AN ABSTRACT OF THE THESIS OF

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Title: Rhizosphere Nitrogen Fixation ($C_2H_2$ Reduction) Associated with the Major Species of the Tall Grass Prairie

Abstract Approved: James M Mayo

Three aspects of rhizophere nitrogen fixation ($C_2H_2$ reduction) in the tall grass prairie were investigated in this study. First, a survey was done to determine how many plant species have nitrogen fixing ability associated with their roots. Over 40 species from the POACEAE, FABACEAE and ASTERACEAE were tested. More than 30 of those species gave a positive response. Second, the effect of moisture stress on rhizosphere nitrogen fixation was studied. Xylem Pressure Potential (XPP) and rhizosphere nitrogen fixation were measured through the summer for Andropogon gerardi, A. scoparius, Sorghastrum nutans, and Panicum virgatum. As XPP decreased, the rate of nitrogen fixation also decreased. This may be a response to decreased photosynthesis or an increased deactivition of nitrogenase from exposure to oxygen. Third, the response of rhizosphere nitrogen fixation to spring burning was measured. Adjacent burned and unburned sites were chosen for this study. The rate of nitrogen fixation in the burned prairie was consistently higher than in the unburned prairie for all four of the major tall grass species.

# RHIZOSPHERE NITROGEN FIXATION ($C_2H_2$ REDUCTION) ASSOCIATED WITH THE MAJOR SPECIES OF THE TALL GRASS PRAIRIE

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# INTRODUCTION

The tall grass prairie has long been noted for its great productivity of forage grasses. This indicates that large amounts of nitrogen must be available to the plants (Risser et al. 1981). Even though the atmosphere is comprised largely of nitrogen gas, it is unavailable for use directly by plants. In order for plants to utilize nitrogen, it must be in a combined form as either $NH_4^+$ or $NO_3^-$. Although a good nitrogen budget for the tall grass prairie does not exist, some of the inputs of available nitrogen are known. There are two major pathways for converting unavailable $N_2$ gas into nitrogen compounds that can be used by plants. One of these pathways is the conversion of $N_2$ to $NO_3^-$ by lighting. This $NO_3^-$ is transferred from the atmosphere to the soil by precipitation or dryfall. The second pathway is through biological nitrogen fixation. The process of biological nitrogen fixation can be divided into three distinct types; symbiotic nitrogen fixation, non-symbiotic nitrogen fixation, and nitrogen fixation by free living bacteria associated with the rhizosphere of higher plants.

Symbiotic nitrogen fixation is that fixation occurring in bacteroid nodules within the roots of certain species of plants. The occurrence of bacteroid nodules is common among species of the legume family (FABACEAE), and has also been found in several other families. The amounts of combined nitrogen entering the ecosystem through biological nitrogen fixation has not been fully quantified. With bacteroid nitrogen fixation, the difficulty in quantification is due to the scarcity of nodules (Risser et al. 1981) and the rooting depth of some of the prairie legumes. Lead plant (Amorpha canescens), for example, may root to a depth of five meters (Weaver, 1954).

Nonsymbiotic nitrogen fixation is associated with the Cyanobacteria (blue-green algae) and some free living bacteria. With the development of the $15_N$ and acetylene reduction assays, several free living microorganisms once reported as having nitrogen fixing ability were found to be unable to fix nitrogen. Among them are some of the yeasts and fungi (Mulder, 1975). Only prokaryotic microorganisms have been proven to have the ability to fix nitrogen. Nitrogen fixation by cyanobacteria may be important to the prairie ecosystem, but the amounts of nitrogen fixed are generally small. In the Canadian prairie, Alexander (1979) determined the input of nitrogen from cyanobacteria to be 1-2 Kg $ha^{-1}yr^{-1}$. The cyanobacteria Nostoc occurs in the tall grass prairie as an algal crust on the soil surface.

The major emphasis of study in nonsymbiotic nitrogen fixation has involved free living bacteria. The first free living, nitrogen fixing bacteria to be isolated was Clostridium pasteurianum by Winogradsky in 1893. The free living, nitrogen fixing bacteria belong to only a few families and genera. They include: (a) the aerobic azotobacters; (b) the facultative anaerobic klebsiellas; (c) the facultative anaerobic bacilli of the Bacillus polymyxa and B. macerans group; (d) most of the saccharolytic clostrida; (e) the anaerobic sulfate-reducing bacteria of the genera Desulphovibria and Desulphomaculum; (f) the photosynthetic bacteria (Mulder, 1975).

In recent years there has been considerable interest in the association of some of these free living, nitrogen fixing bacteria with the rhizosphere of higher plants, particularly the grasses. This association is due to a zone of enrichment around plant roots. Plant roots exude a variety of compounds into the surrounding soil. Among the compounds

found in root exudates are sugars, amino acids, peptides, enzymes, vitamins, organic acids, nucleotides, and many other miscellaneous compounds (Rovira, 1969). According to Mulder (1975), if the amount of combined nitrogen in the soil is low and the amount of combined carbon is high, the presence of free living, nitrogen fixing bacteria will be favored over those microorganisms not capable of nitrogen fixation.

The best known association of this type is that of the tropical grass Paspalum notatum with the bacteria Azotobacter paspali (Döbereiner, 1970). Similar associations have been found in temperate grasses. Tjepkema and Burris (1976) reported finding nitrogen fixation associated with the roots of Andropogon gerardi, A. scoparius, Spartina pectinata, Stipa spartea, Poa pratensis, Sprobolus heterolepsis, and Panicum virgatum in Wisconsin. Weaver et al. (1980) found associative nitrogen fixation in Cyndon dactylon, Paspalum dilatum, P. notatum, P. urvillei, P. plicatalum, Axonopus affnis, Andropogon gerardi, and A. scoparius in Texas. Associative nitrogen fixation has also been reported for Scripus atrovirens in Massachusetts (Kana and Tjepkema, 1978), Oryzopsis hymenoides in Colorado (Wullstein, 1980) and many other native grasses and forbs. Some agricultural species have also been reported to fix nitrogen. Among them are certain varieties of wheat (Neal and Larson, 1976), corn (Raju, Evans, and Seidler, 1972), and rice (Trolldenier, 1977).

This research had three major objectives. The first objective was to survey a broad range of prairie grasses and forbs for nitrogen fixing ability. There have been no reports of rhizosphere nitrogen by prairie plants in Kansas and more specifically in the Flint Hills region of Kansas. The aim of this survey was to confirm that species reported to

have nitrogen fixing ability in other geographic regions also have that ability in the Flint Hills. This survey also contains species that have not been previously tested for nitrogen fixing ability.

The second objective was to determine the effect of spring burning on rhizosphere nitrogen fixation associated with the major tall grass species. The tall grass prairie is not a true climatic climax community, but is a fire maintained sub-climax community. In the absence of fire, the grassland is quickly invaded by cool-season grasses, woody shrubs and trees. Historically, the prairie was burned periodically by fires started by lightning or by the Plains Indians. Fire was used by these early men to attract large herbivores, bison and elk, in order to hunt them. Spring burning is now used to control woody vegetation, to increase forage production and quality, and to improve distribution of use by cattle. These range practices result in increased cattle gains (Owensby and Smith, 1973). Figure 1 illustrates the difference in appearance between burned and unburned prairie in the spring.

Hulbert (Pers. Comm. 1982) found that applying nitrogen fertilizer increased flower stalk and leaf productivity in the major tall grass species. This indicates that increases in soil nitrogen following burning could be responsible in part for the increase in forage production. This increase in nitrogen may be due to an increase in rhizosphere nitrogen fixation. It should be noted, however, that fertilizing native tall grass prairie is not an accepted practice.

The third objective of this study was to measure the effect of moisture stress on rhizosphere nitrogen fixation. Moisture stress is a common environmental factor affecting the prairie ecosystem. Adequate moisture is not only essential for plant growth, but has also been

Figure 1. Tallgrass prairie in spring showing difference between burned (foreground) and unburned (backbround) sites.

shown to have an influence on rhizosphere nitrogen fixation (Vlassek, Paul, and Harris, 1973). Moisture stress in that study as well as in other similar studies was measured as percent soil moisture content. Measuring soil moisture content does not accurately reflect moisture stress. Since water moves from an area of high energy to an area of low energy, not from high concentration to low concentration, a better measure of moisture stress would be to measure the free energy of water. The free energy of water is referred to as water potential ($\psi$). Percent soil moisture is also a poor measure of moisture stress because soils exhibit hysteresis. This is especially true of the clay soils common in the Flint Hills. When comparing percent soil moisture to $\psi$, the soil does not wet and dry on the same curve. Instead, it exhibits a series of curves. Thus, one measure of soil moisture content could represent more than one $\psi$, resulting in large errors.

In this study, moisture stress was measured as the $\psi_\rho$ in the xylem of the plants. In the xylem:

$$\psi = \psi_\rho + \psi_\pi$$

where $\psi_\rho$ is the pressure potential and $\psi_\pi$ is the osmotic potential. Since $\psi_\pi$ is negligible in the xylem:

$$\psi = \psi_\rho$$

$\psi_\rho$ can be determined by measuring the Xylem Pressure Potential (XPP).

