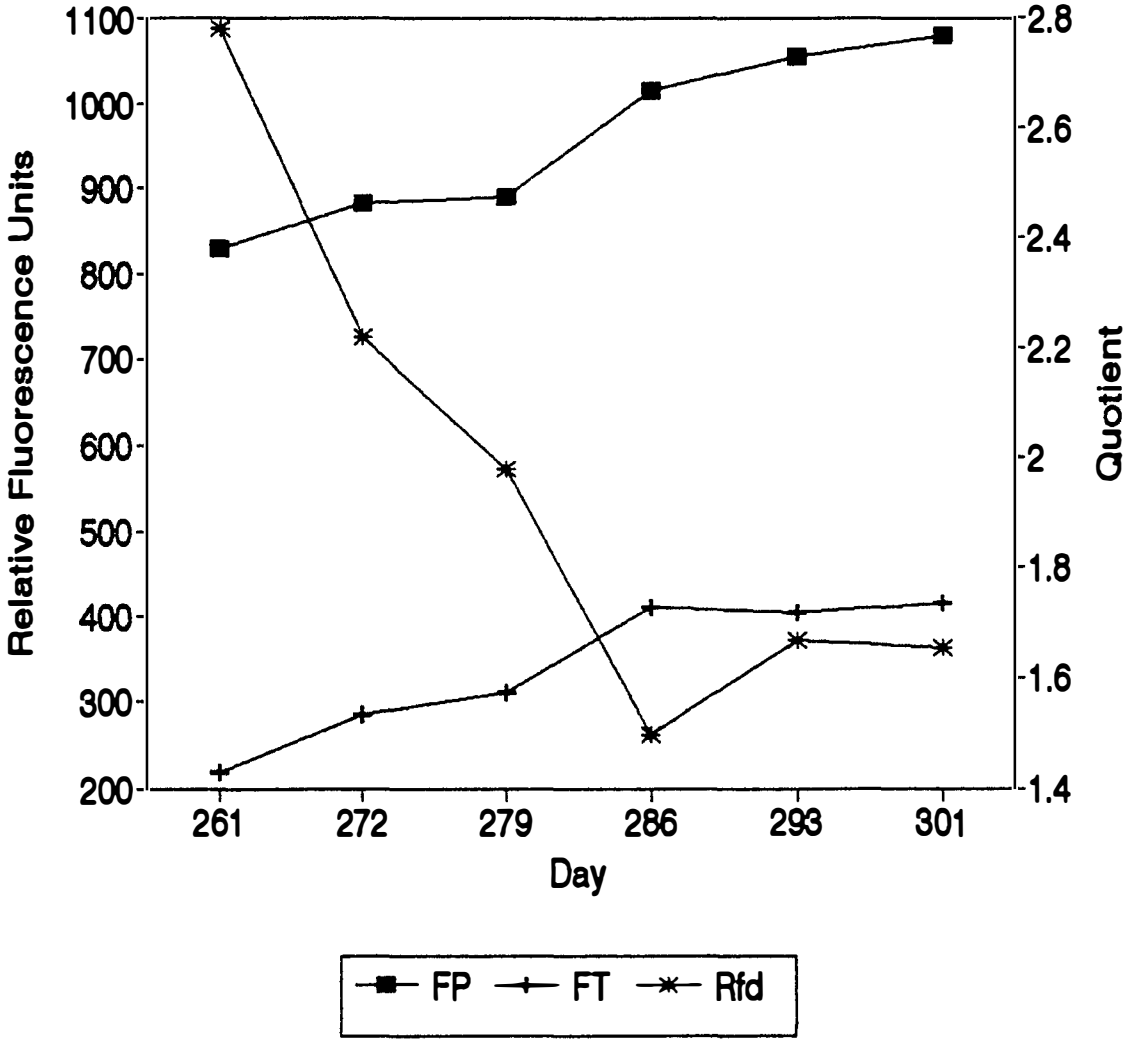

Figure 10. Mean F_P, F_T, and R*fd* values for autumn. Data smoothed using moving average function of Quattro Pro. changes in photosynthetic activity.

