PHOTOSYNTHETIC RESPONSES OF WHEAT TO LEAF RUST AND ALUMINUM STRESS

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Bachelor of Science

University of Arkansas

Fayetteville, Arkansas

1999

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2001

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ALUMINUM STRESS

Thesis Approved:

Thesis Advisor

Dean of the Graduate College

ACKNOWLEDGEMENTS

I would like to extend the sincerest thanks to my major advisor, Dr. Bjorn Martin for all of his patience, support, and wisdom throughout the duration of my Masters project. I would also like to thank my committee members Dr. Robert Hunger and Dr. Brett Carver for their expertise and guidance. I would like to thank Pamela Tauer and Craig Siegerest for their assistance. I have been graced with a wonderful family, and they have been by my side all of my academic career and I want to thank them for their eternal support. I would also like to thank my husband Stephen, for his support and encouragement. I have been blessed, and I thank God for making this possible.

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LIST OF ABBREVIATIONS

A	Photosynthetic carbon assimilation rate, μmol CO_2 m^{-2} s^{-1}
A_{amb}	Photosynthetic rate at 350 μmol CO_2 L^{-1} air, μmol CO_2 m^{-2} s^{-1}
A_{max}	Maximum photosynthetic rate, μmol CO_2 m^{-2} s^{-1}
Al	Aluminum
C_i	Internal [CO_2] in leaf
Chl_{tot}	Total chlorophyll
g_s	Stomatal conductance to water, mol m^{-2} s^{-1}
F_m	Maximum chlorophyll fluorescence (dark-adapted)
F_m'	Maximum fluorescence in the light
F_o	Minimum fluorescence in dark
F_o'	Minimum fluorescence in light
F_p	Peak fluorescence
F_v	Variable fluorescence, F_m - F_o
F_v/F_m	Maximum quantum efficiency of PS II
HRW	Hard Red Winter Wheat
HRSW	Hard Red Spring Wheat
NIL	Near-isogenic line
PAR	Photosynthetically active radiation, μmol photons $m^{-2}s^{-1}$

PS I, PS II	Photosystems I and II, respectively
RH	Relative humidity
Q_A	Primary electron acceptor in PSII
q_N	Nonphotochemical fluorescence quenching coefficient
q_P	Photochemical fluorescence quenching coefficient
Y	Effective quantum yield of Photosystem II

SUMMARY

Wheat is a globally economic crop. Its importance can be dated back to 1200 BC. The world's reliance on wheat will only grow in the future due to increasing population numbers, and the demand for better yields will only continue to rise. Nature and man have both provided obstacles that prevent many crop production systems from reaching maximum yields. Many research programs focus on the sink limitations of the harvestable plant part such as yield, whereas this study focused on photosynthetic source limitation. The photosynthetic responses of wheat were studied under the influence of two separate but concurring stresses in the southern Great Plains, leaf rust infection and aluminum toxicity.

Fungal diseases have plagued grain crops since their domestication, and continue to reduce grain quality and production. Yield losses as high as 42% have been attributed to wheat leaf rust (*Puccinia triticina*). Infection by *P. triticina* is often seasonal and conditional. Leaf rust causes severe damage to both the juvenile and the adult plant. Visual symptoms appear as chlorotic and necrotic spots on the leaf surface, and rust colored pustules of the fungus are found in these areas. In light of the visual symptoms, we investigated the effects this fungus has on the photosynthetic apparatus of the wheat plant.

Acid soils, are a problem in many areas around the globe. Many virgin soils, already acidic, have worsened due to biological and agricultural factors. Breeders continue to assess the problem. Tolerance has been introduced into many cultivars that were once susceptible to acidic soils. There are no symptoms specific to aluminum stress, and effects on susceptible plants such as stunted growth and chlorosis are merely

secondary problems resulting from nutrient and water deficiencies. A classic symptom of aluminum toxicity in wheat is a stunted root system, resulting in greatly reduced aboveground biomass. We investigated the response of photosynthesis of wheat to aluminum toxicity in this study to address this issue.

Two methods were used to measure photosynthesis: gas exchange and chlorophyll fluorescence. Gas exchange methods have long been widely used to measure photosynthesis, and modern portable equipment has become a powerful tool in physiological research both in the field and the laboratory. The gas-exchange system that was used here measures carbon dioxide (CO_2) and water vapor exchange rates between the leaf and the surrounding air with the aid of infrared gas analysis technology. From the transpiration rate (E) and leaf area, the stomatal conductance (g_s) is computed, and g_s combined with the CO_2 exchange rate (A) allows computation of the internal CO_2 concentration (C_i) of the leaf by well-established methods.

Chlorophyll fluorescence is another useful and noninvasive tool for measuring photosynthetic activity in leaves of green plants. Kinetics of fluorescence emission upon exposure of dark-adapted leaves in light reflects the initial phases of the induction of photosynthesis. Fluorescence properties obtained upon light exposure of dark-adapted leaves have long been used as an intrinsic indicator of responses to stress. In general, these measurements reveal the maximum dark-adapted efficiency of photosynthesis and the transition that occurs upon exposure to light. More recently it has become possible to measure steady state fluorescence properties of leaves continually exposed to normal light. We measured fluorescence of intact leaves in the light and the dark to obtain as complete a picture as possible of photosynthetic responses of wheat plants to rust

infection and aluminum toxicity. The strength of our approach is to combine simultaneous measurements of whole leaf photosynthesis (gas exchange) with measurements of partial photosynthetic processes going on inside the chloroplast (fluorescence).

We investigated the possible source-related limitations of leaf rust-affected and aluminum-stressed wheat using susceptible and resistant lines as experimental material. Information gathered from gas exchange and fluorescence measurements provided an understanding of if and how rust and aluminum reduced the photosynthetic source. Hopefully this information will also be helpful to breeders, providing insight as to how selection in regards to the sink has affected the photosynthetic source factors in the plant.

When the spring wheat Thatcher and its near-isoline containing the leaf rust resistant gene, Lr19, were inoculated with leaf rust urediniospores both chlorophyll content and photosynthesis rates were dramatically decreased for Thatcher and considerably less for Thatcher Lr19. Photosynthetic rates also decreased when expressed on a per unit chlorophyll basis, indicating that the loss of chlorophyll content was not the sole reason for reduced rates. Nonphotochemical quenching increased with rust infection for Thatcher, while photochemical quenching decreased. Loss of chlorophyll was most probably the major cause for lower photosynthesis, but damage to the thylakoid membranes and/or stroma components were probably also involved.

The responses of the HRW wheat near-isolines OK91G105 (tolerant of acidic soil), OK91G108 (susceptible to acidic soil), and the parent lines Atlas 66 and Century were investigated in acidic soil containing high amounts of aluminum and in the same soil which had been limed. Photosynthetic rates and chlorophyll contents of the unlimed

soil treatments were not affected by lower pH and higher aluminum content. Fluorescence parameters of OK91G105, OK91G108, and Century did not vary between soil treatments. However, Atlas 66 showed higher quantum use efficiency and nonphotochemical quenching in nonlimed soil treatments when compared to the limed treatment. From this study it appears as though Atlas was the only line that was affected by soil pH and aluminum content at the level detected here. Atlas performed better in the unlimed soil than in the limed. All other treatments were unaffected.

CHAPTER ONE

INTRODUCTION

Leaf Rust and Wheat

The relationship between an obligate parasite and its host is complex. Physiological changes can be direct results of the pathogen (primary stress), or indirect consequences of those effects (secondary stress). A net reduction in photosynthetic activity is a common symptom of many diseases, and several investigators have examined how fungal parasites affect photosynthesis (Livne, 1964; Owera et al., 1981; Goodman et al., 1986; Moll et al., 1995). Chlorosis is typical of senescing as well as rust-infected leaves. A loss of green color is indicative of a reduction in chlorophyll in that area. The chlorophyll content of rust-infected leaves is reduced, which may reduce the net photosynthetic activity (Goodman et al., 1986). However, it is possible that even if chlorosis occurs, the light reactions are unaffected under normal growth conditions due to high chlorophyll content.

Owera et al. (1981) reported a correlation between green area and chlorophyll loss in rust-infected barley. Roberts and Walters (1988) also noted a similar result, concluding that the decline in photosynthesis was due to chlorophyll loss in pustulated areas. Therefore, the response of photosynthesis to stress may vary depending on the basis on which photosynthesis is expressed. Thus, net photosynthesis may show a different trend when it is expressed on a basis of a unit leaf area as compared to on a unit of chlorophyll.

Gas Exchange

Modern gas exchange equipment is versatile, and makes monitoring photosynthetic activity easy. We used a LI-6400 Portable Photosynthesis Machine (LI-COR, INC., Lincoln, NE), which gives the user many options to customize measurements. The system has a chamber that nondestructively clamps onto the leaf. Once the leaf is within the chamber a controlled environment is created where temperature, CO_2 concentration, air flow rate, relative humidity (RH), and light intensity may all be controlled. Carbon dioxide and water vapor exchange rates between the leaf and surrounding air are measured with the aid of infrared gas analysis technology.

We chose to perform manual CO_2-response and light-response curves, in conjunction with a fluorometer. The first gas exchange measurement was on a leaf that had been dark adapted for a 20-min period in order to attain a dark respiration value, and fluorescence data were collected simultaneously for dark-adapted (relaxed) leaves. The CO_2-response curves were obtained simply by collecting a sequence of CO_2 measurements at a constant light intensity (800 μmol m^{-2} s^{-1} PAR), by varying the CO_2 concentrations in the air. Curves were constructed from the data by plotting the internal CO_2 concentration values (C_i) against the photosynthesis values (net photosynthesis in this study). Once the curve has been fitted with a regression equation, there are several important factors that can be investigated. The calculated slope is representative of the carboxylation efficiency of the plant, and the ceiling of the curve is the light and CO_2 saturated photosynthesis rate. The photosynthesis value at ambient CO_2 (350 μmol CO_2 mol^{-1} air) represents what is expected of a plant in its natural setting.

Light-response curves provide information about the performance of photosynthesis as well. While constructing a light-response curve a leaf is surrounded by a constant CO_2 concentration (350 μmol CO_2 mol^{-1} air) and exposed to a range of light intensities. Photosynthesis is recorded at each level of light intensity, and the dark respiration can be added to each of these values to create the gross photosynthetic rate. The photosynthesis values (gross photosynthesis in this study) are plotted on the vertical axis against light intensity on the horizontal axis. A similar regression equation is used for the light-response curves as for the CO_2-response curves. The slope represents the quantum yield of CO_2 fixation of the plant. The maximum photosynthesis rate recorded at the highest light intensity is the A_{max}.

Photosynthesis is not the only parameter that is monitored by gas exchange equipment. Internal CO_2 concentrations in the leaf (C_i) stomatal conductance (g_s) and transpiration are also tracked simultaneously. Gas exchange devices are very useful to study the activity of photosynthesis, and provide information once response curves are constructed.

Chlorophyll Fluorescence

Fluorescence is another excellent tool that takes an in-depth look at photosynthesis. Light energy is absorbed by chlorophyll molecules for photosynthesis (Bolhar-Nordenkampf and Oquist, 1993). Absorbed light may also be lost as heat or re-emitted as fluorescence (Ouzounidou, 1993). When green leaves are illuminated, chlorophyll molecules become excited. This excitation energy drives photosynthesis (Seaton and Walker, 1990). A red light photon (670 nm) contains sufficient energy to

boost an electron in a chlorophyll molecule from the ground state to the first excited singlet state (Bohlar-Nordenkampf and Oquist, 1993). The excitation energy is transferred across the pigment bed to a reaction center where photochemistry in the form of a charge separation occurs with a rate constant, k_p, of 10^{-8} seconds (Bolhar-Nordenkampf and Oquist, 1993). This takes place in photosystem II (PS II) during the primary photochemical step of photosynthesis. If stable charge separation does not occur, then the excess light must be released by other means to allow excited chlorophyll to return to the ground state. Some energy is lost by radiationless deactivation (heat), and a smaller fraction is lost as fluorescence (red light emission) (Seaton and Walker, 1990). PS I does not fluoresce at room temperature, so all fluorescence recorded comes from PS II (Schreiber et al., 1998).

Fluorescence occurs when an electron in the first excited singlet state decays to the ground state. The energy difference between the first singlet state and the ground state of chlorophyll is equivalent to that of a red light photon. Therefore fluorescence from a living green plant is red. Only a small percentage (2.5-5.0%) of the absorbed light is lost from a leaf in this manner. If photosynthesis is inhibited so that de-excitation by photochemistry is reduced, then de-excitation through fluorescence and thermal processes increases.

Much headway has been made in the last twenty years in understanding fluorescence. It was realized in the 1980s that fluorescence was influenced not only by the early photochemistry of photosynthesis. Fluorescence changes with changing carbon assimilation also, so fluorescence induction curves show distinct phases over the first few seconds or minutes when dark-adapted leaves are exposed to light. Fluorescence is

strongly influenced by the reduction /oxidation state of the electron transport system, which is regulated through feedback by the enzymatic dark reaction (Seaton and Walker, 1990). This relationship became clear upon the resolution of different fluorescence quenching mechanisms.

The progress in interpretation of fluorescence data in the last twenty years is largely due to new developments in quenching analysis. Quenching is a term that denotes all processes that lower fluorescence yield below its maximum (Krause and Weis, 1991). Q refers to an electron acceptor in PS II that has the property of regulating fluorescence emission (Seaton and Walker, 1990). The primary electron acceptor of PS II is called Q_A, because fluorescence is quenched when it is chemically oxidized. This means that when the first stable acceptor, Q_A, is in an oxidized state it 'quenches' fluorescence by passing electrons on through the photosystems. In darkness Q_A occurs in its oxidized form, and the reaction centers are said to be 'open' because Q_A is able to accept an electron from the charge separation in the reaction center of PS II. When the reaction centers are open (Q_A is oxidized) photochemical charge separation and the stabilization of Q_A can take place (Schreiber et al., 1998). When Q_A is reduced to Q_A^-, the reaction center becomes closed and the probability of fluorescence is high. A decrease in the rate of charge separation occurs when reaction centers are closed, and as a result more excited electrons decay to the ground state by means of fluorescence.