# MATERIALS AND METHODS

## Study Areas

This research was done at three study areas. The majority of the research was done at the Konza Prairie Research Natural Area near Manhattan, KS. The Konza Prairie covers approximately 3500 ha, most of which is native, unplowed tall grass prairie. The area also includes small areas of farmland and riparian woods. Management of the area is designed to approximate the tall grass prairie as it was before settlement by European man.

The Konza Prairie is divided into four replicates of a number of burning treatments. Those treatments are: burning after one, two, four, and ten years; burning after a wet year; burning after a dry year; and unburned. The treatment areas are divided such that each area is a small, but complete watershed. These burning treatments offered an excellent opportunity for sampling different burn treatments that have similar soils and are of the same range type.

The F. B. and Rena G. Ross Natural History Reservation (RNHR) near Emporia, KS, was also used as a study area. The Ross Reservation offered not only burn treatments, but also allowed for sampling of a grazed and burned area and an adjacent area that was unburned and ungrazed. The Ross Reservation was also used to sample plants for the survey portion of the research.

The largest part of the survey was conducted at The Land Institute, Salina, KS. The Land Institute is owned and operated by Dr. Wes Jackson. The research at The Land Institute is aimed at developing a perennial grain crop. A better understanding of nitrogen fixation by native grasses and forbs would be highly advantageous in developing a

self-sustaining perennial grain crop. For this reason, Dr. Jackson allowed sampling in The Land Institute herbary. The Land Institute's herbary is a large collection of prairie forbs and grasses offering an excellent opportunity to sample a variety of prairie plants for the survey portion of this research. This sampling was done with the help of Ms. Laura Jackson.

Acetylene Reduction Technique

Nitrogen fixation was measured using the acetylene reduction technique. This assay is based on the fact that the nitrogenase enzyme system not only reduces $N_2$ to $NH_4^+$, but will also reduce acetylene ($C_2H_2$) to ethylene ($C_2H_4$). This was first shown by Schöllhorn and Burris (1966) and by Dilworth (1966). They were studying the mechanism of nitrogenase and were testing a number of gases as nitrogenase inhibitors. When testing acetylene as an inhibitor, they found that it did inhibit nitrogenase, but after a time, the acetylene disappeared. It was then discovered by using $^{14}C$ labelled acetylene that the acetylene was being reduced to ethylene. Ethylene is not reduced further and does not inhibit nitrogen fixation or acetylene reduction. The nitrogenase enzyme system has such a high affinity for acetylene that it is not necessary to exclude $N_2$. The presence of ethylene is easily detected using gas chromatography.

The acetylene reduction assay has allowed nitorgen fixation to be studied not only in the laboratory but also in the field. Samples can be taken in remote areas, injected with acetylene, and returned to the laboratory for analysis with a gas chromatograph. In this study a portable gas chromatograph was constructed and taken to the study area. Field studies of nitrogen fixation have been done in remote areas such as the Arctic (Stutz and Bliss, 1970) and in the South American tropics (Döbereiner, 1970).

Root Collection

Previous researchers using the acetylene reduction assay collected samples as intact cores of soil (Weaver et al. 1980; Kana and Tjepkema, 1978). This method does not ensure sufficient live root material to detect nitrogenase activity or measure it accurately. This method also makes it difficult to be sure of the identity of the species of roots within the core. This is especially true in the grasslands because of the tendency for roots of neighboring plants to become intertwined. It is also possible for ethylene to be metabolized by soil organisms before it diffuses out of the soil core (Witty, 1979).

The method of root collection chosen for this research involved digging up the root system of a plant and removing only those roots obviously alive. Only live roots and adhering soil were taken in the sample in order to insure the presence of sufficient root exudate in the enrichment zone to support nitrogen fixing bacteria. By using only living roots, the results could be reported on a per gram of root basis. If dead roots had been included in the sample, it would have lowered the reported rate of nitrogenase activity.

As the roots were removed from the plants, they were placed in sample vials and immediately closed with serum stoppers to prevent dessication of the sample material. In some cases, a few drops of water were added to the vial to maintain the humidity in the vial.

On each sampling date, an attempt was made to collect at least six samples of a species from each of two burn treatments. One sample from each set of samples was designated as a control and was not injected with acetylene. This control was designed to measure any ethylene that might normally be produced by the roots or soil microorganisms.

Acetylene Injection

Within two hours after roots were collected, acetylene was injected into the vials through the serum stopper. Transfer of acetylene from the bottle of compressed acetylene was accomplished by using a small innertube as a low pressure tank. The valve stem core of the innertube was removed and a serum stopper was fitted over the valve stem. Epoxy was used to seal the stopper to the valve stem to prevent leaking of the acetylene. To remove any gases originally in the innertube, a hypodermic needle was fastened to the end of a vacuum line. The needle was then inserted through the serum stopper on the innertube. The vacuum then pulled out any gas that was in the innertube. By fitting a hypodermic needle onto the outlet of the regulator on the tank of compressed acetylene and inserting that needle through the serum stopper of the innertube, an easy transfer of acetylene could be accomplished. The innertube was then filled to a very low pressure.

Before injecting acetylene into the sample vials, a five ml sample of air was removed from the vial with a syringe. Then an equivalent volume of acetylene was injected into the vial. This was done to maintain the same pressure in the sample vial.

Early in the research, it was discovered that the acetylene in the innertube was contaminated with a small amount of ethylene. The source of contamination was not known but was believed to be the rubber of the innertube. To correct for the contamination by ethylene, an acetylene "blank" was prepared for each set of samples. This was done by removing five ml of air from an empty vial and then injecting five ml of acetylene into the empty vial. This provided a control that could be subtracted from the results for each sample. This is a common problem since

ethylene is nearly ubiquitous (Mayo, Pers. Comm.).

Incubation

As soon as possible after acetylene injection, the samples were incubated in darkness. Dark incubation was chosen since it most closely represents light conditions in the soil. It is not known whether light affects any of the processes involved with nitrogen fixation.

Incubation directly in the soil at the site of collection was considered. This incubation would have been the closest possible simulation of the natural conditions. Incubation in the laboratory was chosen, however, because of the logistics involved with *in situ* incubation. Incubation directly in the soil would have involved returning to each collection site after the incubation time had elapsed. This would mean marking each sample site and then relocating them following incubation. Because of the distances between study areas and between sample sites, *in situ* incubation was rejected.

An incubation time of two days was chosen. Two days allowed sufficient time for a measurable concentration of ethylene to be produced without using up all of the available carbohydrate. The available carbohydrate was considered to be the limiting factor in the process of nitrogen fixation in the sample vial.

The samples were incubated in air at a temperature of 23-26 C. Previous research (van Berkum and Sloger, 1982) has indicated that greater rates of acetylene reduction can be achieved by incubating in an atmosphere of reduced oxygen. This is because nitrogenase is readily inactivated by $O_2$. Since the samples in this study were incubated in air the results are a conservative estimate of nitrogen fixation in the soil.

The literature is unclear as to a reasonable estimate of $O_2$ concentration at the root surface. Mulder (1975) incubated samples at an $O_2$ concentration of four percent. In the laboratory, one set of samples was incubated at an $O_2$ concentration of approximately four percent. This was done to give an indication of what the results would have been if all the samples were incubated at reduced $O_2$ concentration.

To do this, the sample vials containing root material were flushed with $N_2$ gas and then stoppered. Oxygen was then added to the vial to approximate a four percent concentration. A set of samples incubated in air was run at the same time. One ml samples of gas were removed from the vials at intervals throughout the incubation period and measured for ethylene production.

Gas Chromatography

Production of ethylene in the sample vials was measured using gas chromatography. The gas chromatograph used in this study was a portable model constructed from plans designed by Mallard et al. (1977). This gas chromatograph (Figure 2) was designed specifically for the purpose of measuring nitrogen fixation by the acetylene reduction technique. It uses a 12 volt power supply that makes it quite portable and very useful in a field study situation.

The three major components of this gas chromatograph are the diffusion column, the sensor, and a microampmeter. The column used in this study was 3.0 mm OD by 44 cm stainless steel, packed with 22 cm of Poropak R and 22 cm of Poropak N (Alltech, Deerfield, IL). A tee fitting at one end allows for an inlet for the carrier gas, $N_2$, and also an injection port. The other end of the column is attached to a Swagelok fitting that has been soldered to a brass detector block. The

Figure 2. Gas chromatograph, innertubes for acetylene and ethylene and flasks used for calibration.

brass block serves as a heat sink and as a housing for the sensor. The sensor is one commonly used in many smoke detectors. This sensor has a sintered semi-conductor molded around a small filament heater. When the filament is heated in the presence of combustible gases, the resistance of the semi-conductor is lowered. The change in the resistance is then indicated by the microampmeter.

The gas chromatograph was housed in a metal instrument case. For added protection and to increase portability, a wooden case lined with styrofoam was constructed and the gas chromatograph was then placed inside. Openings in both cases were provided for access to controls, microampmeter, carrier gas inlet, injection port, power terminals, and recorder output. It was soon discovered that the cases were too well sealed. This prevented the test gases from escaping the unit rapidly. This increased the recovery time of the sensor between injections because the trapped gases remained in contact with the sensor for a longer time. This problem was overcome by placing a small fan in the gas chromatograph such that it drew fresh air from outside the case and directed it on the sensor and the brass detector block. This not only kept the test gases clear of the sensor but also pressurized the air inside the unit and forced the test gases out more rapidly. The fan did not affect the sensitivity of the detector, but it did speed up the recovery of the sensor between injections.