There were no treatment x day differences apparent in any of the fluorescence parameters. A few of the measurements and ratios did show sensitivity to seasonalAs mentioned earlier, F_V/F_P and F_V/F_O were highly correlated to the seasonal changes in chlorophyll content (Figure 9). While no chlorophyll content measurements were taken from seedlings it appears that chlorophyll content increases as the growing season progresses. Figure 11 shows the seasonal fluctuation in F_V/F_P in the large trees and asteeply increasing value for F_V/F_P in seedlings. F_O seems to be the most sensitive to seasonal changes in photosynthesis. The inverse relationship between F_O and reaction center efficiency can be seen in the seasonal changes in Figure 12. Declining F_O values during spring expansion have been reported by Kiknadze (1971) from linden and birch (species not given).

Physiological Relationships

There was good agreement between the chlorophyll fluorescence results and physiological measurements made by other researchers on this project. Net photosynthesis measurements were made during the 1993 growing season and reported by Hanson *et al.* (in press). Further references to net photosynthesis are from Hanson *et al.* unless otherwise indicated.

Mature tree foliage demonstrated reduced levels of net photosynthesis compared to subambient trees. Ambient exposed mature trees had photosynthesis levels 25% below those of subambient treated trees while twice ambient treatments resulted in as much as

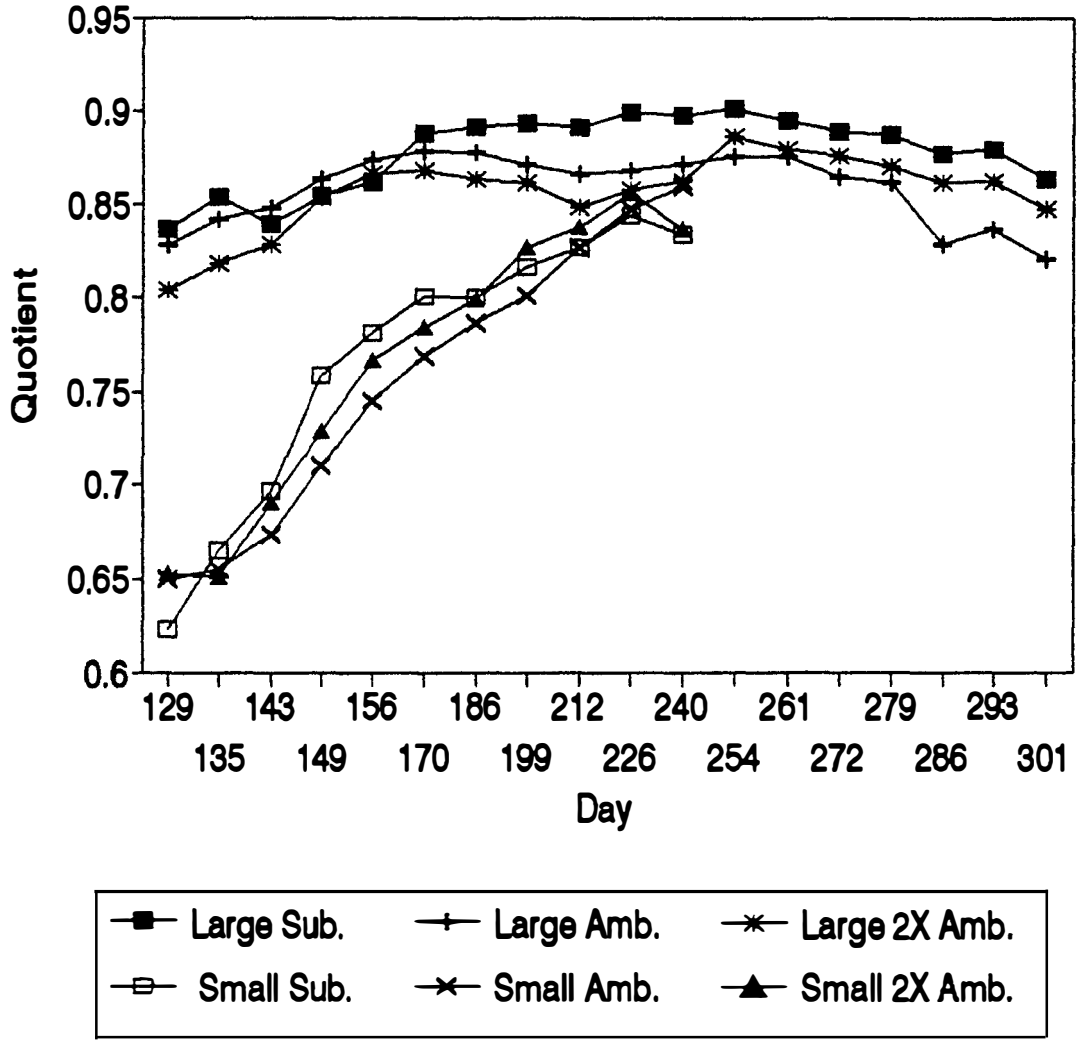

Figure 11. Mean F_V/F_P by day for each treatment. Data smoothed using moving average function of Quattro Pro.