Energy used for electron transport causes photochemical quenching (q_P). Energy that does not drive photosynthesis or cause fluorescence results in nonphotochemical quenching (q_N). Nonphotochemical quenching involves thermal de-excitation stimulated by the light-induced proton gradient across the thylakoid membrane, and the transfer of

electrons to PS I by 'spillover' (Havaux et al., 1991). Nonphotochemical quenching has also been found to be correlated with the 'energization' of the thylakoid memebrane (Schreiber et al., 1997).

Pulse modulated chlorophyll fluorometers are able to measure fluorescence in full sunlight if needed, without disturbance of changing actinic light. A weak modulated light source is used in conjunction with a fluorescence system, which only monitors the fluorescence emitted at the particular modulation frequency (Bolhar-Nordenkampf and Oquist, 1993). To generate a Kautsky curve another light source is also needed. To induce trap closure high intensity (saturating) light pulses of 5-20,000 μmol m^{-2} s^{-1} are given from a third light source, and this is used for quenching analysis (Bolhar-Nordenkampf and Oquist, 1993). Upon application of a sufficiently strong light pulse, Q_A becomes fully reduced and q_P is suppressed. The remaining quenching is nonphotochemical (Schreiber et al., 1995).

Saturating light pulses given to dark-adapted leaves close PS II reaction centers by reducing Q_A to Q_A^-, resulting in emission of the maximum fluorescence (F_m) (Schreiber et al., 1995; Seaton and Walker, 1990; Bolhar-Nordenkampf and Oquist, 1993). When a leaf is in continuous light the fluorescence level induced by a saturating pulse falls short of its maximum value in the dark due to nonphotochemical quenching (q_N). The maximum light adapted fluorescence is called F_m' (Seaton and Walker, 1990). In the dark, prior to the pulse, when Q_A is oxidized and the reaction centers are open, the minimum fluorescence yield is observed (F_o). The difference between F_m and F_o, is called the maximum variable fluorescence (F_v), and F_v/F_m is a measure of the maximum quantum efficiency of PS II photochemistry. For most dark-adapted plants, Bjorkman and

Demmig (1987) reported the F_v/F_m to be about 0.8. Rust-affected bean plants have been found to have a decrease in F_v/F_m, suggesting inefficiency of PS II (Moll et al., 1995). Figure 1 is a quenching curve that demonstrates how the different parameters discussed above are calculated.

The chlorophyll fluorescence induction curve is most often referred to as the 'Kautsky' curve. The curve consists of two phases. The fast kinetics is completed in less than 2 s, followed by the slow kinetics spanning several minutes. The initial fast phase represents events in primary processes of PS II leading up to maximal reduction of Q_A^- at F_p, the peak fluorescence yield. The slow phase occurs after F_p has been reached, and is influenced by reduction of the plastoquinone pool, energization of thylakoid membranes, and indirectly carbon metabolism (Bohlar-Nordenkampf and Oquist, 1993).

Rationale

Significant yield reductions can occur due to wheat leaf rust. We hope to help reduce these losses by understanding the physiological effects of rust on wheat. By pinpointing the source limitation, much grain may be saved. If the complex physiological relationships between pathogen and host can be further understood, that insight could be applied in traditional breeding or biotechnological crop improvement programs. Rust pathogens are difficult to control due to their ability to change rapidly, so it may be more feasible for the farmer to use information gained as part of a preventative program, than to stop the fungus after it has taken a substantial part of the crop.

OBJECTIVES

Preliminary experiments in our laboratory and existing literature (Goodman et al., 1986) suggested that a reduction in photosynthesis in leaf rust-affected leaves was a direct result of chlorophyll loss. Hence, the objectives of this study were to determine (i) if rust infection reduces leaf gas exchange rates, and if so (ii) determine if a loss of effective leaf area and/or biochemistry of the mesophyll is the cause. By combining gas exchange and chlorophyll fluorescence measurements, it should be possible to determine where leaf rust attacks the photosynthetic machinery.

Leaf rust resistance of Thatcher Lr19 is via the hypersensitive response. The plant is infected by the pathogen, and the germ tube penetrates the epidermis, resulting in chlorotic spots called flecks. The pathogen does not progress past this point. Infection is not prevented, but spread of the infection is stopped by the necrotic fleck. Our last objective of this experiment was to compare the photosynthesis in resistant and susceptible lines inoculated with leaf rust.

The experiments were designed to test the following hypotheses:

1. Leaf rust infection reduces photosynthetic CO_2-fixation rates.
2. Leaf rust infection alters CO_2-and light-response curves in terms of initial slope and maximum rate; hence carboxylation efficiency and quantum yield are lowered.
3. Leaf rust infection alters the kinetics and magnitude of fluorescence properties, disrupting the balance between photochemical and nonphotochemical mechanisms of dissipating excitation energy.
4. Photosynthesis is unaffected by rust infection in resistant lines.

MATERIALS AND METHODS

Experimental Material

One of the ways wheat is classified is by the time that it is planted. Spring wheat is planted in the spring and harvested in the summer. Winter wheat (planted in fall and harvested in early summer) is planted in most of the United States, whereas spring wheat is restricted to the northern sections of the United States (the Dakotas, Montana, etc.) and Canada. Oklahoma, and surrounding states, plant hard red winter (HRW) wheat. Leaf rust-resistant varieties are available in both HRW wheat, and in hard red spring wheat (HRS). The experimental cultivar chosen was Thatcher, a spring wheat.

Our interest was to observe the changes in photosynthetic activity, and determine the damage zone(s) due to leaf rust infection in both resistant and susceptible lines. It is very difficult to compare responses in two materials if the genetic background differs, which introduces confounding factors that we cannot control. Using isolines is the best way to investigate a response without adding differences in genetic background. True isolines, lines differing by only one gene, are rarely available, but near-isolines (NILs), differing in a chromosomal segment containing multiple genes, are readily available. Leaf rust resistant near-isolines are not available in HRW wheat; therefore, we chose to use spring wheat near-isolines Thatcher and Thatcher Lr19 in this study. Thatcher is susceptible to leaf rust, whereas Thatcher Lr19 is resistant to the race population used. A bulk mix of *Puccinia triticina* urediospores was used to inoculate wheat. This bulk mixture was collected in May 1999, from ten HRW wheat cultivars (Agseco 7853, Big

Dawg, Champ, Chisholm, Custer, Jagger, Karl 92, 2137, 2163, and 2174) grown at three locations (Apache, Kingfisher, and Lahoma) in Oklahoma. The avirulence/ virulence formula of this bulk mixture as determined on a set of single-gene differentials and selected HRW wheat varieties was: 9 19 26 'Siouxland' (24 + 26) / 1 2a 2c 3 3ka 11 16 17 24 30 'Century'. Inoculation was performing using talc as the carrier. Control plants (noninfected) received talc only.

Planting

Twelve seeds were planted in a pan containing ReadiEarth© (Scotts-Sierra Horticultural Products, Inc., Maryville, OH) potting medium, which had been saturated with water prior to planting. After emergence the seedlings were thinned so that there were nine seedlings equally spaced (three rows of three seedlings/row). Each pan represented a block. Each replication consisted of four treatments: (i) resistant isoline, Thatcher Lr19, infected with leaf rust (ii) uninfected resistant isoline (iii) infected susceptible line, Thatcher, and (iv) uninfected susceptible line.

The pans were placed in growth chambers set for day/night cycles of 14/10 h at 21/18°C. Starting ten days from planting, pans were watered daily with a fertilizer solution containing one teaspoon of Peters 20/20/20 with micronutrients (Spectrum Group, St. Louis, MO) per gallon of water. Prior to that they received water only.

Inoculation Procedure

Plants were inoculated with spores of *P. triticina* at four weeks of age. Talc was used as the spore carrier; a 1:4 spore to talc mixture by volume was used producing an

expected 60-75 uredia/cm^2. Plants were misted with a dilute tween/water solution before application of the spore/talc mixture. Control plants were misted and dusted with talc only.

Following inoculation, the plants were placed in a misting chamber at a PAR value of about 40 μmol $m^{-2}s^{-1}$ for 16 h. The misting period maintained free moisture on leaf surfaces, which is crucial for successful spore germination and infection. After the misting period, the doors of the misting chambers were slightly opened to allow slow drying of leaf surfaces. This drying down period lasted for 8 h, and allowed the plants to slowly equilibrate with the relative humidity of the room. A quick drying may shock the spores and cause germ tubes to rupture. After the inoculation procedure, plants were returned to the growth chambers.

Chlorophyll Content

Chlorophyll content (Chl_{tot}) was determined by collecting the leaf sections of the plants used for gas exchange and fluorescence measurements. Measurements began on 'day 0', prior to inoculation, and continued every other day (days 2,4, etc) to day 12 following inoculation. Samples were gathered on seven even numbered days, from days 0-12, rendering seven samples. The area of the leaf occupying the leaf chamber was determined with the use of a LI-3000 leaf area meter (LI-COR Inc., Lincoln, NE).

The procedure of Arnon (1949) was used to extract the chlorophyll and calculate Chl_{tot}. First the sampled leaf section was ground in 80% acetone with sea sand in a mortar and pestle. The homogenized sample was centrifuged at 5,000 x g for 2-3 min. The absorbancies of the sample were analyzed using a spectrophotometer (Spectronic Instruments Inc., Rochester, NY). A multiple wavelength function was selected using

645 nm, 663 nm, and 720 nm. Chlorophyll *b* and *a* absorption peaks in an 80% acetone solution occur at the first two wavelengths. Chlorophyll does not absorb at 720 nm, so this wavelength was used as a baseline by subtracting it from the values at 645 nm and 663 nm before applying the Arnon (1949) equations. The correction for light scattering obtained this way was very small.

Fluorescence and Gas Exchange Measurements

It is possible that internal changes take place within the leaf as a result of rust infection well before visual symptoms are observed. Flecking can usually be seen within seven days after inoculation. In this particular study flecking was visible between days 4 and 6 following inoculation. To follow the physiological changes that occur upon inoculation, the plants were measured just prior to inoculation (day 0) and every other day thereafter on days 2, 4, 6, 8, 10, 12, constituting seven measuring days. Only two plants could be measured each day, so two treatments of the same line were measured one day, the other line the next day, and so forth. Within this measuring period the typical life cycle of leaf rust was completed, i.e. the rust spores germinated, formed uredia, and released new spores.

Chlorophyll fluorescence was measured simultaneously with gas exchange. An attached leaf was placed inside the LI-6400 leaf chamber. The top of the chamber was constructed of clear plastic that allowed light from an external metal halide lamp to drive photosynthesis. The amount of incident light impinging on the leaf surface was varied by inserting combinations of metal wire screens between the lamp and the leaf chamber. The top of the leaf chamber also had a port for a fiber-optic light pipe connected to the

OS-500 fluorescence equipment. In this configuration simultaneous measurement of both gas exchange and fluorescence emission on the same portion of the leaf could be conducted.

The conditions in the leaf chamber were maintained at 25°C and 50% RH. The CO_2 concentration and light intensity varied with the type of experiment. Initially the CO_2 concentration was set at 350 μmol mol^{-1} and the entire leaf chamber was covered with a heavy black cloth. After 20 min dark respiration was determined. Dark-adapted fluorescence (F_o, F_m and F_v) was measured using a 0.8 s saturating light pulse. Next, the leaf was exposed to 200 μmol m^{-2} s^{-1} actinic PAR for five minutes of fluorescence induction kinetics (Figure 1). Every 14 s saturating light pulses were given to determine how excitation energy was dissipated over time (time-dependence of changes in quenching coefficients q_P and q_N following exposure to light). At the end of the fluorescence induction measurements, the actinic light was turned off, the leaf briefly exposed to far-red light, and the F_o' determined. A difference between the F_o and F_o' reveals a change in minimum fluorescence that is not readily reversible upon darkening of the leaf. Photoinhibition often occurs in stressed leaves and it causes such a change in minimum fluorescence.

Next, PAR was increased stepwise to 800 μmol m^{-2} s^{-1}. After equilibrium, gas exchange and fluorescence were collected at eleven CO_2 concentrations in the air ranging from close to zero to 2000 μmol mol^{-1}. The initial measurement was at 350 μmol mol^{-1} followed by measurements at lower CO_2 concentrations. The CO_2 concentration was then returned to 350 μmol mol^{-1} and subsequent measurements were made at stepwise

increased CO_2 concentrations. CO_2-response curves were generated by plotting photosynthesis rates against calculated internal CO_2 concentrations (C_i).

The regression equation:

$$y = \frac{\phi \times x + A_{max} - \sqrt{(\phi \times x + A_{max})^2 - 4 \times \phi \times A_{max} \times \Theta}}{2 \times \Theta} + R_d$$

was used for the CO_2-response curve , where A_{max}=maximum photosynthesis rate, Θ = convexivity, R_d= dark respiration and ϕ =initial slope. The same equation was used to fit the light-response curves as well, except with the exclusion of the addition of dark respiration.

Light-response curves were generated in a similar manner by controlling the CO_2 concentration in the air at 350 μmol mol^{-1}. Gas exchange and fluorescence data were collected at nine light intensities ranging between zero and 1800 μmol m^{-2} s^{-1} PAR.

After 15 min of dark adaptation of the same leaf, a five-minute fluorescence induction curve (Kautsky curve) was obtained to resolve its O, I, D, P, S, M and T phases (Bohlar-Nordenkampf and Oquist, 1993). For this measurement, 1600 μmol m^{-2} s^{-1} PAR from the external light source was used as actinic light.

The leaf was again dark adapted for 20 min and the fast kinetics of fluorescence induction was determined during a 10s exposure to 200 μmol $m^{-2}s^{-1}$ PAR. This was done to resolve the early components of the induction curve.

Experimental Design

Three replications in time were performed in this study. Repeated measures were conducted in each replication. There were two lines and two inoculation treatments,

inoculated or noninoculated, making a total of four treatments units. A randomized block design was used.

Statistical Analyses

Repeated measures with optimal intra-plant, variance/covariance structure was utilized with a repeated measures statement with SAS (Statistical Analysis System, SAS Institute Inc., Cary, NC) using the Proc Mixed procedure. The variance/covariance structure was evaluated using AIC information and criteria. The autoregressive (AR) structure and variance component (VC) were compared, and whichever value was smaller was the method that was used. The presented material varied in use of AR and VC.