This gas chromatograph is equipped with two sensitivity ranges. Range #1 measures concentrations between $10^{-10}$ moles $ml^{-1}$ to about 4.0 X $10^{-9}$ moles $ml^{-1}$. Range #2 measures concentrations from 0.5 X $10^{-9}$ to about 3.0 X $10^{-8}$ moles $ml^{-1}$. The higher sensitivity of range #1 was required in this study because of the very low rate of nitrogen fixation

by nonsymbiotic bacteria. When testing for nitrogen fixation by legume nodules, range #2 was used because of the greater rate of nitrogen fixation by the symbiotic bacteria in the nodules.

The operation of this gas chromatograph was quite simple. First, the carrier gas was turned on and allowed to run for several minutes before the power was turned on. After the power was turned on, the unit was allowed to warm up for several minutes. When the gas chromatograph had warmed up and the microampmeter needle had stopped drifting, a sensitivity range could be selected and the meter zeroed. The gas chromatograph was then ready for use.

A one ml sample was taken from the sample vial using a one ml hypodermic. The sample was then injected into the gas chromatograph through the injection port. The time of injection was then noted. Gas chromatography is based on the rate at which a specific gas will move through the column while interacting with the packing. Since different gases have different reaction rates, different gases from the same injection arrive at the sensor at different times. Ethylene moves through the column after about 60 seconds. Acetylene takes about 90 seconds to reach the sensor. By noting the time from injection to the peak response on the microampmeter, the gas can be identified as either ethylene or acetylene. The concentration of the gas was determined by the maximum needle deflection (i.e. peak height) of the microampmeter.

Gas chromatography calibration was achieved using a series of ethylene dilutions. Five 250 ml volumetric flasks were made up to final volume of 200 ml by using glass beads. This was done by filling the flask with 200 ml of water and then adding glass beads until the water level just reached the serum stopper.

To make the series of dilutions, one flask was made to a concentration of $10^{-7}$ moles $ml^{-1}$ by adding 0.496 ml of ethylene to 199.5 ml of air. The rest of the dilutions were made from this standard by the following dilutions.

2.0 ml of $10^{-7}$ moles $ml^{-1}$ in 198.0 ml = 1.0 X $10^{-9}$ moles $ml^{-1}$

1.0 ml of $10^{-7}$ moles $ml^{-1}$ in 199.0 ml = 0.5 X $10^{-9}$ moles $ml^{-1}$

0.5 ml of $10^{-7}$ moles $ml^{-1}$ in 199.5 ml = 0.25 X $10^{-9}$ moles $ml^{-1}$

0.2 ml of $10^{-7}$ moles $ml^{-1}$ in 199.8 ml = 0.10 X $10^{-9}$ moles $ml^{-1}$

By injecting these dilutions in series and recording the meter deflection (peak height), a calibrating curve could be determined. The instrument was calibrated once during each day of use.

Drying Samples

After each sample had been tested for ethylene with the gas chromatograph, the sample material was removed from the sample vial and placed in a small paper bag. Each bag was then labelled with date of collection, date of testing, species, type of material (root, rhizomes, nodules, or soil), study area, location in study area, and sample number. The samples were then dried to a constant weight at 70 C and then weighed.

Weighing Samples

When the samples had sufficiently dried, 10-12 sample bags were removed from the oven and placed in a dessicator with dry-rite. This allowed the samples to cool to room temperature before weighing without allowing them to take up moisture from the air. After the samples had cooled, one sample was taken from the dessicator at a time. The sample was quickly separated into root material and soil. The root material was placed in a covered petri dish and weighed.

Xylem Pressure Potential

Moisture stress was measured as Xylem Pressure Potential (XPP). Water in the xylem of plants is under tension. When a stem or leaf is cut; the water in the xylem is drawn back from the cut surface. This is due to the tension on the water in the xylem. By exerting an external pressure on the severed plant part (eg. a leaf), water in the xylem can be forced back to the cut surface. The negative of the pressure required to force the water back to the surface is the Xylem Pressure Potential. Since the water is under tension, the XPP is reported as a negative number.

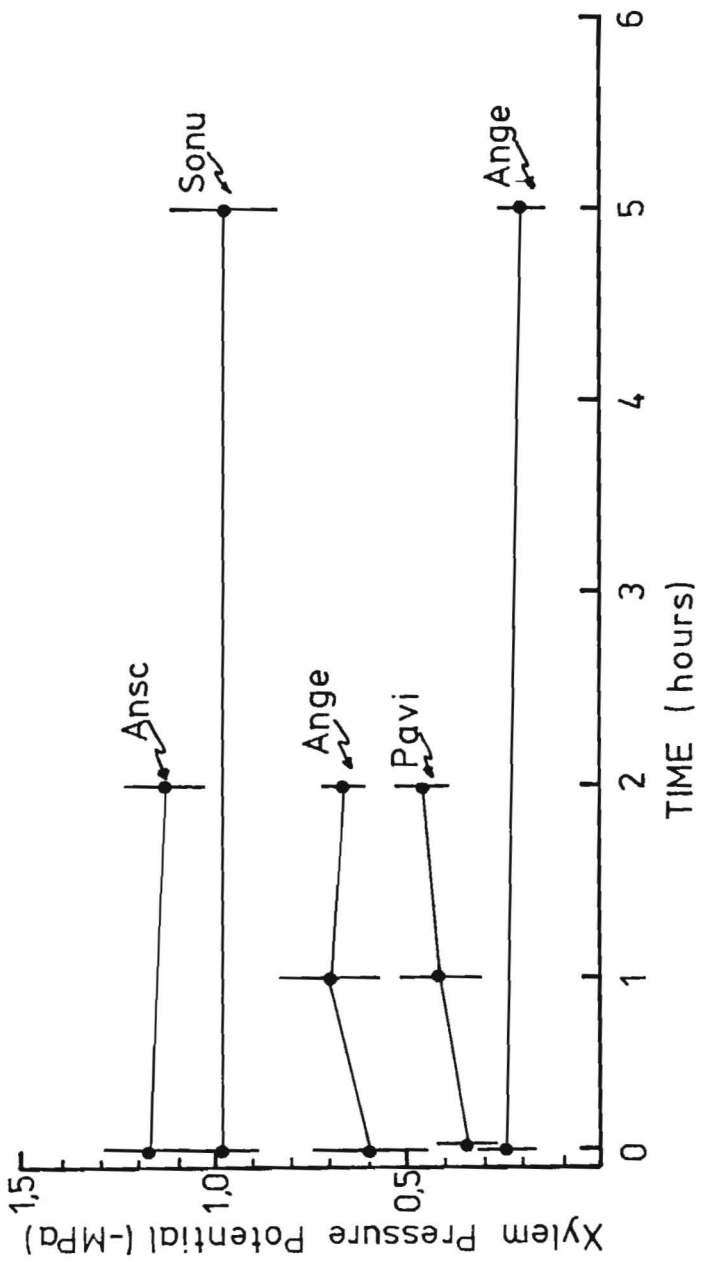
The Scholander Pressure Bomb (P.M.S. Instruments, Corvallis, Oregon) works on this principle. A cut leaf is placed in the pressure chamber with the cut end exposed through the lid. The chamber is then pressurized with $N_2$ gas while observing the cut end of the leaf with a magnifying lens. When water is seen at the cut surface, the $N_2$ gas is shut off and the pressure in the chamber is read from the pressure gauge. The negative of this pressure is the XPP.

In this study seven leaves from seven different plants of each species being studied were collected at each sampling site. The leaves were then placed in pint plastic containers with wet paper towels. This kept the samples from losing any significant amounts of water before the samples were read (Figure 3). Each container held only leaves from one species from one site. The containers were labelled according to species and location. The samples were then taken to the laboratory to measure the XPP.

Leaf samples were taken at dawn and mid-day along two transects. Each transect was run once every two weeks. Leaf samples were also

taken with root samples on some occasions.

Figure 3. XPP of leaves of Andropogon gerardi (Ange), A. Scoparius (Ansc) Panicum virgatum (Pavi), and Sorghastrum nutans (Sonu) stored in plastic containers. Time course shows little change in XPP for leaves stored for up to five hours. Vertical bars are 95 % confidence intervals.


Xylem Pressure Potential (-MPa)
1,5
1,0
0,5
0
1
2
3
4
5
6
TIME (hours)
Ansc
Ange
Pavi
Sonu
Ange

## RESULTS AND DISCUSSION

### Survey

Throughout the summer of 1982, a survey to determine the extent of nitrogen fixing ability associated with the rhizosphere of prairie plants was undertaken. This survey was designed to test a large number of prairie species, accessions and genetic crosses for nitrogen fixing ability. The survey centered on three families of prairie plants: the POACEAE, FABACEAE and ASTERACEAE. Tables 1, 2, and 3 list the plants tested in each family and the number of samples having a positive response (i.e. acetylene reduction) and the number having no response. The tables also indicate the type of material tested. Table 4 lists accessions of *Tripsacum dactyloides* and genetic crosses tested. The accession numbers were assigned by The Land Institute for plants collected from different areas. The crosses are also from The Land Institute.