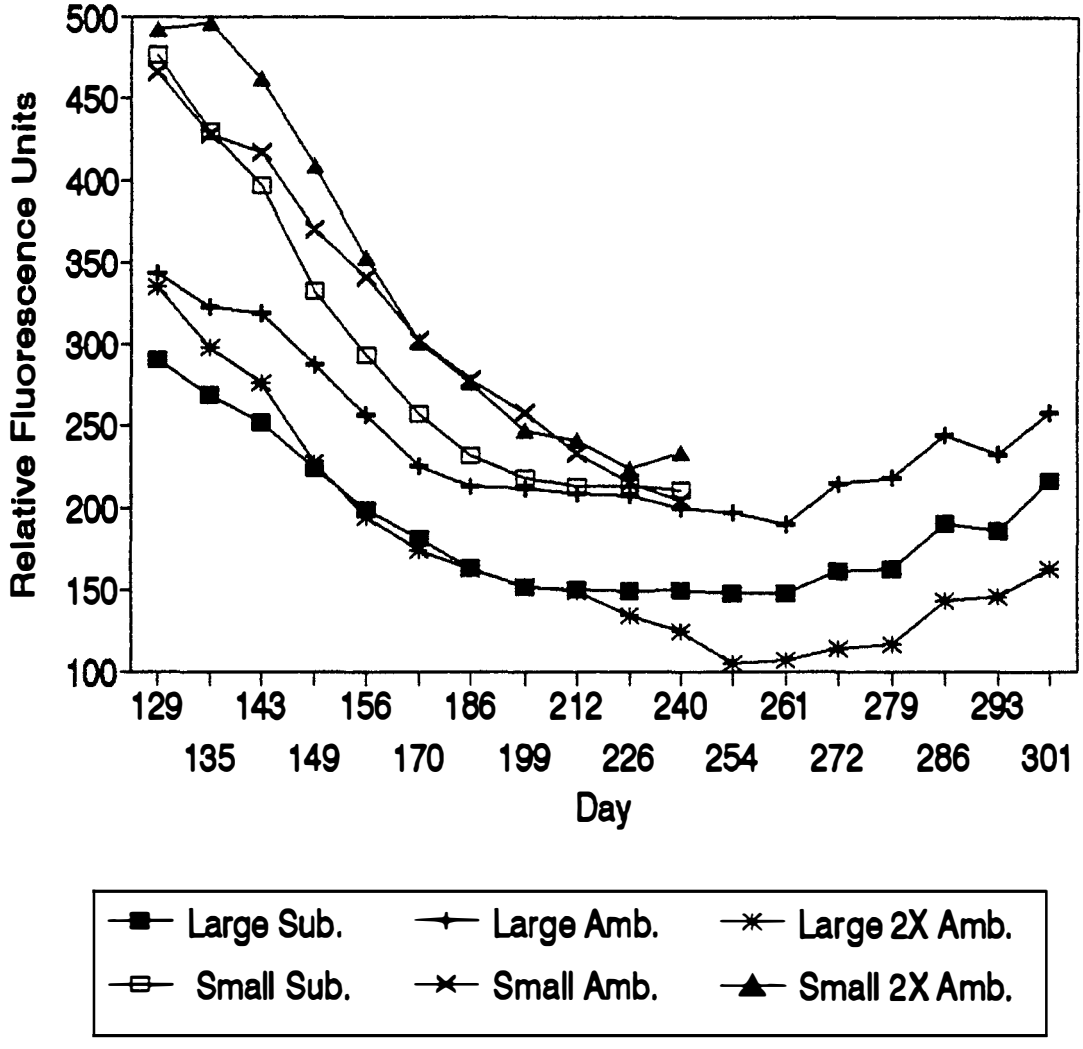

Figure 12. Mean F_O by day for each treatment. Data smoothed using moving average function of Quattro Pro.

a 50% decrease. Reich and Amundson (1985) reported similar linear decreases in net photosynthesis of red oak with respect to both O_3 concentration and dose. Although not linear, the chlorophyll fluorescence measurements do indicate significant disruption of photosynthesis in mature trees. Because of the proposed response types involving homeostatic and restitution phases it is not possible to make quantitative correlations between chlorophyll fluorescence and net photosynthesis.

Although no direct analyses of net photosynthesis were performed for comparisons between trees and seedlings, the physiological measurements indicated reduced net photosynthesis in seedlings. This conclusion was supported by the chlorophyll fluorescence measurements (Table 3). The seedlings were also not as sensitive to differences between treatments. Analysis of treatment x chamber size effects exhibited F_P, F_V, F_T, F_Q, and $T_{1/2}$ were statistically different in mature trees versus seedlings (Table 4). Although not statistically significant ($P = 0.15$), F_O also indicated reduced photosynthesis and sensitivity to treatment in seedlings.

Ozone sensitivity has been linked to leaf conductance (Reich, 1987). Although leaf conductance of seedlings in this study was less than that of mature trees, calculation of internal ozone uptake did not produce a better correlation to net photosynthetic activity than external exposure measures. Reich (1985) concludes that within species, external exposure is linearly related to net photosynthesis and that comparisons of internal dose should be used for interspecific comparisons.