RESULTS

Gas Exchange

Figure 2 demonstrates that A_{amb} decreased over time in infected Thatcher with leaf rust, and either remained unchanged or only slightly decreased in uninoculated Thatcher and in Lr19 when compared to the controls. The means and corresponding standard errors of A_{amb} are plotted versus time for all four treatments. A_{amb} values were significantly lower for infected treatments of Thatcher when compare to the control beginning on day 6. There was an isolated significant difference between noninfected and infected Thatcher Lr19 on day 10 (Table 1). There was no notable difference in uninoculated Thatcher or Thatcher Lr19.

Stomatal conductance (g_s) values were also investigated at ambient CO_2 (Table 1) The g_s values remained unchanged for all treatments over time. Large variations among plants or replications may have been responsible for this.

The CO_2 response curves of the three replications are displayed in Figures 3 and 4. The slope value and the maximum value were the parameters of interest in these particular curves. There was no significant change in the slope between uninfected and infected plants for either line (Table 1). The photosynthesis rate at saturating light and CO_2 was also observed (Figures 3 and 4). There was a decrease in this value due to infection for both Thatcher and Thatcher Lr19 when compared to noninfected plants, but the decrease was greater in Thatcher than in Thatcher Lr19.

The light response curves of all three replications are shown in Figures 5 and 6. Gross photosynthesis values were used to construct the light response curves. Gross photosynthetic values are calculated by adding dark respiration values to each

photosynthesis value. The slope of the light response curves was unaffected by rust infection for either line (Figure 2).

Maximum photosynthesis (A_{max}) values in the form of means and standard errors of the three replications are shown in Figure 7. A_{max} values were recorded at the highest light intensity possible (1600 μmol $m^{-2}s^{-1}$ PAR). A_{max} values decreased significantly for infected plants when compared to the noninfected, with Thatcher being the most affected line (Tables 1 and 2). A dramatic reduction in A_{max} was evident for Thatcher infected when compared to the noninfected plants starting on day 6 (Table 2 and Figure 5). Infected Thatcher Lr19 plants experienced lower rates when compared to the noninfected plants on the last two measured days, 10 and 12.

Fluorescence

The fluorescence parameters of F_v/F_m, q_P, q_N, and Y (Figure 1) were also examined. The parameters were automatically calculated by the fluorometer using F_o, but F_o' was virtually identical to F_o. There were significant differences over time in q_P, q_N and Y values for noninfected Thatcher and infected Thatcher (Table 1). There was a noticeable change in the three parameters over time with rust infection (Figures 8, 9, 10). Infected Thatcher plants experienced higher q_P, q_N, and Y than noninfected Thatcher plants starting on day 8 (Table 3). There were no differences in fluorescence parameters between noninfected and infected Thatcher Lr19. No change in F_v/F_m was observed for either line (Table 3).

Chlorophyll Content

Total chlorophyll content (Chl_{tot}) declined significantly over time for Thatcher control (Figure 11), perhaps caused by aging of leaves throughout the twelve-day period. There was a significant to highly significant difference between inoculated and uninoculated Thatcher beginning on day 4 and persisting to day 12 (Table 2). A_{amb} and A_{max} were calculated on a per unit chlorophyll basis (mmol CO_2 mol Chl^{-1} s^{-1}). Infected Thatcher showed a significant decease in A_{amb} /Chl starting at day 6 and lasted day 12 (Table 2), while the other three treatments remained unchanged. There was no difference in A_{max}/Chl_{tot} between infected and noninfected Lr19, other than on day 12 (Table 2). Inoculated and uninoculated Thatcher significantly differed on days 8 through 12.

DISCUSSION

Gas Exchange

Both A_{amb} and A_{max} were reduced by rust infection in Thatcher Lr19, but even more so in susceptible Thatcher. Chlorophyll content (Chl_{tot}) was also reduced, as suggested by previous literature. However, lower chlorophyll content alone was not sufficient to explain the reduced photosynthesis because when normalized on a unit chlorophyll basis both A_{amb} and A_{max} (mmol CO_2 mol^{-1} Chl s^{-1}) were significantly reduced in infected Thatcher when compared to noninfected Thatcher. Chlorotic leaf areas containing pustules are the cause for loss of chlorophyll. Reduction in photosynthesis rate per unit chlorophyll suggests rust impaired photosynthesis not only by reducing the effective leaf area in proportion to chlorophyll loss, but also by lowering the photosynthetic efficiency on a per unit chlorophyll basis.

The photosynthesis rates from the CO_2-response curves at saturating light and CO_2 were reduced with rust infection for both infected treatments (Figures 3 and 4). The most dramatic reduction was evident in susceptible Thatcher. A reduction in the light and CO_2 saturated photosynthetic value from the CO_2-response curves suggests that the capacity to regenerate RuBP, under control of the electron transport and photophosphorylation, was reduced.

No significant changes in chlorophyll content were observed in infected Thatcher Lr19. The small amounts of green area loss, called flecking evidently were not sufficiently large to result in a significant decrease in Chl_{tot}. A_{max} values decreased significantly on days 10 and 12 for infected Thatcher Lr19 plants. Flecking is the first

and only symptom in a hypersensitive response to rust infection. Flecking usually occurred at day 6, with no visible changes thereafter. The small stress of initial infection of the pathogen may have accelerated the senescing process, resulting in a decrease in maximum photosynthetic capacity on days 10 and 12 following infection.

Figure 4 shows that there may be a small decrease in photosynthesis at light and CO_2 saturation for Thatcher Lr19. Again, a decrease in this photosynthetic rate (under light- and CO_2-saturation) usually entails a reduction in the electron transport capacity or photophosphorylation. Photosynthesis rates at ambient CO_2 levels were unaffected. Therefore it seems that ambient photosynthesis was unaffected in infected Thatcher Lr19 plants, and problems were incurred first when the leaf was under saturating CO_2. At high CO_2, electron transport represented a greater limitation in infected leaves than in uninfected leaves.

Neither the slopes of the CO_2- or light-response curves were reduced with leaf rust infection in either line. Therefore, the carboxylation efficiency and quantum yield of photosynthesis of infected leaves were not reduced with rust infection. It appears that reduced photosynthetic rates may have been due to a reduction in light harvesting with fewer photons being captured to drive the process. Fewer photons absorbed could be the reason for lowered electron transport rate.

Chlorophyll Fluorescence

The nonphotochemical quenching coefficient (q_N) increased under the stress of rust infection, whereas the photochemical quenching coefficient (q_P) remained constant until days 10-12 at which it also increased in infected Thatcher when compared to

noninfected Thatcher plants. Progression of infection caused an increased proportion of the captured electrons not driving photosynthesis. The effective quantum yield of PS II, Y, decreased significantly with rust infection in infected Thatcher when compared to the noninfected Thatcher plants (Figure 10). The differences in these parameters for infected and noninfected Thatcher plants suggest that an increasing amount of excitation energy was dissipated as heat rather than powered photosynthesis. The maximum quantum efficiency (F_v/F_m) was unaffected.

Previous literature suggests that rust reduces photosynthesis by lowering the chlorophyll content. This is consistent with the similarity in timing of the appearance of visual symptoms, reduced chlorophyll content, and lowered photosynthesis rate (A_{amb} and A_{max}) on a leaf area basis. All the parameters showed a significant reduction that occurred in concert with visual symptoms and worsened throughout infection for susceptible plants. The resistant Thatcher Lr19 individuals showed changes in photosynthetic parameters due to rust infection as well, but at later days after infection and to a much lesser extent. However, lowered chlorophyll content is not sufficient to be the sole explanation of reduced photosynthesis. Both A_{amb} and A_{max} were reduced even when normalized on a per unit chlorophyll basis. Thatcher Lr19 showed a reduction in photosynthetic rates on days 10 and 12, but there was no significant loss in chlorophyll. Also, changes in fluorescence suggest damage to the photosynthetic machinery due to rust infection.

We chose to look at near- isolines, Thatcher and Thatcher Lr19, that were susceptible and resistant to leaf rust infection. Investigating near-isolines allows researchers to study differences in plant response without introducing compounding

factors. Often times there is a price to be paid, such as reduced yield, for resistance. If there are yield reductions, then reduced photosynthesis could be a likely cause. The uninfected treatments of Thatcher and Thatcher Lr19 did not differ photosynthetically. The infected treatments in this study differed in response to leaf rust, as expected. Infected Thatcher Lr19 experienced a decrease in maximum photosynthetic rates twelve days after infection, but photosynthesis under normal conditions were unaffected. Photosynthetic rates, both ambient and maximum, in Thatcher were greatly reduced by rust infection much before day 12. There was also a significant loss of green leaf area in Thatcher that did not occur in Thatcher Lr19 indicating that chlorophyll loss was the primary reason for reduced photosynthetic rates. Leaf rust caused additional damage to the thylakoid and/or stroma.

LITERATURE CITED

Arnon, D. L.1949. Copper enzymes in isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24:1-15.

Bolhar-Nordenkampf, H.R. and G. Oquist. 1993. *In* Photosynthesis and Production in a Changing Environment: A Field and Laboratory manual. Chapman and Hall, London.

Bjorkman, O. and B. Demming. 1987. Photon yield of O_2-evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta 170:489-504.

Goodman, R.N., Z. Kiraly and K.R.Wood. 1986. The Biochemistry and Physiology of Plant Disease. Univ. of Missouri Press, Columbia.

Havaux, M., R. J. Strasser and H. Greppin. 1991. A theoretical and experimental analysis of the q_P and q_N coefficients of chlorophyll fluorescence quenching and their relation to photochemical and nonphotochemical events. Photosynth. Res.27:41-55.

Kraus, G.H. and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis: The basics. Annu. Rev. Plant Physiol. Mol. Biol. 42: 313-49.

Livne, A. 1964. Photosynthesis in healthy and rust-affected plants. Plant Physiol. 39:614-621.

Moll, S., P. Serrano and C. Boyle. 1995. *In vivo* chlorolophyll fluorescence in rust-infected bean plants. Angew. Bot. 69:163-168.

Montalbini, P. and B.B. Buchanan.1974. Effect of rust infection on photophosphorylation by isolated chloroplasts. Physiol. Plant Path. 4:191-196.

Ouzounidou, G. 1993. Changes in variable chlorophyll fluorescence as a result of Cu-treatment: response-relations in *Silene* and *Thlaspi*. Photosynthetica. 29(3): 455-462.

Owera, S.A.P, J.F. Farrar and R. Whitbread. 1981. Growth and photosynthesis in barley infected with brown rust. Physiol. Plant Pathol. 18: 79-90.

Roberts, A.M. and D.R. Walters. 1988. Photosynthesis in discrete regions of leek leaves with the rust *Pussinia allii* Rud. New Phytol. 110:371-376.

Schreiber, U. and W. Bilger. 1987. Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. *In*: Plant Response to Stress, pp.27-53. Tenhunen, J.D., F.M. Catarino, O.L. Lange, and W.C. Oechel (eds). Springer Verlag, Munchen, Wien, and New York.

Schreiber, U., W. Bilger and C. Neubauer. 1995. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. *In* Ecophysiology of Photosynthesis. Ecological studies, Vol.100. E.D. Schulze and M.M. Caldwell (eds). Berlin, Germany.

Schreiber, U., W. Bilger, H. Hormann and C. Neubauer. 1998. Chlorophyll fluorescence as a diagnostic tool: basics and some aspects of practical relevance. Photosynthesis- A Comprehensive Treatise. A.S. Raghavendra (ed). Cambridge University Press, Cambridge, U.K.

Seaton, G.R. and D.A. Walker. 1990. Chlorophyll fluorescence as a measure of photosynthetic carbon assimilation. Proc. R. Soc. Lond. B. 242:29-35.

CHAPTER 2

INTRODUCTION

Aluminum Toxicity and Wheat

Soil acidity is characterized by high amounts of H^+ in the soil solution. Aluminum becomes increasingly more soluble at pH levels lower than 5.5. Aluminum content is strongly affected by chemical, physical, and biological properties of the soil. For example, clayey soils are more prone to aluminum accumulation than sandy soils (Srivastava and Gupta, 1976). Application of KCl, $CaCl_2$, gypsum, and nitrogen (N) tend to elevate aluminum availability, whereas applications of phosphorus (P) or magnesium (Mg) tend to decrease the available forms of aluminum. Insoluble aluminum phosphates form when P is added to the soil, alleviating the threat of aluminum toxicity. Generally, soils high in organic matter are less apt to accumulate aluminum due to the strong binding of aluminum to organic acids in the soil.

Aluminum hydrolyzes in acidic soils, further decreasing the pH level with H^+ released at each hydrolysis. Therefore the Al species and content will vary with pH (Srivastava and Gupta, 1996; Brady and Weil, 1996). The surface layer of agricultural soils are acidified further when essential nutrients are removed with harvest (Carver and Ownby, 1995).

Aluminum is not an essential element for most higher plants. Exceptions to this are tea, ferns, and some hydrophytes (Marschner, 1995). Acid soils are phytotoxic to most agriculturally important plants. The signs of aluminum stress are difficult to distinguish from other nutrient disorders. This is because aluminum indirectly induces

nutrient deficiencies of N, K, Ca, Mg, and Mo as a result of decreased uptake from the soil. Aluminum-stressed plants may exhibit leaf chlorosis, purpling of tissues, leaf curling, or interveinal chlorosis caused by deficiencies of N, P, Ca, and Mg, respectively (Foy et al., 1974; Srivastava and Gupta, 1996; Carver and Ownby, 1995).

Thickened roots, often with brown tips, are indicative of aluminum stress long before symptoms are visible in the aerial plant tissue (Carver and Ownby, 1995; Rengel, 1997). The growth restriction prevents roots from reaching water and available nutrients that are deeper in the soil. Since the detrimental effects of aluminum exposure are primarily observed in the root system (Marschner, 1995), most research has focused on this area. All tolerance mechanisms known thus far are root related.