A negative response in this type of survey does not indicate that the plant has no rhizosphere nitrogen fixing association. A negative response only indicates that at the time of sampling, nitrogenase activity was at or near zero. The plant may not have a rhizosphere association for nitrogen fixation or environmental factors may have limited nitrogenase activity. Nitrogenase activity can be restricted by moisture stress, low amounts of combined carbon, high amounts of combined nitrogen, temperature extremes, and other factors.

### Response of Nitrogen Fixation to Burning

The nitrogenase activity of *Andropogon gerardi*, *A. scoparius*, *Panicum virgatum*, and *Sorghastrum nutans* in burned and unburned areas

Table 1. Type of material tested and number of samples positive (+) and negative (-) for acetylene reduction by species of POACEAE.

| Species | Type of Material | #(+) | #(-) |
|---|---|---|---|
| Agropyron elongatum | root | 1 | 1 |
| A. smithii | root | 2 | 0 |
| Andropogon gerardi | root | 59 | 9 |
| A. hallii | root | 1 | 0 |
| A. ischaemum | root | 0 | 1 |
| A. scoparius | root | 9 | 2 |
| | soil | 1 | 0 |
| Bouteloua gracilis | root | 0 | 1 |
| Elymus canadensis | root | 0 | 1 |
| Koleria cristata | root | 1 | 0 |
| Panicum virgatum | root | 9 | 2 |
| Phalaris arundinaceae | root | 1 | 0 |
| Sorghastrum nutans | root | 13 | 2 |
| Spartina pectinata | root | 1 | 0 |
| Tridens flavus | root | 0 | 1 |
| Tripsacum dactyloides | root | 7 | 2 |
| Zea diploperennis | root | 1 | 2 |

Material from The Land Institute, Konza Prairie, and RNHR

Table 2. Type of material tested and number of samples positive (+) and negative (-) for acetylene reduction by species of FABACEAE.

| Species | Type of Material | #(+) | #(-) |
|---|---|---|---|
| Amorpha canescens | root | 1 | 0 |
| | nodules | 1 | 0 |
| Astragalus sp. | nodules | 1 | 0 |
| Baptisa australis var. minor | root | 1 | 0 |
| | root cortex | 0 | 1 |
| | nodules | 1 | 0 |
| Cassia marilandica | roots | 0 | 2 |
| Desmanthus illinoense | nodules | 1 | 0 |
| Desmodium illinoense | nodules | 1 | 0 |
| Lathyrus sylvestris | nodules | 1 | 0 |
| Lespedza bicolor | nodules | 1 | 0 |
| L. capitata | nodules | 1 | 0 |
| L. stuevei | nodules | 1 | 0 |
| Melilotus officianalis | nodules | 1 | 0 |
| Petalostemon purpureum | nodules | 1 | 0 |
| P. candidum | nodules | 1 | 0 |
| Psoralea sp. | nodules | 1 | 0 |
| Schrankia nuttallii | nodules | 1 | 0 |

Material from The Land Institute, Konza Prairie, and RNHR

Table 3. Type of material tested and number of samples positive (+) and negative (-) for acetylene reduction by species of ASTERACEAE.

| Species | Type of Material | #(+) | #(-) |
|---|---|---|---|
| Aster ericoides | root | 1 | 0 |
| Englemannia pinnatifida | root | 0 | 1 |
| Helianthus grosseratus | root | 0 | 1 |
| H. maximilliani | root | 0 | 1 |
| Heliopsis helianthoides | root | 0 | 1 |
| Liatrus pycnostachya | root | 0 | 1 |
| Ratibida pinnata | root | 1 | 0 |
| Silphium actinatum | root | 0 | 1 |
| S. speciosum | root | 0 | 1 |
| Solidago canadensis | root | 2 | 1 |
| S. gigantea | root | 2 | 0 |
| S. petiolaris | root | 1 | 1 |

Material from The Land Institute and Konza Prairie

Table 4. Number of samples positive (+) and negative (-) for acetylene reduction by accessions of *Tripsacum dactyloides* and genetic crosses.

| Species or cross | Accession # | #(+) | #(-) |
|---|---|---|---|
| *Tripsacum dactyloides* | 09 | 0 | 1 |
| | 18 | 1 | 0 |
| | 27 | 0 | 1 |
| | 37 | 0 | 1 |
| | 39 | 1 | 0 |
| | 84 | 0 | 1 |
| | 86 | 1 | 0 |
| | 94 | 0 | 1 |
| | 100 | 1 | 0 |
| | 320 | 0 | 1 |
| (*Zea mays* X Trda) X *Zea mexicana* | - | 0 | 1 |
| (*Zea mays* X Trda) X *Zea mays* | - | 0 | 1 |

Trda - *Tripsacum dactyloides*

Material from The Land Institute

is shown in Table 5. Nitrogenase activity associated with the rhizosphere of Andropogon gerardi and A. scoparius from burned areas was significantly greater than those plants from unburned areas. For Panicum virgatum and Sorghastrum nutans, nitrogenase activity in the burned areas was also greater, but these differences were not statistically significant, perhaps due to insufficient sampling. They do indicate a trend of increased nitrogenase activity following burning.

The effect of spring burning of rhizosphere nitrogen fixation has not been previously reported in the literature. As shown by this study, nitrogen fixation is increased by burning. This increase may be responsible in part for the increased production of the tall grass prairie following burning. The cause of this increased nitrogenase activity is not fully understood. However, there are some possible explanations.

During root collection, it was noted that plants in burned areas began new root growth earlier in the season than did plants from the unburned areas. This is probably due to earlier warming of the soil and less water stress in the spring in the burned areas (Knapp, 1984). The new root growth indicates increased photosynthesis resulting in more carbohydrate exuded into the rhizosphere which will increase the number of free living, nitrogen fixing bacteria in the rhizosphere. Increased nitrogenase activity may be due to this increase in number of nitrogen fixing bacteria.

Ecotypes of Andropogon gerardi

On July 8, 1982, I discovered what appeared to be two ecotypes of Andropogon gerardi growing in a burned unit of the Konza Prairie. The two different big bluestems were growing sympatrically on an upland site. One was dark green in color and had wide leaves. This ecotype

Table 5. Rhizosphere acetylene reduction by four prairie grasses in annually burned (B) and unburned (UB) prairie. (n moles $C_2H_4$/g root)

| | SPECIES | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ange* | | Ansc* | | Pavi* | | Sonu* | |
| Date | B | UB | B | UB | B | UB | B | UB |
| 5-26 | 14.79 | - | 8.32 | - | 13.35 | - | 8.73 | - |
| 6-08 | 20.63 | 0.00 | 14.52 | 0.00 | 12.52 | 0.00 | 23.72 | 0.00 |
| 6-14 | 7.80 | 7.47 | 8.28 | 0.00 | 10.14 | 0.00 | 16.64 | 5.41 |
| 6-15 | 17.25 | 9.6 | - | - | - | - | - | - |
| 6-16 | 14.46 | - | - | - | - | - | - | - |
| 6-17 | 35.56 | 5.0 | 24.57 | 3.07 | 47.23 | 2.37 | 30.83 | 9.87 |
| 6-25 | 8.80 | 14.60 | - | - | - | - | - | - |
| | 7.50 | 1.95 | - | - | - | - | - | - |
| | 14.62 | 1.59 | - | - | - | - | - | - |
| | 48.39 | 5.50 | - | - | - | - | - | - |
| | 7.37 | 12.67 | - | - | - | - | - | - |
| 6-30 | 3.36 | 13.17 | 27.91 | 2.24 | 7.25 | 7.20 | 3.82 | 6.74 |
| 7-08 | 9.09 | - | - | - | - | - | - | - |
| | 13.44 | - | - | - | - | - | - | - |
| | 13.37 | - | - | - | - | - | - | - |
| | 13.28 | - | - | - | - | - | - | - |
| | 9.50 | - | - | - | - | - | - | - |
| 7-12 | 4.57 | - | - | - | - | - | - | - |
| | 2.87 | - | - | - | - | - | - | - |
| | 32.35 | - | - | - | - | - | - | - |
| | 11.54 | - | - | - | - | - | - | - |
| | 34.70 | - | - | - | - | - | - | - |
| 7-14 | 43.95 | - | - | - | - | - | - | - |
| | 22.73 | - | - | - | - | - | - | - |
| | 0.00 | - | - | - | - | - | - | - |
| | 12.73 | - | - | - | - | - | - | - |
| 7-19 | 9.37 | - | - | - | - | - | - | - |
| | 20.75 | 1.96 | - | - | - | - | - | - |

Table 5. (Continued)

| | SPECIES | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ange* | | Ansc* | | Pavi* | | Sonu* | |
| Date | B | UB | B | UB | B | UB | B | UB |
| 7-21 | 1.09 | - | - | - | - | - | - | - |
| 7-24 | 11.74 | 23.38 | - | - | - | - | - | - |
| | 21.20 | 15.66 | - | - | - | - | - | - |
| | 68.40 | - | - | - | - | - | - | - |
| 9-29 | 1.57 | 6.13 | - | - | - | - | - | - |
| Mean | 16.9 ± 5.4[a] | 8.48 ± 3.9 | 16.7± 11.3 | 1.3[a] | 18.1 | 2.4 | 16.8 | 5.5 |

*Ange = Andropogon gerardi; Ansc = Andropogon scoparius;
Pavi = Panicum virgatum; Sonu = Sorghastrum nutans

[a]Significant difference at the 95 % level

was designated Ange Dg. The second was lighter green and had narrow leaves. This ecotype was designated Ange Lg. Figure 4 shows both ecotypes growing together.