4. CONCLUSION

Synthesis of Results

F_P, F_V, F_T, and F_Q were significantly different between treatments. F_O, and $T_{1/2}$ were not significant but produced similar response patterns. None of the fluorescence parameters exhibited linear changes with increasing ozone treatment. The peaks and valleys that were produced indicate two response types from the different parts of the photosynthetic machinery. F_O and F_T demonstrate the first response type where perturbation occurs at ambient concentrations of ozone. Increased amounts such as those found in the twice ambient treatments activates a repair or restitution mechanism re-establishing a homeostasis. The second response type is shown by F_P and $T_{1/2}$. Ambient concentrations induce a change resulting in homeostasis. Greater amounts of ozone exceed the ability of the plant to compensate and injury is evidenced. These patterns are supported by the significant differences exhibited by F_V and F_Q.

These response patterns also indicated that ozone has a multisite, multiphasic effect on photosynthesis. Increased F_O shows a reduction of competent reaction centers. Increasing $T_{1/2}$ demonstrates reduction in the electron transfer rate from the reaction centers to the primary electron acceptor, Q. The decrease in F_P is due to the disruption of the thylakoid membrane. The leaky membrane prevents the build up of protons in the intramembranous space thus reducing the back pressure on electron transfer. This allows for the more rapid flow of electrons away from Q consequently reducing F_P. Higher F_T

values indicate effects of ozone beyond Q, including stromal dark reactions. Although not occurring in phase, the combination of these effects is a reduction of net photosynthesis.