Less than 10% of the total 50-200 mg Al/kg total dry matter is found in the shoots of most crop plants (Srivasta and Gupta, 1996; Zhang and Taylor, 1988). Older leaves contain ten times the amount of aluminum found in younger leaves. Therefore it is believed that aluminum is not mobile within the plant system. Detrimental effects observed in shoots are likely caused by reduced root growth. Less root mass means less surface area that is able to absorb essential nutrients and water. It is likely that aluminum exposure hinders absorption and translocation of nutrients. Moustakas et al. (1995) reported a decrease in Ca, Mg, K, and Fe in shoots of aluminum tolerant and susceptible lines in nutrient solutions (pH 4.5) of varying Al levels. The tolerant cultivar, Yecora E was found to have higher amounts of nutrients in all plant tissues compared to the susceptible variety Nestos. N, P, and Fe in maize shoots were found to decrease significantly with increasing Al concentrations (Lidon et al., 1999). It is expected that total dry weights of aluminum stressed plants will be less than non-stressed plants. Ohki

(1986) reported no decreased dry weights of susceptible plants at low aluminum concentrations, but did at higher concentrations.

Ohki (1986) was one of the first in studying the negative effects of aluminum stress on photosynthesis by investigating transpiration and chlorophyll content in wheat and sorghum. Ohki concluded that chlorophyll content in wheat was more sensitive to aluminum exposure than sorghum. A general decrease in chlorophyll content is expected with many nutrient deficiencies, which may in turn decrease photosynthesis. Ohki (1986) observed a decrease in photosynthesis rate, presumably caused by lower chlorophyll content.

Moustakas et al. (1995) investigated the negative effects of aluminum stress via chlorophyll fluorescence. They reported a decrease in F_o, representing a decrease in efficiency of light usage. F_I and F_P increased significantly, the increase in F_P representing increased Q_A reduction. They attributed the alterations of the kinetics of the induction curves of both susceptible and the more tolerant variety to alterations of thylakoid functioning.

Tolerant germplasm has been identified and is effective against Al containing soils. There are two basic forms of resistance: prevention of aluminum uptake, and sequestration after the aluminum has penetrated the plasmalemma. Exudation of malate and citrate from the plant into the rhizosphere has been noted to aid in Al tolerance (Miyasaka et al., 1991; Delhaize et al., 1993). Exudation of malate is the predominant mode of tolerance exhibited in wheat (Tang et al., in press). Organic acids chelate Al, making it unavailable to the plant. Henderson and Ownby (1991) observed that excess mucilage produced on the root cap in ten wheat cultivars was correlated with Al

tolerance. The mucilage slows the aluminum movement allowing the organic acid/Al ratio to rise.

Atlas 66 is a HRW wheat variety highly tolerant to aluminum. This valuable trait prompted the use of this germplasm as a donor parent in developing tolerant near-isolines (NILs) to Century and Chisholm. Atlas 66 is most effective because of exudation of malate and phosphate. Tang et al. (in press) quantified the genes transferred to NILs of Century and Chisholm. The tolerant (T) NILs did not perform as well as Atlas 66 in any of the treatments, and Century tolerant (T) lines performed better than Chisholm (T) lines in nutrient solutions. The conclusion from these studies was that only one gene was transferred, and only malate exudation occured in these lines. One Century-T line was used in this study, OK91G105, due to superior performance.

In light of all of this, further research is needed to define the overall effects of aluminum stress on photosynthesis in tolerant and susceptible wheat germplasm. No photosynthetic work has been conducted on the available tolerant and susceptible near-isolines. We feel that we have developed a protocol that is useful to investigate the photosynthetic response to Al toxicity in tolerant and susceptible wheat lines. Gas exchange measurements on intact leaves provide a window into the overall process of photosynthesis, and chlorophyll fluorescence measurements add considerable detail. These measurements could provide an indicator of whether or not tolerance mechanisms present in wheat cultivars prevent other nutrient deficiencies as well. Many physiological investigations have used nutrient solutions as the growth media. A comparison of physiological responses in soil could be insightful because they more realistically predict cultivar performance in the field.

Rationale

Aluminum limits plant growth around the globe. Agricultural land is problematic in Europe, with some 60 % of Polish agricultural land affected (Aniol, 1984 cited by Carver and Ownby, 1995), and areas in Australia, Canada, South America, and South Africa have become increasingly more acidic in recent years (Carver and Ownby, 1995). The southern Great Plains are greatly affected by surface soil acidity, primarily due to continuous wheat production that has accelerated acidification of virgin soils. Oklahoma State University provided free soil tests to Oklahoma farmers in 1996 as a result of two consecutive years (1995 and 1996) of subnormal yields. Of the submitted samples Zhang et al. (1988) reported that 39% of the wheat fields had pH values below 5.5, the critical value for wheat.

The most intensive wheat production region in the United States is the southern Great Plains. This region also contains large areas of acidic topsoil. Southeastern Oklahoma, receiving the highest amount of rainfall in the state (140 cm/yr), has naturally acidic soils that have not been further affected by cultivation (Carver et al., 1998) probably because liming has always been a common practice in this area. Lack of free lime in the soil and poor liming practices along with continuous wheat production have rendered central Oklahoma the problematic area of the state.

To address the problem of aluminum toxicity in acid soils, tolerant wheat germplasm, OK91G105 and OK91G108 were developed and released. Tolerant germplasm can survive and produce yields, but are no solution to the problem of acid

soils. Much headway has been made, but for maximum benefit more must be learned about the physiology of aluminum toxicity.

OBJECTIVES

The objectives if this study were to (1) examine the photosynthetic response of HRW wheat tolerant and susceptible near-isolines exposed to acid soils containing Al, and (2) to locate the site(s) of damage to the photosynthetic apparatus, if photosynthetic activity is reduced.

MATERIALS AND METHODS

Experimental Material

Two NILs derived from the winter wheat lines Century and Atlas 66 were selected for this study: OK91G105 the aluminum tolerant (T) isoline, and OK91G108 the susceptible (S) isoline. The two isolines as well as the parent lines were included in this study. Two soil treatments were used: nonlimed and limed. The four lines in each soil type equaled eight treatment units. Aluminum-stressed plants were grown in acid soil (pH=4.5), and control plants were grown in the same soil that was limed up to the optimum pH for wheat, 5.5.

Soil media

Acidic treatments consisted of autoclaved field soil in a 1 L pot. The same soil was used for the control treatments, except lime and water were added two weeks prior to planting so that the pH was $\geq$ 5.5 at the time of planting. Three seeds of each line were planted and then thinned to one seedling per pot. All of the lines were planted in separate pots, watered regularly and grown for four weeks in a growth chamber set for day/night cycles of 14/10 h at 21/18°C receiving a light intensity of 300 μmol m^{-2} s^{-1} PAR. Seven replications were performed over time.

Acid soil was gathered from a continuous wheat field in Garfield County, Oklahoma. The soil was sterilized and random samples were collected prior to planting to determine nutrient content and pH. Nitrogen (N), phosphorus (P), and potassium (K)

levels were sufficient, and no added fertilizer was needed. The mean pH was 4.5. Random samples were collected following plant growth to determine pH.

Chlorophyll Content

The same procedure was followed for this study as discussed in Chapter 1. Chlorophyll extractions were performed on frozen leaf tissue, as in the rust experiment.

Fluorescence and Gas Exchange Measurements

The same procedure was used for this experiment as discussed in Chapter 1. Measurements were performed four weeks after planting. Subsequent to measurements, leaf segments were collected and leaf area measured. There were seven replicates of gas-exchange measurements, but only four replications of fluorescence measurements due to equipment failure.

Tissue and Soil Analysis

Individual plants for this study were harvested at the end of measurements for determination of total dry weights and shoot analysis of Al, Ca, Mg, K, and P contents. Roots and shoots were dried in an oven at 39°C. Dry weights were recorded, and then samples were ground and passed through a 1mm screen. A minimum weight of two grams is required for tissue analysis. In many cases this was not available; therefore replicated samples of treatments containing less than that amount were combined. Samples were sent to the Soil, Water, and Forage Analytical Laboratory located at Oklahoma State University for analysis.

Experimental Design

There were six replications in time for gas exchange measurements and four for fluorescence. Each of the four lines were grown in the two soil treatments, nonlimed (acid) and limed. A radomized block design was used with 8 treatment units. Treatment units for the aluminum experiment were (i) aluminum tolerant NIL OK91G105 grown in acid soil, (ii) OK91G105 grown in limed soil, (iii) tolerant Atlas 66 grown in acid soil, (iv) Atlas 66grown in limed soil, (v) acid susceptible NIL OK91G108 grown in acid soil, (vi) OK91G108 grown in limed soil, (vii) susceptible. Century grown in acid soil, and (viii) Century grown in non-acid soil. Seeds were pre-germinated on moist filter paper in a petri dish two days prior to planting.

Statistical Analysis

The proc mixed procedure for analysis of variance in SAS (Statistical Analysis System, SAS Institute Inc., Cary, NC) was used for the seven replications of gas exchange data, and four replications of fluorescence data. Covariance structure was evaluated using variance components (VC), and the Satterthwaite method was used to determine degrees of freedom

RESULTS

Soil type did not affect A_{amb}, A_{max}, g_s, and the initial slope of the CO_2-response curve (Table 4). Lower slopes of the CO_2- response curves were found for Century

grown in nonlimed soil than in the limed soil. Acid soil treatments significantly lowered initial slopes of the light response curves of Atlas (Table 4). No other gas-exchange-related parameters (A_{amb},A_{max}, g_s, or slope of CO_2-response curves) were affected by acid soil.

Chlorophyll content was not affected by soil pH. Because of the manner in which this study was conducted there was no change in chlorophyll content, most probably due to the absence of drought stress on the wheat plants. If drought stress had been present, there may have been noticeable chlorosis and a much different response. As it was, chlorophyll content of both susceptible and tolerant lines did not decrease in the presence of acid soil.

There were no changes in photosynthesis or chlorophyll content, but there were differences in growth. All lines grown in nonlimed soil had less growth than the lines grown in limed soil (Tables 5 and 6).

Seven replications were measured, but only four replications were used to analyze fluorescence parameters due to equipment malfunction. The q_p values did not differ with soil pH (Table 4). The q_N and Y values were higher for Atlas 66 grown in acid soil the in the limed (Table 4). Fv/Fm was also unaffected by soil pH.

Random samples of the limed and nonlimed soil were collected and analyzed for N, P, K, and Al. Sampling was conducted after the plants had been harvested. There were no significant differences in the nutrient content of the soil after harvest (Table 8). The wheat plants had received adequate nutrients according to the pre-plant soil test (Table 7). Al saturation differed between the two treatments. The nonlimed soil had an average Al saturation of 14.4%, and only 9.0% for limed soil. The pH values of the

samples collected after the plants were harvested did not differ between the two soil treatments (Table 8).

Nutrients and Al content was also analyzed in dry shoot material. Samples were analyzed for N, Ca, P, and Al content. There were no differences in nutrient content in the shoots grown in unlimed and limed soil.

DISCUSSION

Gas Exchange and Chlorophyll Content

Photosynthesis was unaffected by aluminum (low pH) in susceptible and tolerant lines under the conditions used in this experiment. Chlorophyll content was also unaffected in acid and lime treatments.

There appeared to be no damage caused to the photosynthetic system by exposure to acid soil. However, the initial slope of the CO_2-response curves suggested that Century grown in acid soil had decreased carboxylation efficiency compared to those grown in limed soil (Table 4). Evidently it was not enough to lower photosynthetic rates at ambient CO_2. When photosynthetic parameters were expressed per unit chlorophyll (mmol CO_2 mol^{-1} Chl s^{-1}) there were no significant differences in either soil treatment of any of the four lines tested. Quantum use efficiency was also decreased in Atlas 66 grown in limed soil, indicated that captured light was used more efficiently in more acidic soil conditions.

The aim in using acidic field soil in this study rather than nutrient solution was to examine plants in a growth medium most similar to field conditions. Detrimental effects of aluminum are most evident from pre-emergence to the seedling stage. Four-week-old plants were studied, yet the measured leaf area in the leaf chamber at this stage was still very small, often times less than 3.0 cm^2. Plants were grown in a growth chamber with ideal temperature, relative humidity, and water. Growing the wheat plants in this environment might have removed compounding factors found in a field setting. Drought and temperature stress are normally present in the field and may be necessary to produce

the usual field symptoms. Individuals grown in acid soil in this study were smaller than those grown in limed soil. Chlorosis did not occur in any of the acid treatments in this study, perhaps because no drought stress was present.

Chlorophyll Fluorescence

Atlas 66 plants grown in acid soil was the only line that experienced changes in fluorescence parameters. Nonphotochemical quenching (q_N) was higher than in the limed treatment. The fluorescence yield of PS II (Y) was also considerably higher in the acid treatment than the limed.

No changes in the kinetics of the fast and slow phases of the induction curves were noted. This suggests that the photochemistry of PS II was unaffected by presence of aluminum.

Soil Analysis

Random samples of the limed and nonlimed soil were gathered and analyzed for N, P, K, and Al content. The soil was gathered and analyzed after the plants had been harvested. Nitrogen (NO_3-N) levels were lower for nonlimed (acidic) soil than for limed soil. P and K values remained the same for both soil treatments. Adequate nutrients were available to the plants according to preplant soil tests (Table 7).

Aluminum poses a threat to wheat when the saturation level surpasses 15%. The saturation level is simply the Al content divided by the total amount of nutrients. The average Al saturation found in nonlimed soil was not as high as expected. Average Al

saturation in nonlimed soil was 14.4%, and 9% for limed soils. The difference in Al saturation levels inevitably had an impact on plant growth.

Unexpectedly, the pH values of the soil that had sustained the wheat plants for four weeks did not vary between nonlimed and limed treatments, even though it had differed prior to planting. We cannot explain how this happened. Dolomitic lime was applied two week prior to planting. Wheat seeds were planted in limed soil once the pH remained above 5.5. The pH values of the nonlimed soil were about 4.5 prior to planting (Table 7). There was not as great a difference in pH values of the two soil treatments as desired. This may have affected the results of the experiment, but at this point it is not certain to what extent. However, we followed the customary practice to collect soil from the field at the time of the year when it is not supporting a crop. In light of this we chose to rely on the soil test performed prior to harvest. The nutrient content was the same, but the pH differed (nonlimed pH = 4.7, limed $\geq$ 5.5).