XPP and acetylene reduction of these potential ecotypes of A. gerardi were sampled separately. These results are shown in Table 6. These results show that Ange Dg had a higher rate of nitrogenase activity through most of the sampling period. The XPP of Ange Dg was lower than the XPP for Ange Lg. This indicates that Ange Dg was experiencing greater moisture stress. This probably is due to the stomates of Ange Dg staying open longer thus allowing for increased $CO_2$ assimilation. The increase in photosynthesis could allow for more photosynthate to be translocated to the roots and then exuded. This might increase the energy available to the rhizosphere for nitrogen fixation.

Ange Lg had a higher XPP. This suggests that it is conserving water by closing its stomates sooner. This could reduce $CO_2$ assimilation and in turn reduce the amount of energy available for nitrogen fixation. This suggests that Ange Lg is adapted to survive better in a dry year. In a wet year like 1982, Ange Dg would do better.

Moisture Stress

Figure 5 shows dawn and mid-day XPP for Andropogon gerardi from all burned and unburned sites. Throughout the season, the plants were not under much moisture stress in both burned and unburned prairies. Plants from the unburned prairie did have lower XPP than did plants from the burned prairie in the early part of the season (Knapp, 1984). Figure 6 shows dawn and mid-day XPP for A. gerardi from adjacent burned and unburned sites. The burned area had been burned annually and the unburned area had not been burned in recent years. Early in the season,

Figure 4. Potential ecotypes of Andropogon gerardi. Ange Lg (center) surrounded by Ange Dg.

Table 6. Rhizosphere acetylene reduction and XPP of Andropogon gerardi ecotypes growing sympatrically in annually burned prairie.

| | n moles $C_2H_4$/g root | | XPP (-MPa) | | |
|---|---|---|---|---|---|
| Date | Dark green | Light green | Dark green | Light green | |
| 7-08 | 11.73 ± 2.8 | 1.54 ± 1.95 | - | - | Mean max XPP = .17 MPa<br>Mean min XPP = 1.34 MPa |
| 7-12 | 17.21 ± 1.9 | 4.03 ± 10.9 | 1.31 ± 0.6 | .84 ± .109 | XPP significant |
| 7-14 | 15.9 ± 2.2 | 3.29 ± 5.5 | - | - | No XPP taken |
| 7-21 | 1.09 | 3.22 | 1.12 ± .16 | 1.20 ± .16 | Only single samples of acetylene reduction |
| 7-23 | - | - | 0.56 ± .09<br>1.82 ± .06 | .22 ± .02<br>1.48 ± .08 | Dawn<br>Midday Significant |
| 7-29 | - | - | .18 ± .10<br>1.60 | .13 ± .07<br>1.65 | Dawn<br>Midday -- Single Samples |
| 8-06 | - | - | .125 ± .07<br>1.6 ± .24 | .20 ± .09<br>.68 ± .31 | Dawn<br>Midday |

Figure 5. Dawn and mid-day XPP for Andropogon gerardi from all burned and unburned prairie sites. Vertical bars are 95 % confidence intervals.

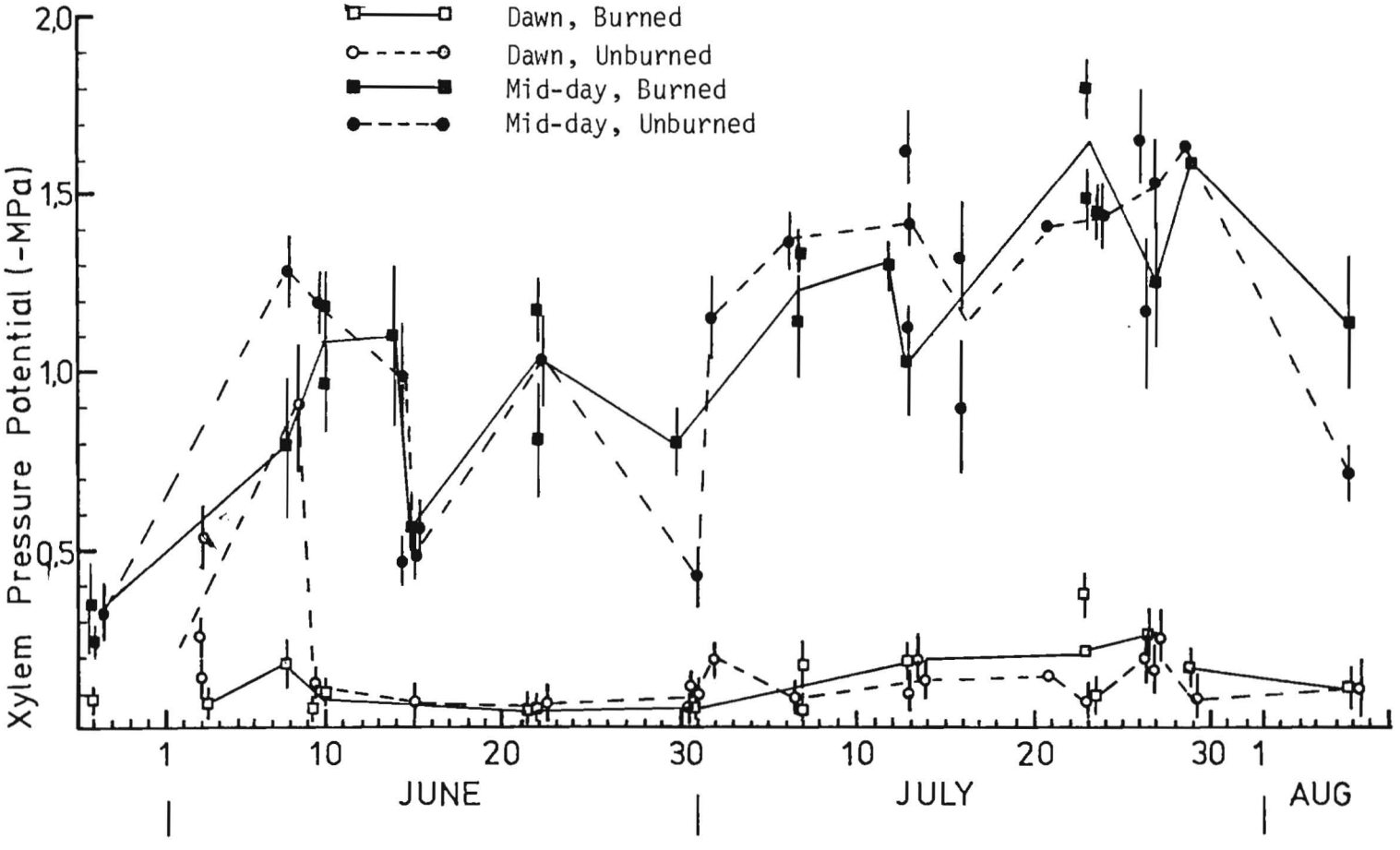
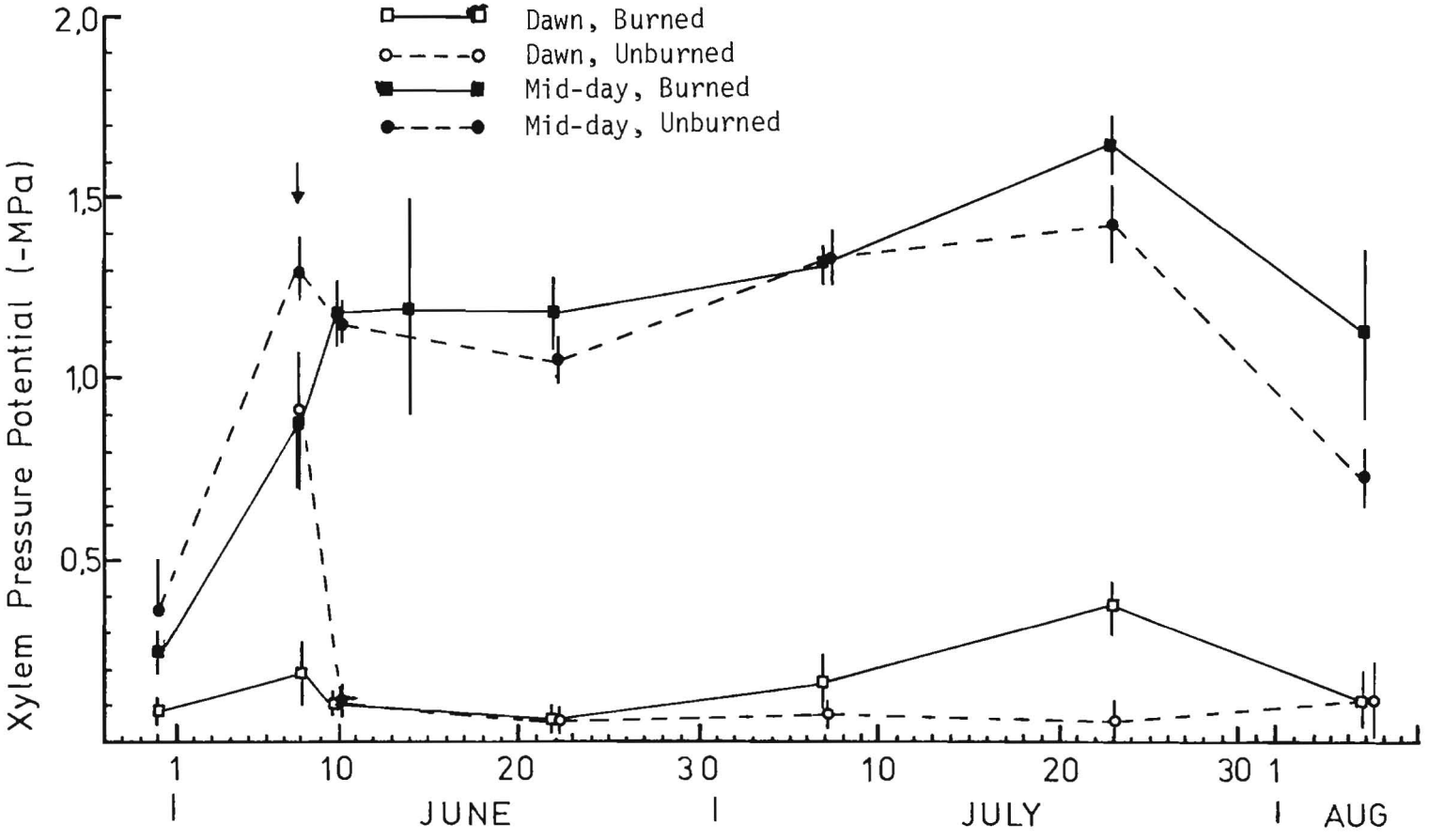