The often used fluorescence ratios of F_V/F_P, F_V/F_O and R*fd* were not significant and did not produce patterns similar to other parameters. This may indicate that these ratios are not capable of indicating changes that result from multiphasic, multisite ozone effects.

Differences between fluorescence parameters from mature trees versus seedlings were significant for all measurements and ratios except F_O. The disparities were due to reduced photosynthetic capability of the seedlings.

Diurnal effects (temperature and ozone concentration) on fluorescence parameters were significant. Across all treatment x chamber size combinations only F_T exhibited a strong, consistent, significant correlation. The experimental design permitted the discernment of treatment effects from these diurnal influences.

Chlorophyll content of leaves from mature trees did not significantly vary between treatments. There was a significant difference in chlorophyll concentration as spring expansion and fall senescence progressed. Treatment x day effects demonstrated that trees receiving ambient and twice ambient concentrations of ozone declined more rapidly during the autumn senescence. F_V/F_P and F_V/F_O were strongly correlated with chlorophyll

content. The non-significance of these ratios between treatments may be due to their inability to detect photosynthetic changes not associated with changes in chlorophyll content.

There was no significant difference in expansion rates between treatments. Differences between mature trees and seedlings was due to the delayed expansion of seedling leaves. None of the fluorescence parameters demonstrated significant treatment x day effects. Seasonal variations in F_V/F_P and F_V/F_O appear to be associated with chlorophyll content changes. F_O exhibits the most sensitivity to photosynthetic changes associated with seasonal development and senescence.

The multiphasic, multisite ozone effects made direct correlation between fluorescence measurements and net photosynthesis measurements impossible. This may be the factor that has confounded previous attempts to correlate fluorescence and photosynthesis. Clearly, the fluorescence measurements do indicate disruption of photosynthetic processes that would result in the linear decrease in net photosynthesis reported by Hanson *et al.* (in press) but not in a manner that permits direct comparisons. These varied ozone effects may also be responsible for the diversity in results obtained from attempts to assess ozone sensitivity using fluorescence and at least partially for the confusion about the exact pathology of ozone damage.

Objective

Chlorophyll fluorescence measurement is a useful tool in assessing the stress caused by the effects of tropospheric ozone on *Quercus rubra.* As a nondestructive, intrinsic probe, it provides insight into the condition of the photosynthetic apparatus. This study establishes that variations in ozone concentration do significantly affect various fluorescence parameters. The efficacy of field usage is dependent on the statistical and sampling design to limit the confounding effects of diurnal changes in temperature and ozone concentration. The singular limitation of chlorophyll fluorescence is the inability to directly correlate fluorescence and net photosynthesis. This deficiency may be overcome once the apparent multiphase, multisite nature of ozone damage is more clearly understood.

Further Research

The results of this study open a plethora of potential future research. Perhaps the most obvious is the repetition of this experiment among other species including those that have been reported to be more sensitive or less sensitive to ozone. Experiments in which the difference between treatments represents a more gradual gradient may give insight into the multiphasic, multisite response that occurred in this work. Fluorescence measurements taken in conjunction with defense mechanism research (SOD, glutathione, ascorbate, etc.) may explain the variation in response apparent in this study. Direct, simultaneous measurement of chlorophyll fluorescence and net photosynthesis may provide a basis for direct correlation between these two parameters.

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APPENDIX

Table 5. Mean leaf measurements (mm) during spring expansion.

Chamber Size	Day	Treatment		
		Subambient	Ambient	2X Ambient
Large	129	167	174	156
	135	170	176	160
	143	170	176	160
	149	170	176	160
Small	136	136	129	130
	143	151	134	140
	149	153	134	141
	156	153	134	141

VITA

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