Plant Analysis

There were visible differences in plant size. All lines produced highly significant differences in terms of shoots dry weight (Table 5). Plants grown in nonlimed soil, regardless of susceptibility to Al, produced less photosynthetic area. The means of the shoot dry weights (Table 6) showed that OK91G108 produced the least amount of leaves, and Atlas 66 produced the most. Photosynthesis and the amount of chlorophyll content did not differ between soil treatments, but plant growth did. The amount of nutrients found in the shoots did not differ by soil treatment (Table 9). The amount of nitrogen left

in the soil postharvest was greater in the limed soil than the nonlimed (Table 8). The retarded plant growth may have been due to an inefficiency of nutrient usage.

Acid soil had no effect on the photosynthetic rates of either tolerant or susceptible wheat lines used in this study. Carboxylation efficiency was lower for Century grown in acid than limed soil, but it was not enough to create lower gas exchange rates under ambient conditions. Fluorescence parameters did not vary by soil treatment for OK91G105 and OK91G108. However, Atlas seemed to perform better photochemically in the unlimed soil, but gas exchange rates remained the same for both soil treatments.

LITERATURE CITED

Brady, N.C. and R.R. Weil. 1996. The Nature and Properties of Soil. 11th Edition. Prentice Hall. Upper Saddle River, New Jersey.

Carver, B.F. and J.D. Ownby. 1995. Acid soil tolerance in wheat. *In* Adv. Agron. 54:117-173.

Carver, B.F., G.V. Johnson, W.R. Raun and E.G. Krenzer. 1998. Increasing acidification of U.S. Southern Great Plains. In Proceedings of a workshop to develop a strategy for collaberative research and dissimination of technology in sustainable crop production in acid savannas and other problem soils of the world. May 4-6, 1998. E. Shaffer (ed.). Purdue Univ.

Delhaize, E., P.R. Ryan and P.J. Randell. 1993. Aluminum tolerance in wheat (*Triticum aestivum* L.) II. Aluminum-stimulated excretion of malic acid from root apices. Plant Physiol. 103: 695-702.

Foy, C.D., H.N. Laever, J.W. Schwartz and A.L. Fleming. 1974. Aluminum tolerance of wheat cultivars related to the region of origin. Agron. J. 66:751-758.

Henderson, M. and J.D. Ownby. 1991. The role of root cap mucilage secretion in aluminum tolerance in wheat. Curr. Top. Plant Biochem. Physiol. 10:134-141.

Lidon, F.C., M.G. Barreiro, J.C. Ramalho and J.A. Lauriano. 1999. Effects of aluminum toxicity on nutrient accumulation in maize roots: implications on phosynthesis. J. Plant Nutr. 22 (2): 397-416.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. Second Edition. Academic Press, Inc. San Diego, CA.

Miyasaka, S.C., J.G. Buta, R.K. Howell and C.D. Foy. 1991. Mechanism of aluminum tolerance in snapbeans: Root exudation of citric acid. Plant Physiol. 96: 737-743.

Moustakas, M., G. Ouzounidou and R. Lannoye. 1995. Aluminum effects of photosynthesis and elemental uptake in an aluminum-tolerant and non-tolerant wheat cultivar. J. Plant Nutr. 18 (4): 669-683.

Ohki, K. 1986. Photosynthesis, chlorophyll, and transpiration responses in aluminum stressed wheat and sorghum. Crop Sci. 26: 572-757.

Rengel, Z. 1997. Mechanisms of plant resistance to toxicity of aluminum and heavy metals. *In* Mechanisms of environmental stress resistance in plants. Amarjit S. Basts and Ranjit K. Basra (eds). Harwood Academic Publishers, Netherlands.

Srivastava, P.C. and U.C. Gupta. 1996. Trace Elements in Crop Science. Science Publishers, Inc. Lebanon, New Hampshire.

Tang, Y., B.F. Carver, M.E. Sorrells, L.V. (in press). Kochian and D.F. Garvin.Physiological genetics of aluminum tolerance in wheat cultivar Atlas 66. Crop Sci.

Zhang, G. and G.J. Taylor. 1988. Effect of aluminum on growth and distribution of aluminum in tolerant and sensitive cultivars of *Triticum aestivum* L. Commun. Soil Sci. Plant Anal. 19:1195-1205.

Zhang, H., G. Johnson, G. Krenzer and R. Gribble. 1998. Soil testing for an economically and environmentally sound wheat production. Commun. Soil. Sci. Plant Anal. 29 (11-14) 1708-1717.

Table 1. Statistical summary for photosynthetic parameters as affected over time

	Thatcher Infected	*Thatcher Control*	*Thatcher Lr19 Infected*	*Thatcher Lr19 Control*
A_{amb} (μmol m^{-2} s^{-1})	< 0.001**	.885	.034*	.599
A_{max} (μmol m^{-2} s^{-1})	< 0.001**	.496	.015*	.600
g_s (mol m^{-2} s^{-1})	.379	.610	.138	.587
Slope CO_2	.093	.219	.534	.886
Slope Light	.004**	.507	.815	.410
q_P	.005**	.107	.391	.491
q_N	.001**	.885	.174	.400
Y	.005**	.718	.567	.584
Fv/Fm	.995	.008	.100	1.00
Chl_{tot} (μmol m^{-2})	< 0.001**	.007**	.175	.438
A_{amb}/ Chl_{tot} (mmol mol^{-1} Chl s^{-1})	.004**	.045*	.020*	.660
A_{max} /Chl_{tot} (mmol mol^{-1} Chl s^{-1}l)	.113	.170	.002**	.886

* = $p < 0.05$
** = $p < 0.01$

Table 2. Statistical summary by day for gas exchange of Thatcher and Thatcher Lr19 treatments

	Thatcher							*Thatcher Lr19*						
Day	*0*	*2*	*4*	*6*	*8*	*10*	*12*	*0*	*2*	*4*	*6*	*8*	*10*	*12*
A_{amb}	.398	.868	.399	.000**	.000**	.000**	.000**	.857	.934	.497	.506	.560	.004**	.131
A_{max}	.082	.434	.067	.009**	.000**	.000**	.000**	.786	.807	.533	.189	.966	.047*	.003**
Chl_{tot}	.434	.395	.023*	.000**	.001**	.000**	.000**	.704	.959	.079	.269	.194	.274	.908
A_{amb}/Chl	.443	.445	.548	.045*	.001**	.014*	.000**	.856	.904	.406	.077	.492	.066	.176
A_{max}/Chl_{tot}	.130	.210	.615	.672	.002**	.007**	.014*	.941	.733	.654	.460	.264	.134	.002**

* = p < .05
** = p < .01

Table 3. Statistical summary by day for Thatcher and Thatcher Lr19for rust treatments

	Thatcher							*Thatcher Lr19*						
Day	*0*	*2*	*4*	*6*	*8*	*10*	*12*	*0*	*2*	*4*	*6*	*8*	*10*	*12*
q_P	.697	.363	.478	.339	.002**	.012*	.020*	.534	.568	.865	.683	.966	.020*	.178
q_N	.323	.831	.574	.097	.004**	.001**	.002**	.812	.827	.868	.570	.944	.005**	.703
NPQ	.267	.539	.515	.235	.001**	.006**	.019*	.812	.827	.868	.568	.944	.005**	.699
Y	.323	.831	.574	.097	.004**	.001**	.002**	.379	.835	.811	.874	.800	.073	.523
F_v/F_m	.935	.928	.001**	.827	.558	.587	.951	.941	.846	.760	.952	.632	.905	.816

*= $p<0.05$ **= $p<0.01$

Table 4. Statistical summary of the response of the photosynthetic parameters to acid soil

	LINE			
Parameter	*OK91G105-T*	*OK91G108-S*	*Atlas 66*	*Century*
A_{amb}	.670	.727	.432	.983
A_{max}	.094	.884	.235	.737
g_s	.125	.793	.251	.303
slope(CO_2)	.878	.757	.928	.049*
slope (light)	.328	.627	.005**	.369
q_P	.167	.587	.116	.267
q_N	.650	.491	.002**	.503
Y	.782	.259	.049*	.240
Chl_{tot}	.991	.485	.095	.769

*=p<0.05
**=p<0.01

Table 5. Statistical summary of the response of the four different lines to acid soil in regards to dry weights of shoot and roots

	OK91G105-T	*OK91G108-S*	*Atlas 66*	*Century*
Shoot	0.0002^{**}	0.0001^{**}	0.0008^{**}	0.0002^{**}
Root	0.0178^{*}	0.0041^{**}	0.0330^{*}	0.0120^{*}

$^{*}= p<0.05$ $^{**}= p<0.01$

Table 6. Means and stand errors for shoot dry weights

	Shoot weights (g)			
	OK91G105-T	*OK91G108-S*	*Atlas*	*Century*
Nonlimed Soil	.65 ± .09	.38 ± .05	.89 ± .25	.65 ± .19
Limed Soil	1.60 ± .19	1.74 ± .23	1.35 ± .25	1.46 ± .23

Table 7. Soil nutrient and pH status prior to planting

PH	*NO_3-N* (lbs/acre)	*P* (lbs/acre)	*K*
4.7 ± .04	54 ± 1.6	112 ± 2.2	345 ± 5.0

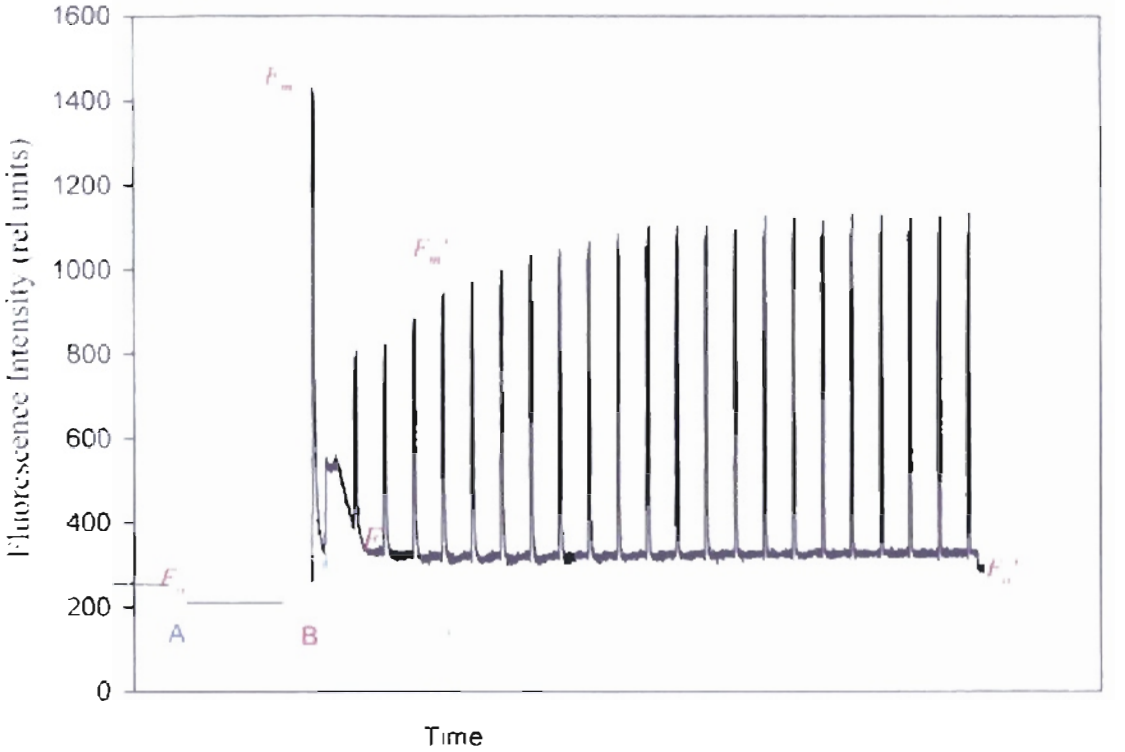
Table 8. pH, soil nutrient, and aluminum status of soil (post-harvest)

	pH	*NO_3-N* (lbs/acre)	*P* (lbs/acre)	*K* (lbs/acre)	*Al sat. (%)*
Nonlimed	5.3 ± 0.1	6.3 ± 2.2	97.7 ± 3.5	295.0 ± 36.4	14.4 ± 1.7
Limed	5.2 ± 0.1	12.7 ± 1.3	105.0 ± 1.7	306.7 ± 19.1	8.9 ± 0.6

Table 9. Means and standard errors of nutrient and al content in shoots

	Al	*Ca*	*P*	*N*
Nonlimed				
105	53.0 ± 17.2	2997 ± 93	3427 ± 343	4.38 ± .24
108	48.0 ± 3.0	2723 ± 214	3823 ± 56	4.29 ± .16
Atlas	40.3 ± 13.2	2317 ± 263	4086 ± 405	4.83 ± .08
Century	35.5 ± 2.5	2532 ± 289	4123 ± 76	4.64 ± .10
Limed				
105	45.7 ± 6.9	4141 ± 477	4329 ± 579	4.43 ± .35
108	39.1 ± 4.9	3458 ± 466	3908 ± 342	4.26 ± .21
Atlas	36.7 ± 5.8	3357 ± 226	3775 ± 248	4.08 ± .47
Century	30.7 ± 3.3	4219 ± 567	4402 ± 614	4.89 ± .08

Figure 1. Induction curve with modulated saturating pulses.



A: Fluorescence level of dark-adapted plant, with reaction centers 'open' (Q_A fully oxidized).

B: Application of saturation pulse

Series of saturating pulses were applied to determine the changes in the quenching coefficients of the sample.