Dawn, Burned
Dawn, Unburned
Mid-day, Burned
Mid-day, Unburned
Xylem Pressure Potential (-MPa)
2,0
1,5
1,0
0,5
1
10
20
30
10
20
30
1
JUNE
JULY
AUG

Figure 6. Dawn and mid-day XPP for Andropogon gerardi from adjacent burned and unburned sites. Vertical bars are 95 % confidence intervals.


Dawn, Burned
Dawn, Unburned
Mid-day, Burned
Mid-day, Unburned
Xylem Pressure Potential (-MPa)
2,0
1,5
1,0
0,5
1
10
20
30
10
20
30
1
JUNE
JULY
AUG

plants from the unburned area were under greater moisture stress than those from the burned area. On June 8, new root growth was noted on plants from the unburned area. After that time, plants from both areas experienced similar moisture stress until the middle of July. During July there was periods of drought after which plants from the burned site experienced greater moisture stress than did plants from the unburned area.

Andropogon scoparius exhibited a similar pattern of XPP during the season on the same burned and unburned sites (Figure 7). Plants from the unburned prairie had a lower XPP early in the season than did A. gerardi during the same period.

Sorghastrum nutans and Panicum virgatum also had a similar pattern of XPP on the same burned and unburned sites (Figures 8 and 9). However, plants from the unburned area remained drier than those from the burned prairie until later in the summer.

All four species had lower XPP in the unburned area until later in the summer when XPP tended to be lower in the burned area. This is probably due to slower root growth in the unburned area because of lower soil temperature. As root growth of unburned plants increased, the XPP increased.

Effect of Moisture Stress on Nitrogen Fixation

Nitrogenase activity ($C_2H_2$ reduction) and XPP for Andropogon gerardi were followed through the summer. Figure 10 indicates that nitrogenase activity does respond to moisture stress. At the end of May, XPP is high indicating little moisture stress. Nitrogenase activity at that time was also high. As XPP began to decrease after June 8, nitrogenase activity also decreased. This pattern continues through

Figure 7. Dawn and mid-day XPP for Andropogon scoparius from adjacent burned and unburned sites. Vertical bars are 95 % confidence intervals.

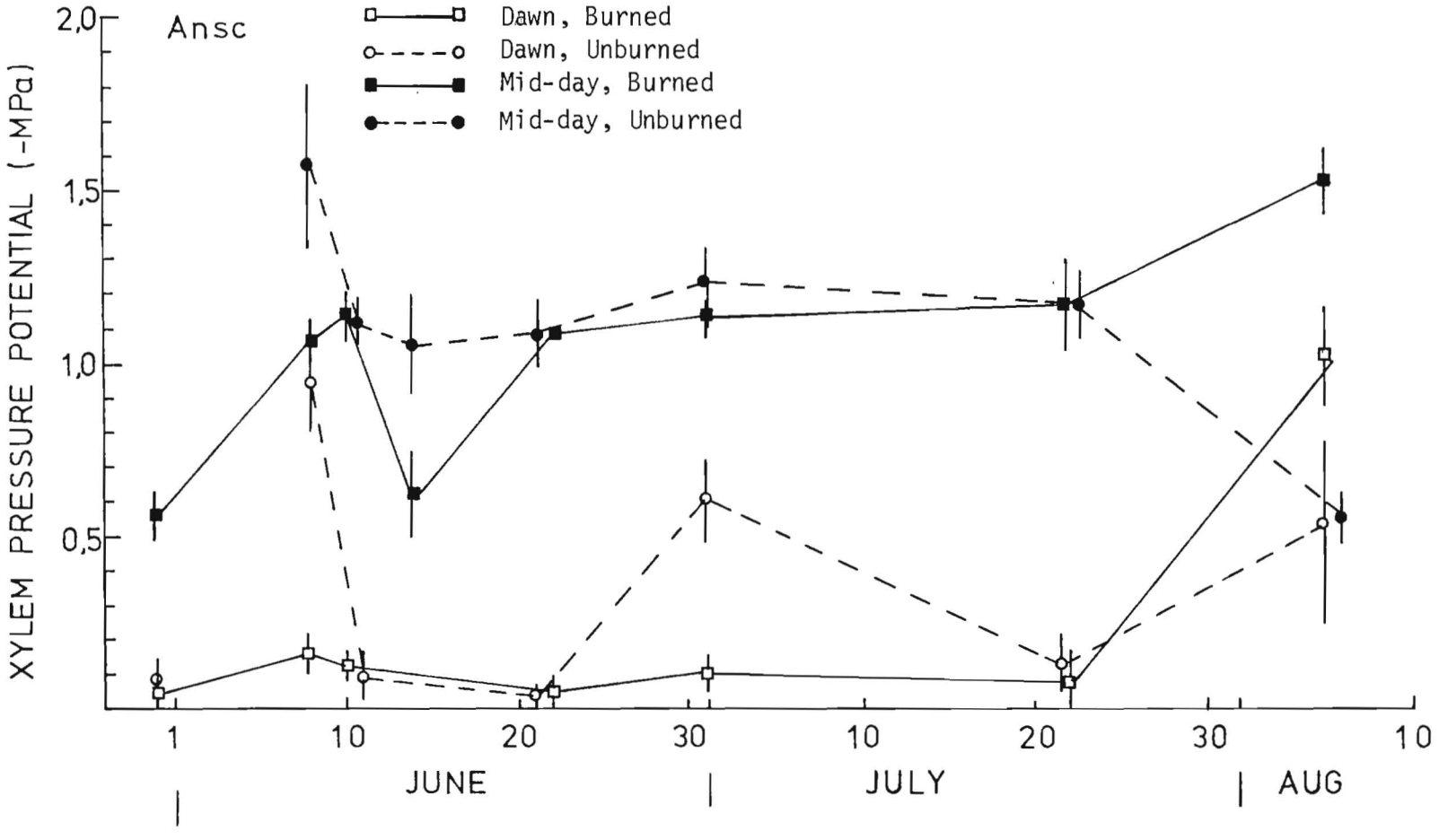

Ansc
Dawn, Burned
Dawn, Unburned
Mid-day, Burned
Mid-day, Unburned
XYLEM PRESSURE POTENTIAL (-MPa)
2,0
1,5
1,0
0,5
1
10
20
30
10
20
30
10
JUNE
JULY
AUG

Figure 8. Dawn and mid-day XPP for Sorghastrum nutans from adjacent burned and unburned sites. Vertical bars are 95 % confidence intervals.