Point at which actinic light was turned on

Equations used to calculate fluorescence parameters:

$F_v = F_m - F_o$ $\qquad q_P = (F'_m - F_s)(F'_m - F_o)^{-1}$

$F_v/F_m = (F_m - F_o)F_m^{-1}$ $\qquad q_N = (F_m - F'_m)(F_m - F_o)^{-1}$

$Y = (F'_m - F_s)F'^{-1}_m$

Figure 2. Changes in ambient photosynthesis (A_{amb}) 2-12 days after leaf rust infection for uninoculated and inoculated Thatcher and Thatcher Lr19

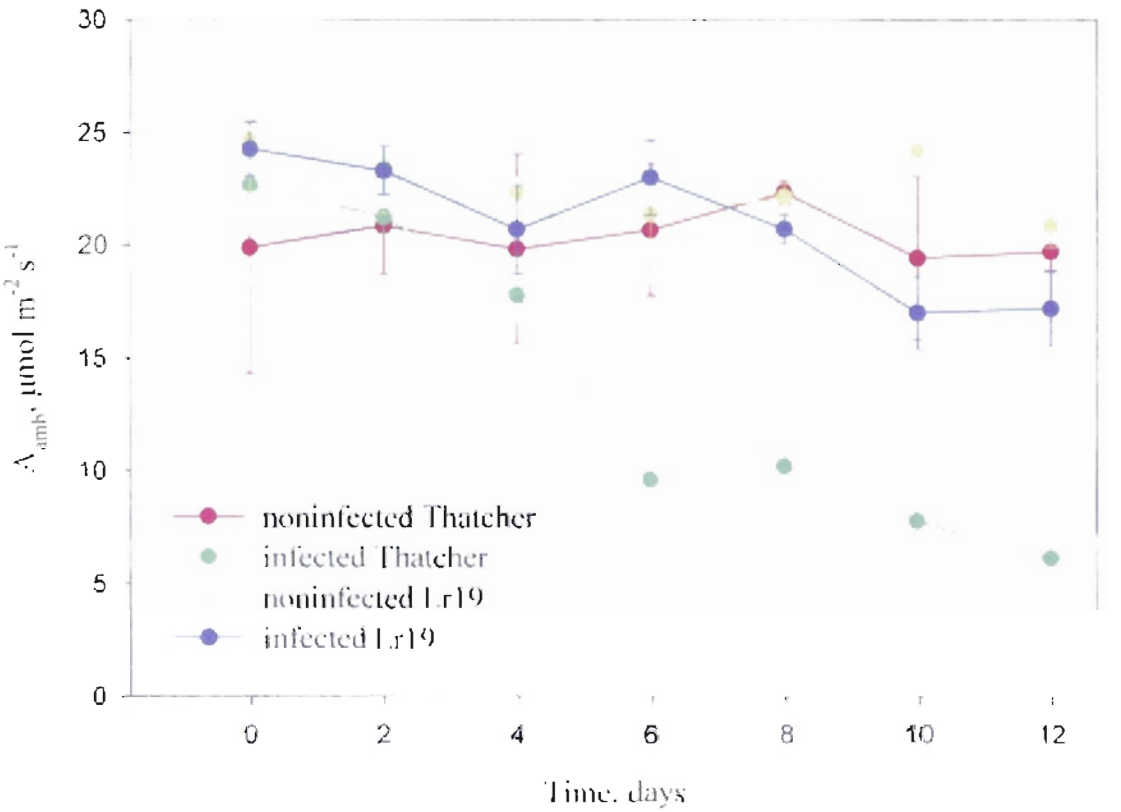


Figure 3. Means and standard errors of CO2-response curves of Thatcher infected and noninfected plants for days 0, 6, and 12

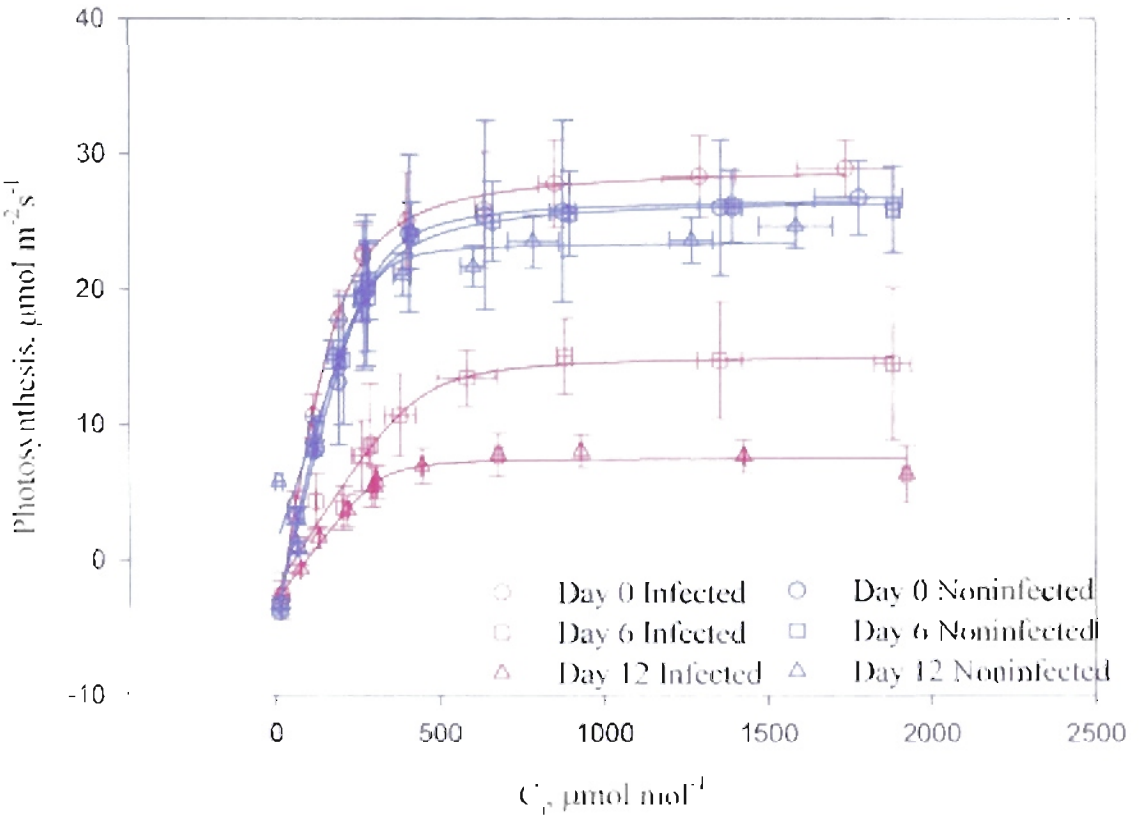


Figure 4. Means and standard errors of CO_2-response curves of Thatcher Lr19 infected and noninfected plants for days 0, 6, and 12

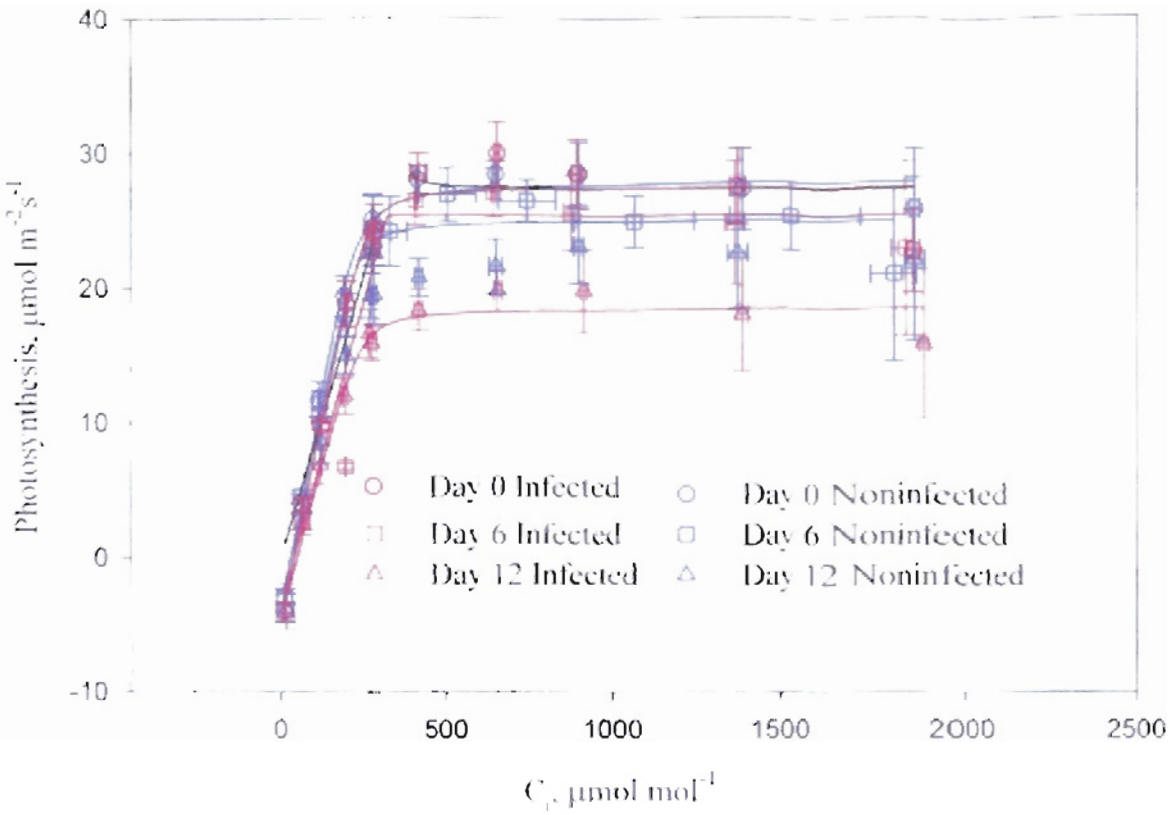


Figure 5. Means and standard errors of light response curves of Thatcher infected and noninfected plants for days 0, 6, and 12

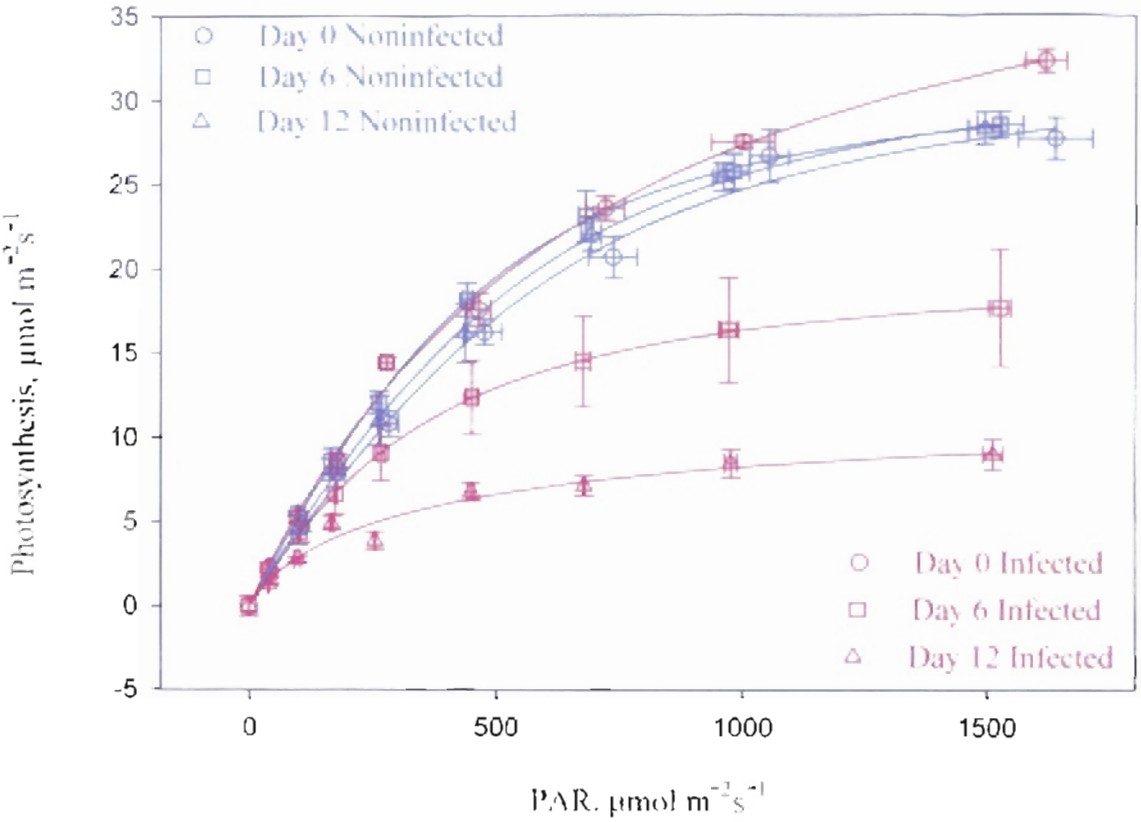


Figure 6. Means and standard errors of light response curves of Thatcher Lr19 infected and noninfected plants for days 0, 6, and 12

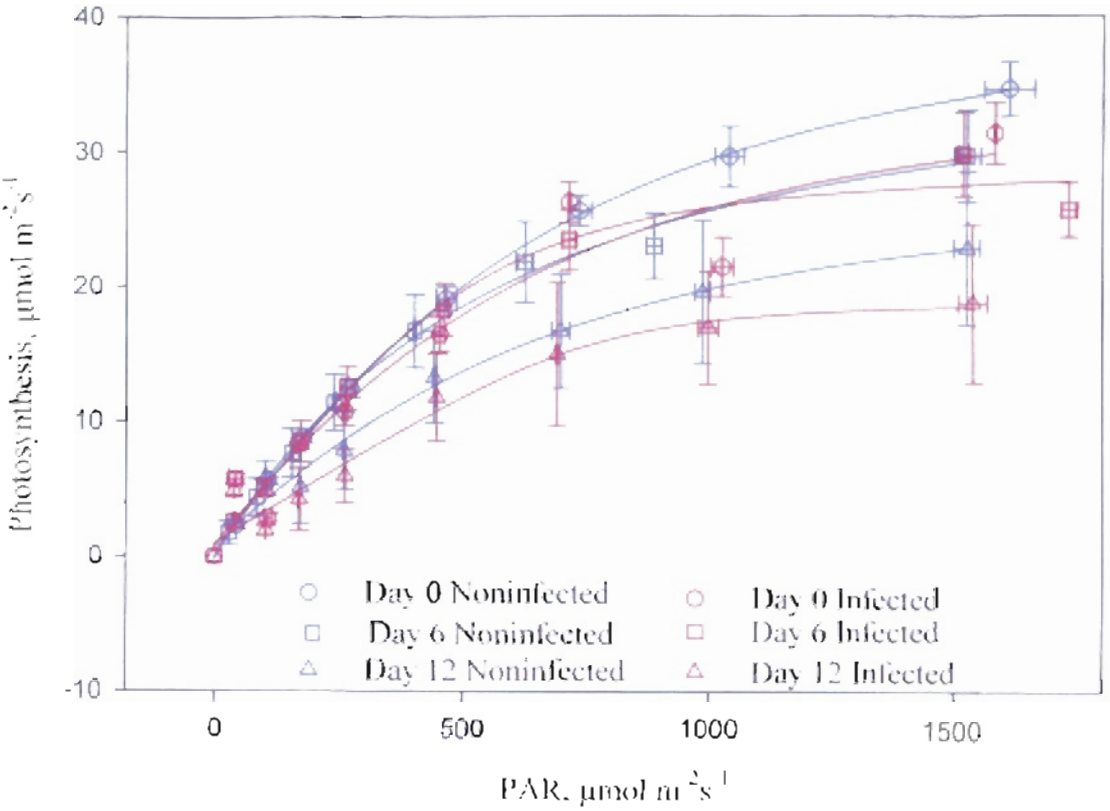


Figure 7. Changes in the photosynthetic rate at saturating PAR value(A_{max}) for Thatcher and Thatcher Lr19 across days 2-12 after leaf rust infection

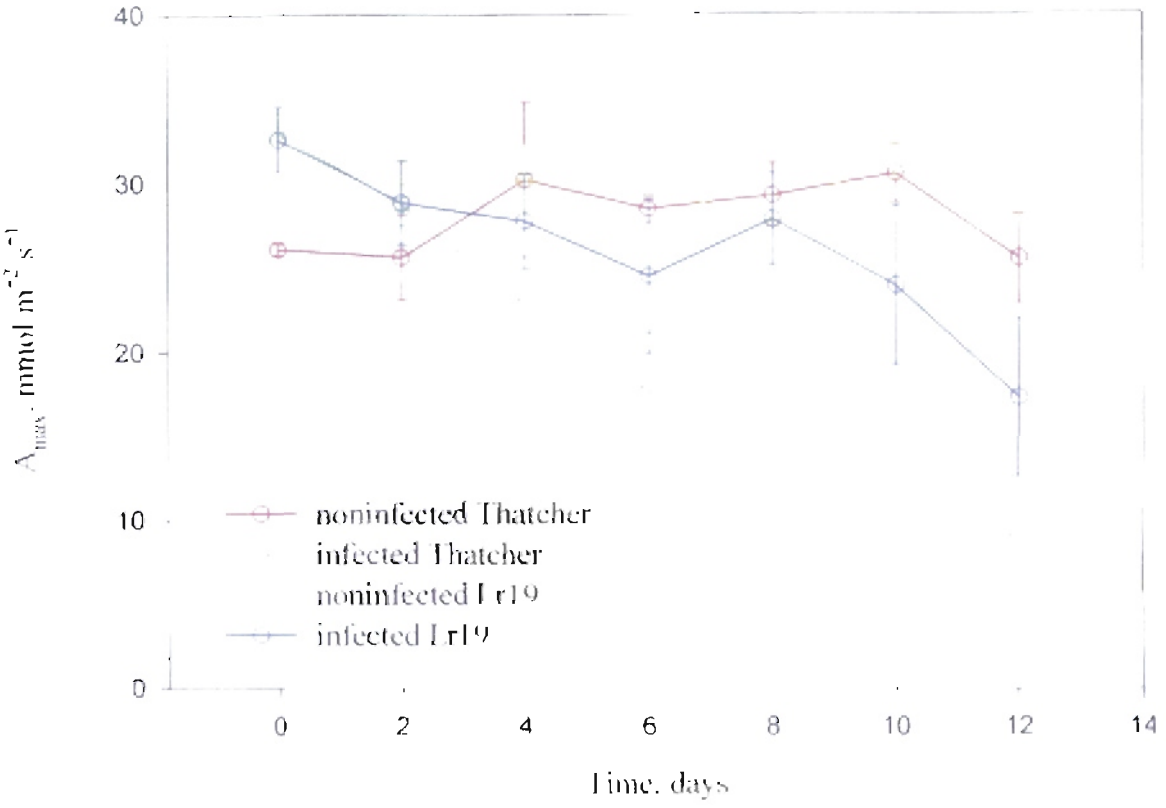


Figure 8. The changes in photochemical quenching (qp) from days 2-12 after leaf rust infection for noninfected and infected Thatcher and Thatcher Lr19

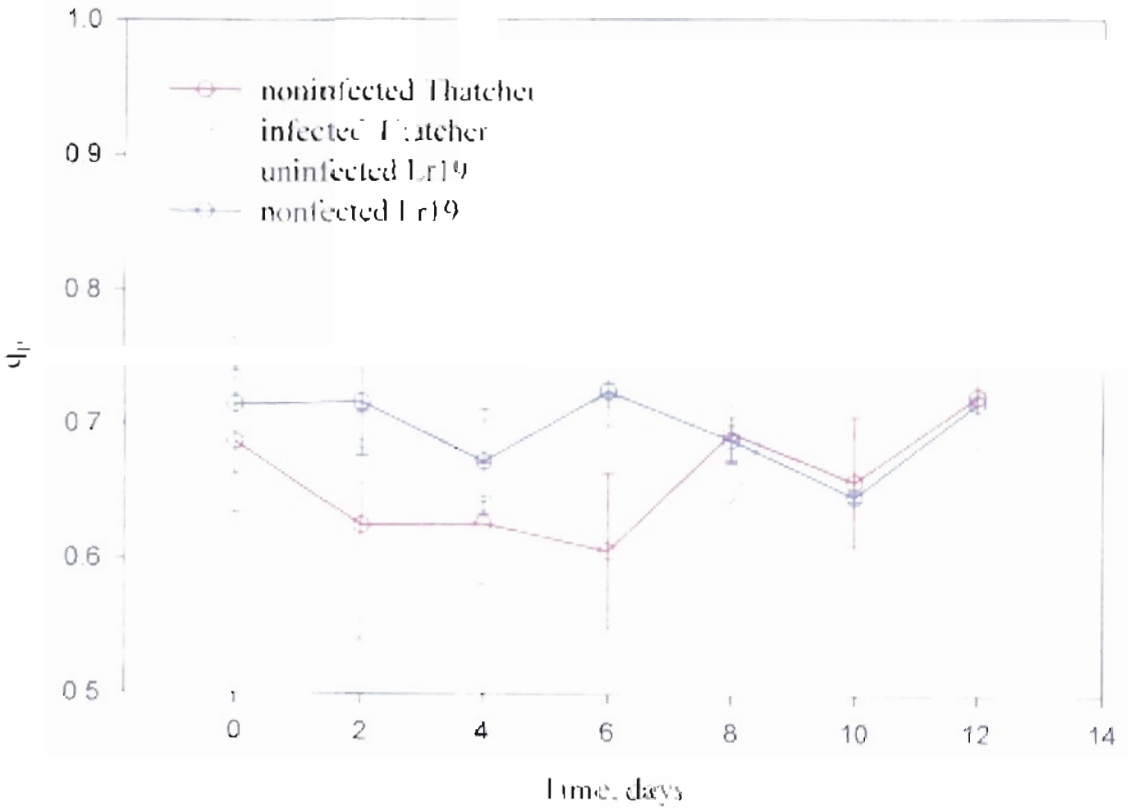


Figure 9. The changes in nonphotochemical quenching (q_N) 2-12 days after leaf rust infection for noninfected and infected Thatcher and Thatcher Lr19

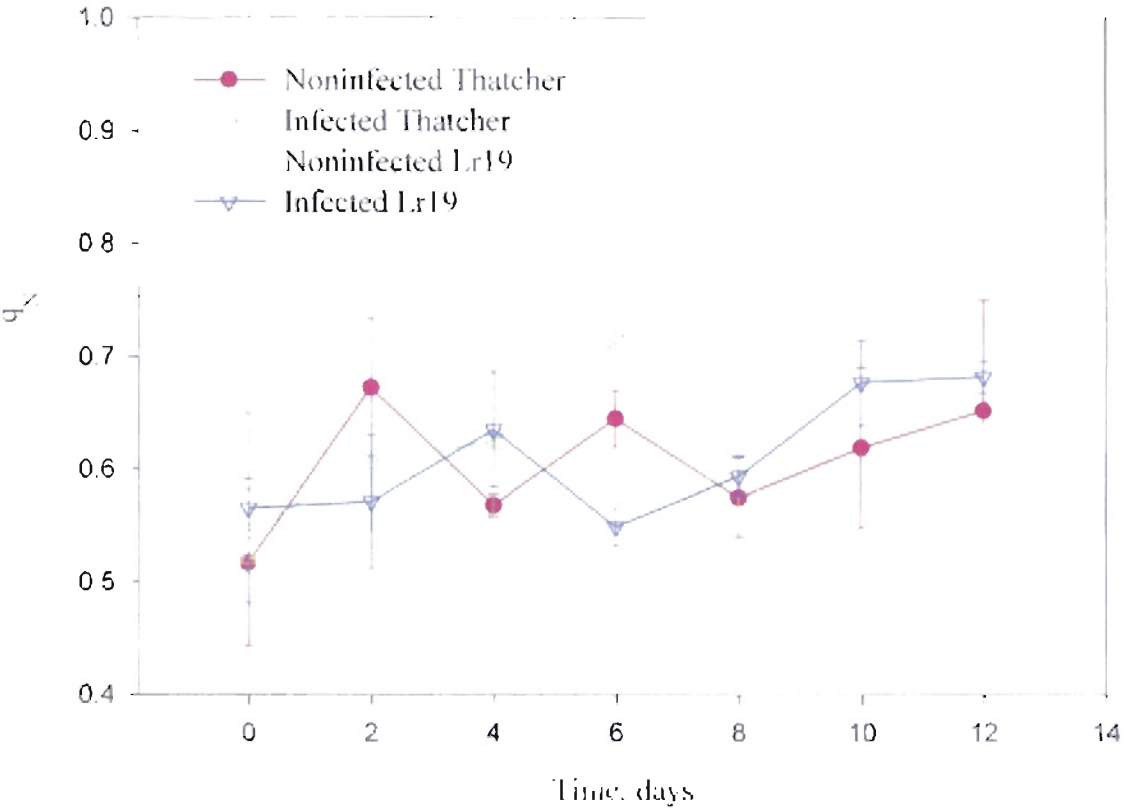


Figure 10. Changes in effective quantum yield (Y) days 2-12 after leaf rust infection in noninfected and infected Thatcher and Thatcher Lr19

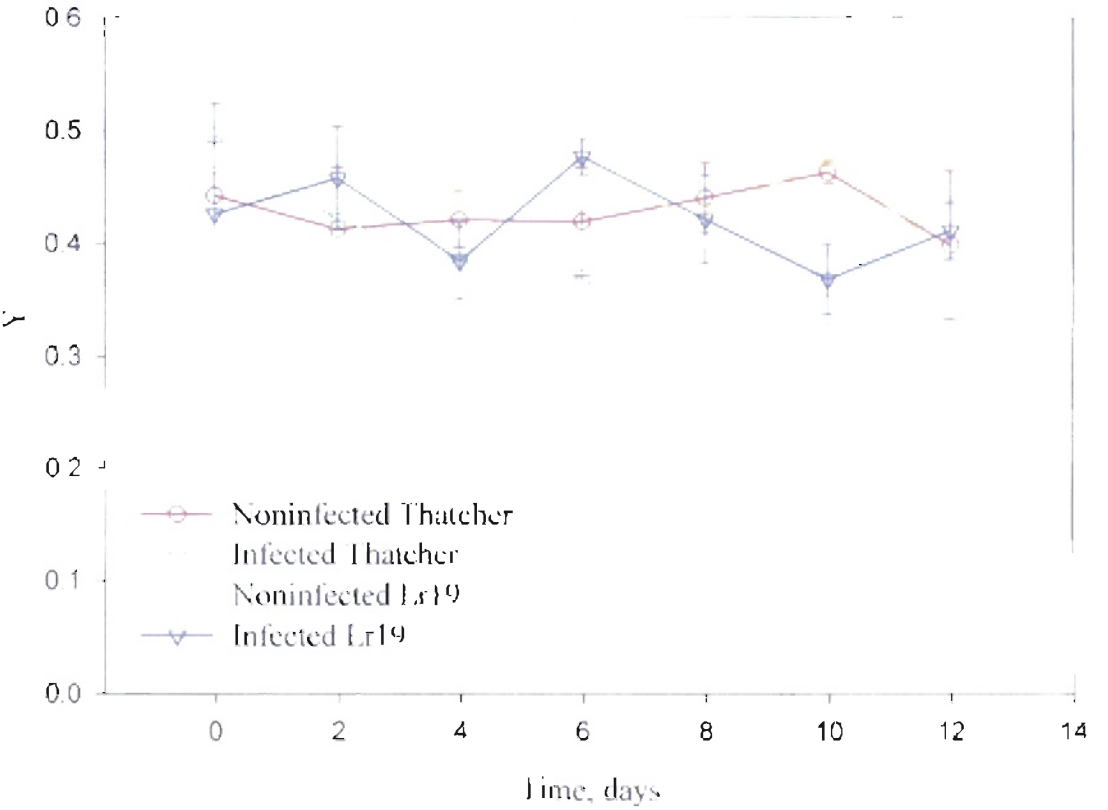


Figure 11. The changes in chlorophyll content (Chl_{tot}) over time for uninoculated and inoculated Thatcher and Thatcher Lr19.