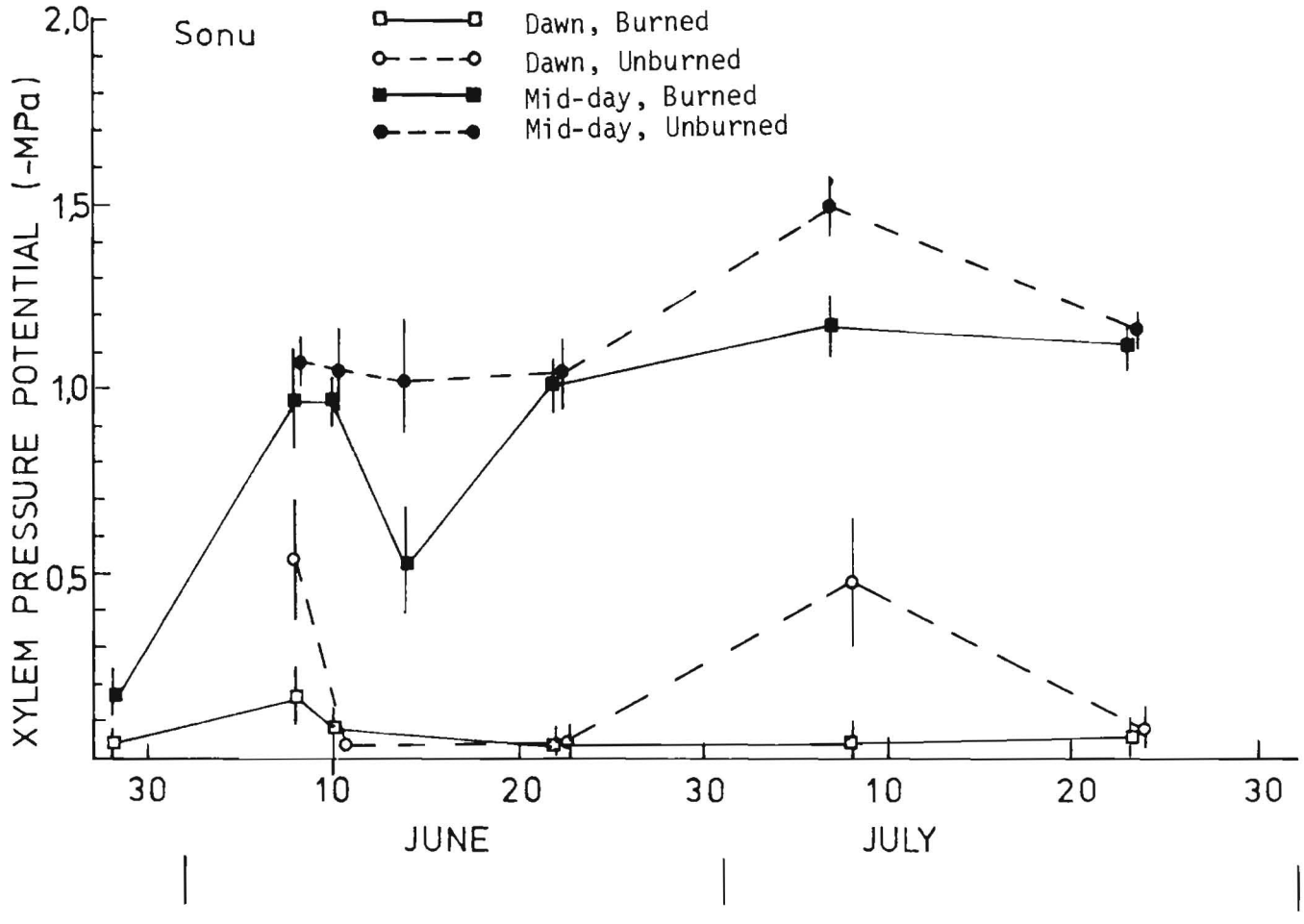

Sonu
Dawn, Burned
Dawn, Unburned
Mid-day, Burned
Mid-day, Unburned
XYLEM PRESSURE POTENTIAL (-MPa)
2,0
1,5
1,0
0,5
30
10
20
30
10
20
30
JUNE
JULY

Figure 9. Dawn and mid-day XPP for Panicum virgatum from adjacent burned and unburned sites. Vertical bars are 95 % confidence intervals.

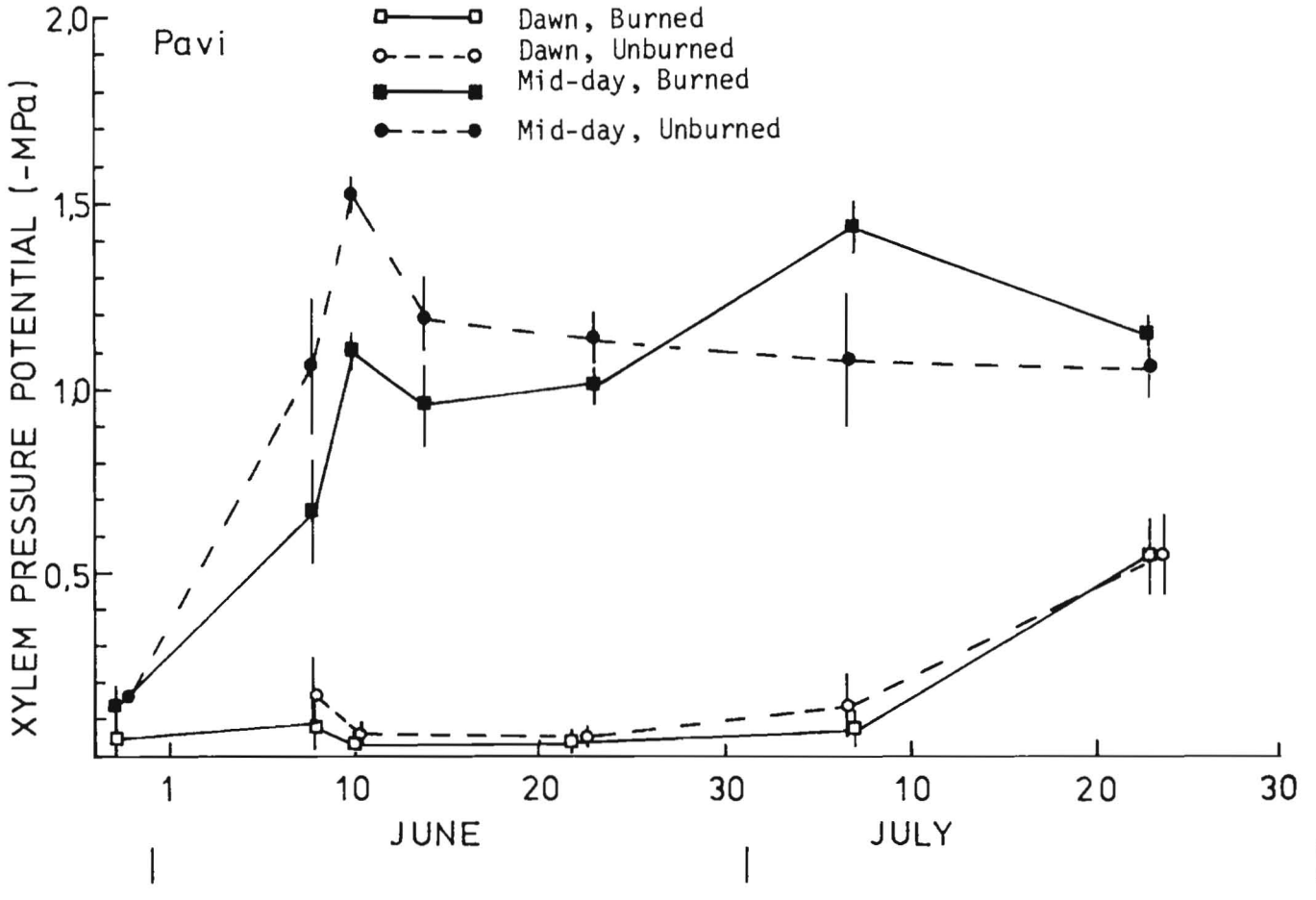

Pavi
Dawn, Burned
Dawn, Unburned
Mid-day, Burned
Mid-day, Unburned
XYLEM PRESSURE POTENTIAL (-MPa)
2,0
1,5
1,0
0,5
1
10
20
30
10
20
30
JUNE
JULY

Figure 10. Nitrogenase activity (ethylene production) and XPP for Andropogon gerardi from burned prairie throughout the summer.

20
15
10
5
n moles $C_2H_4$/g root
ethylene
midday XPP
dawn XPP
XPP (-bars)
26
30
1
5
9
13
17
21
25
29
2
6
10
14
18
22
26
30
May
June
July

the summer. On July 14, the dawn XPP was quite low. This was followed by a decrease in nitrogenase activity to near zero.

Figure 11 more directly illustrates the effect of decreasing XPP on nitrogenase activity. These are single data points of ethylene production and XPP for A. gerardi. Even though these are single data points, they show the tendency for nitrogenase activity to decrease as XPP decreases.

## Effect of $O_2$ Concentration on Nitrogen Fixation

Figure 12 shows the effect of $O_2$ concentration on nitrogenase activity for root samples of the two potential ecotypes of A. gerardi. The samples incubated in air had a linear increase in ethylene concentration through the incubation. Ethylene concentration increased exponentially for the first 24 hours in the samples incubated in four percent $O_2$. After 24 hours, ethylene concentration leveled off. Samples incubated in reduced $O_2$ had a final ethylene concentration as much as eight times greater than those samples incubated in air. This indicates that nitrogenase activity is enhanced be reducing the $O_2$ tension.

These results raise the question of which type of incubation atmosphere more closely represents what is happening in the field. Soil air and atmospheric air have nearly the same $O_2$ concentration (Brady 1974). The problem is knowing what the $O_2$ concentration is at the root surface. van Berkum and Sloger (1982) used concentrations ranging from 0.0 to 20.0 percent. Nowhere in the literature is it indicated that the $O_2$ concentration used for incubation simulated the $O_2$ concentration at the root surface of plants growing in soil.

Kristensen and Lemon (1964) developed a model to determine $O_2$ concentration at the root surface. This model requires that certain

Figure 11. Nitrogenase activity (ethylene production) vs. XPP for Andropogon gerardi from burned prairie.