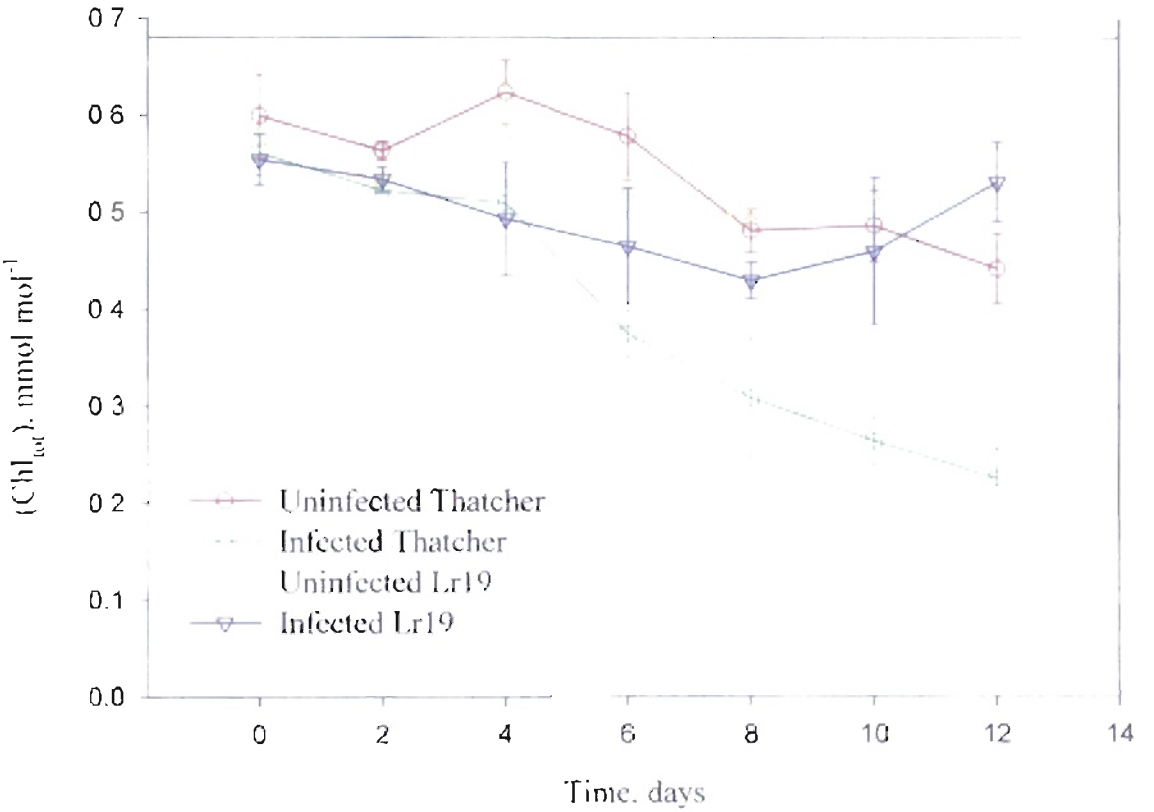


Figure 12. Means and standard errors of CO_2- response of OK91G105 and OK91G108 grown in nonlimed and limed soil treatments

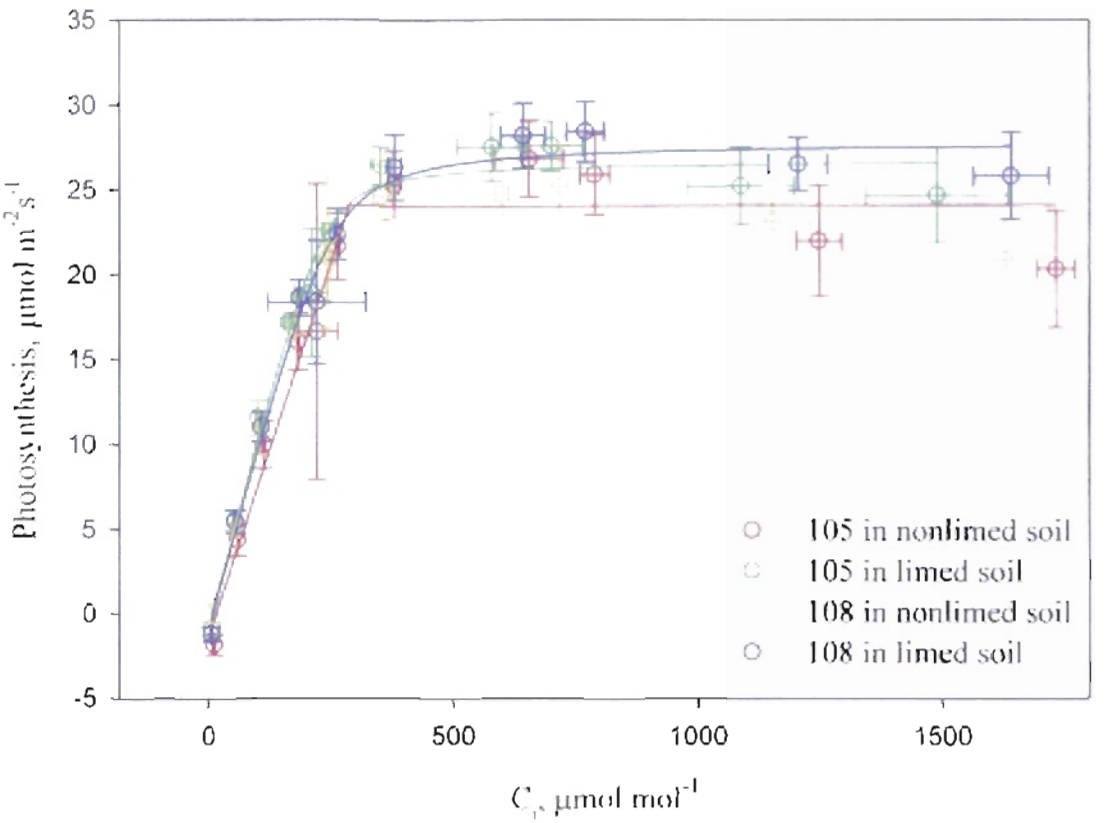


Figure 13. Means and standard errors of CO_2- response of Atlas 66 and Century grown in nonlimed and limed soil treatments

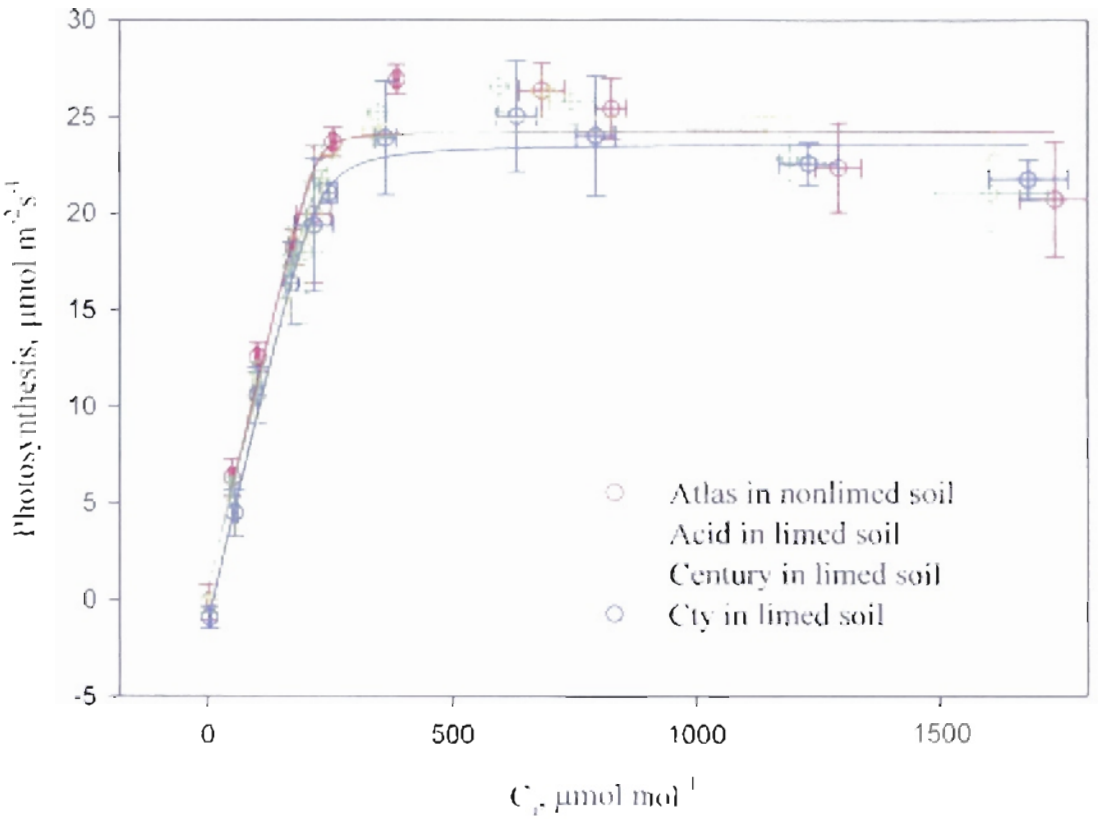


Figure 14. Means and standard errors of light response curves of OK91G105 and OK91G108 in nonlimed and limed soil treatments

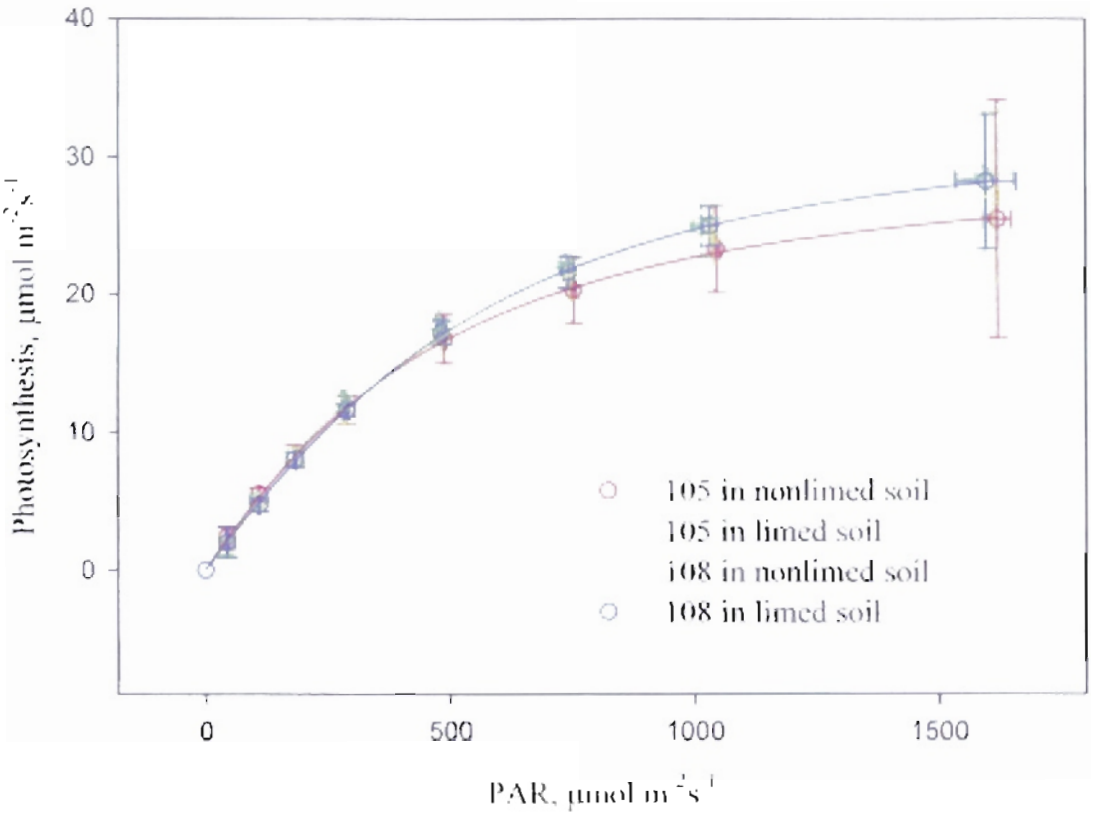
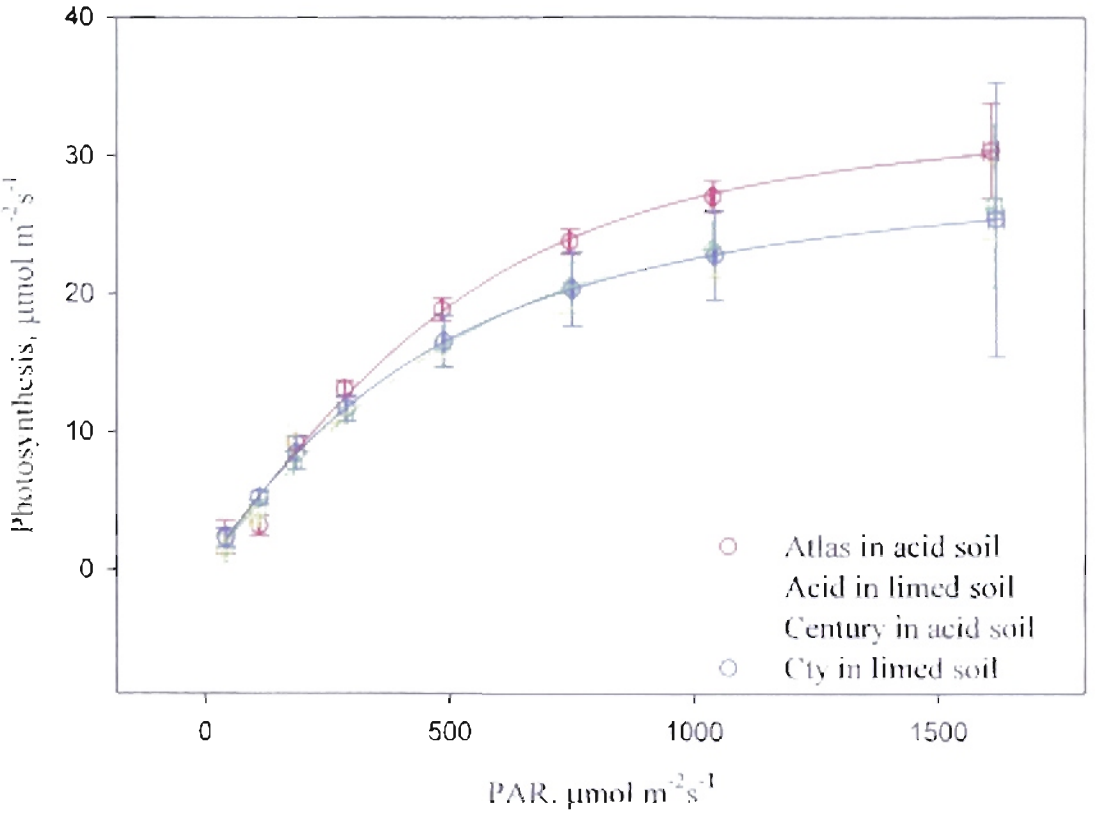


Figure 15. Means and standard errors of light response curves of Atlas 66 and Century in nonlimed and limed soil treatments



VITA

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