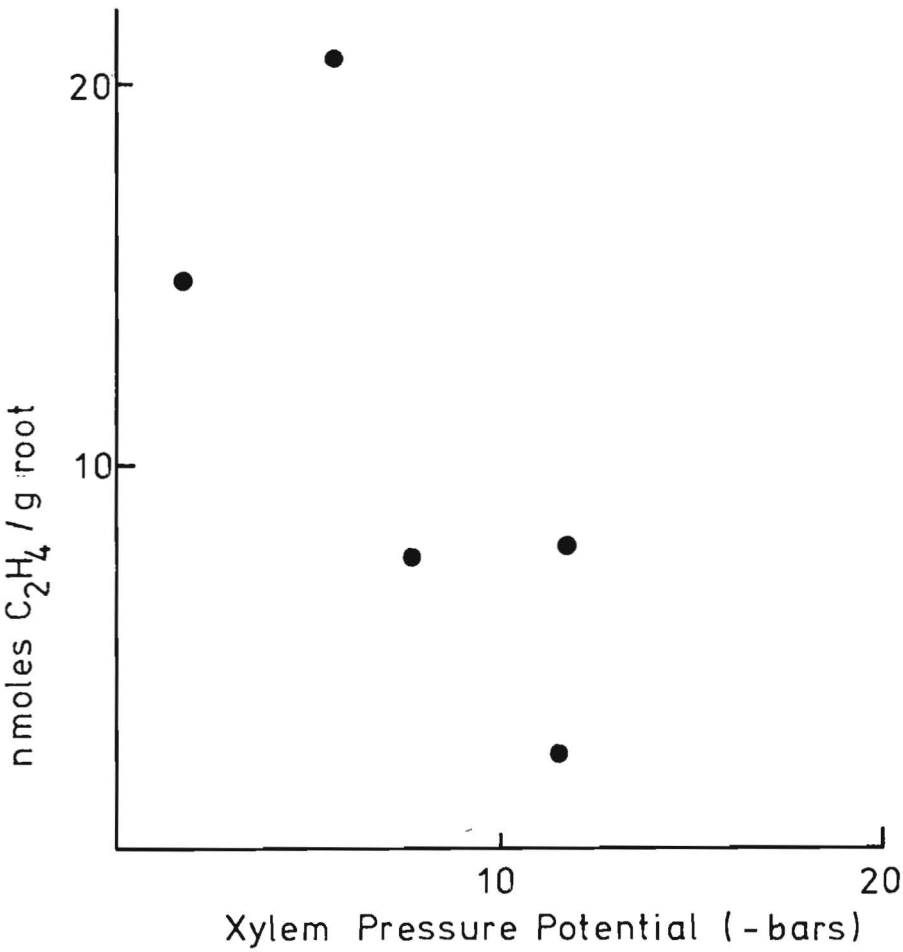
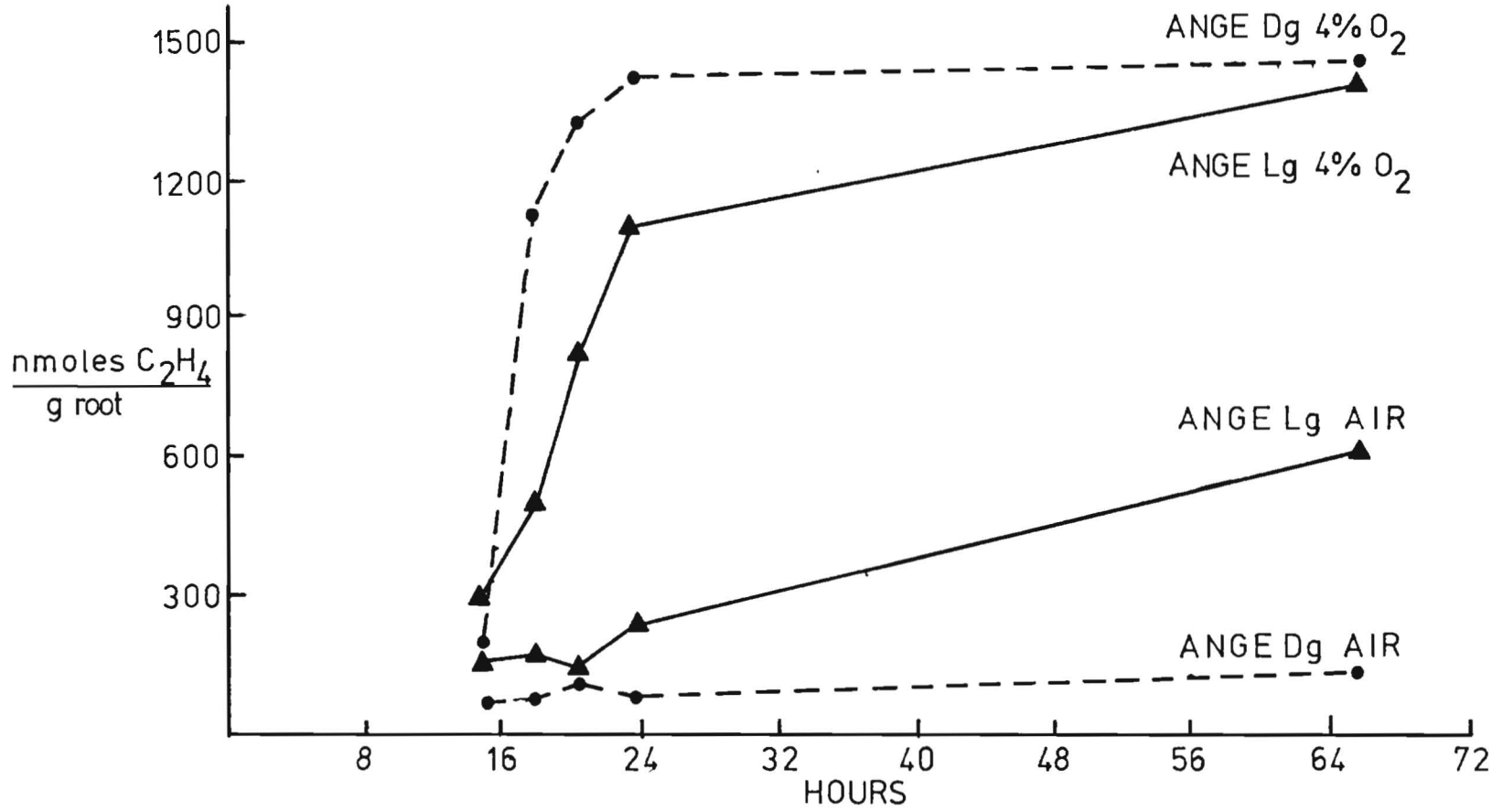

20
10
nmoles $C_2H_4$ / g root
10
20
Xylem Pressure Potential (-bars)

Figure 12. Time course of nitrogenase activity (ethylene production) for two ecotypes of Andropogon gerardi incubated in air and in four percent $O_2$.


ANGE Dg 4% $O_2$
ANGE Lg 4% $O_2$
ANGE Lg AIR
ANGE Dg AIR
1500
1200
900
600
300
nmoles $C_2H_4$ / g root
8
16
24
32
40
48
56
64
72
HOURS

root and soil characteristics must be known. The three root characteristics are its oxygen use rate, the root radius and the critical level of $O_2$ concentration for root growth. The three soil characteristics are the apparent liquid diffusion path length (the distance from the liquid-gas interface to the root surface), the diffusion coefficient for the solid-liquid matrix surrounding the root and the $O_2$ concentration at the liquid-gas interface. Determining $O_2$ concentration at the root surface is beyond the scope of this research, but the model does indicate that the $O_2$ concentration at the root surface will be much less than the $O_2$ concentration of air. This indicates that the reported values of nitrogen fixation in this study are conservative estimates only. These values are adequate for comparison within the study.

## SUMMARY

There are several conclusions that can be drawn from this research.

Rhizosphere nitrogen fixation is associated with many species of prairie plants. Of the 43 species tested, more than 30 had nitrogenase activity associated with their roots.

In measuring XPP, leaves of prairie grasses can be kept in humidity chambers for several hours before measuring without significantly changing the XPP. This allows for a large number of samples to be collected in a short period of time and then taken to the laboratory for XPP measurement.

During the 1982 measurement period, XPP of prairie grasses was not particularly low. The plants were under only moderate stress. Early season water potentials were lower in the unburned areas than in adjacent burned areas. This may be due to lower soil temperature in the unburned areas at that point in the season. This results in reduced early root growth. Later in the season, water potentials were lower in the burned prairie.

Nitrogen activity decreases as XPP decreases. As the season progressed and moisture stress increased, the rate of acetylene reduction decreased. This may be due to lower amounts of carbohydrate in the root exudate. As the plant experiences lower XPP its stomates remain open for only a limited time. This results in a decreased photosynthesis rate and less photosynthate being exuded by the roots.

This phenomenon may also be an affect of $O_2$ concentration. The nitrogen fixing bacteria in the rhizosphere occur on or near the root surface. Normally the root is surrounded by a liquid matrix. This

matrix controls the $O_2$ concentration at the root surface. As this matrix dries, the $O_2$ concentration increases due to contact with the soil air. The nitrogenase is then inhibited by the oxygen.

Potential ecotypes of Andropogon gerardi were found at the Konza Prairie. They have differing rates of rhizosphere nitrogen fixation and differing Xylem Pressure Potential. These differences appear to be the result of adaptations to moisture conditions.

Rhizosphere nitrogen fixation was consistently higher in the burned prairie. This, in part, helps to explain the greater productivity associated with spring burning of the tall grass prairie.

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# LITERATURE CITED


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