DROUGHT STRESS RESPONSES IN A POPULUS HYBRID COMPLEX IN
SOUTHERN ALBERTA

DROUGHT STRESS RESPONSES IN A <u>POPULUS</u> HYBRID COMPLEX IN

SOUTHERN ALBERTA

By

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TITLE: Drought Stress Responses in a <u>Populus</u> Hybrid Complex
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ABSTRACT

Stomatal response of narrow leaf poplar ($\underline{P}$. angustifolia; (PA)), balsam poplar ($\underline{P}$. balsamifera; (PB)), cottonwood ($\underline{P}$. deltoides; (PD)), and a $\underline{P}$. angustifolia X $\underline{P}$. balsamifera hybrid (PX) was studied in the field and in controlled environments. Mild water stress was occurring in both field studies, as evidenced by pre-dawn Ψ values of no less than -0.3 MPa; controlled experiments were more severe, with values dropping below -1.0 MPa. Stomatal conductance patterns differed for each species, and suggest that in terms of tolerating drought stress, PX > PA > PB > PD. These patterns were significantly influenced by microclimate conditions; severe conditions caused partial or complete stomatal closure, even in the presence of low water stress.

Preconditioning plants through brief, repeated exposure to water stress resulted in stomatal closure at approximately -1.0 MPa, a higher level of Ψ than is otherwise indicated for members of the genus $\underline{Populus}$. It may

thus be a valuable component of drought resistance for these species.

Dieback studies indicated dissimilar patterns of dieback between PA and PB; PA experienced dieback on a large number of small branches while that of PB occurred on a small number of main branches. These results suggests that the drought stress response may differ between the two species, and may further indicate that the pattern of dieback is reflective of the physiological response to drought stress.

ACKNOWLEDGEMENTS

This is perhaps one of the most personal acknowledgements you the reader will ever see. It is an acknowledgement of those who aided me professionally, but especially of those who assisted me personally, and of myself; I would not have completed this without the strength to endure. If you don't want to read it don't; those who know me will understand.

I would like to thank my supervisor Dr. Darwyn S. Coxson for introducing me to the wonder of the riparian ecosystem, both in the southern Yukon Territory and in the semi-arid climate of southern Alberta. These ecosystems were not just study sites; they were friend and mentor, and for a brief period of time, home. Who can forget the kraft dinner à la mosquito, the 24-hour Gravol pill, the insanely fast-paced pre-dawn measurement sets, Mr. Moosie and the fertilizer waterfall? I owe my survival of those harried field days to my trustworthy assistants; Biret and April, and especially

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Tracy. Then there's Jen. Oh, the stories I could tell...But she's been there through thick and thin (sometimes very) and I count it a great honour to be a booger. She's made the past year bearable and provided structural support when mine was crashing down around me. She is indeed a rare friend. I just hope we never have another discussion about the shortcomings of SigmaPlot!!!

I owe a special vote of thanks to my co-supervisor Dr. J.N.A. Lott. His professional advice has been extremely helpful and his comments on the manuscript are insightful; he was as dedicated to the completion of this project as I was, and seemingly more so, at times. He is also an excellent listener, hearing more than I say; his understanding of the situation is greatly appreciated and he has managed to motivate me to close this chapter and 'get on with it' as he has so aptly put it.

I would be remiss if I didn't thank the two G boys. One encouraged me to begin this arduous task, the other has supported me in finishing it. They are both extremely

important people in my life, for better or worse. I would also like to thank my family, albeit cautiously, since it was them who encouraged me to continue rather than work at McDonalds! The latter vocation is still a possibility, of course.

I have learned a great deal during the past (what is it, almost three?!) years, and perhaps not coincidentally, much of that learning has taken place in the arena of life, rather than academics. I have discovered that my resilience and self-sufficiency are remarkable, and that owes as much to the unusual situation I was placed in as to my own inherent will to survive. This has not been an easy trip for me, and I am honestly unsure if given another chance, I would choose the route that I did. But it's done, and I'll know better next time.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1

INTRODUCTION

A riparian ecosystem is defined as a river ecosystem;
all of the organisms that are a part of the system are
involved with, located near, or living on the banks of the
river. A major constituent of many riparian ecosystems are
forest communities along river banks. In temperate North
America, these forests consists of willow and poplar species
(Salix and Populus, respectively). Willows and poplars
(Family Salicaceae)are considered to be phreatophytes, deep-
rooted plants that rely on the water table for their water
(Woolf, 1981). These trees often function as keystone
components, providing food and shelter for a variety of
animals including ungulates, raptors, and other predators
(Savoy 1991, Rhodes 1991). Riparian forests also facilitate
the growth of other plant species that require protection
from sun and wind. Without the forest, this complex ecosystem
would cease to exist as it is presently or function in a
greatly degraded form (Rood and Heinze-Milne, 1989).

In southern Alberta along the Oldman River riparian
forests are unique in that they are comprised of three

species of poplar tree that converge and hybridize freely while still maintaining their original integrity (Rood *et al.* 1986). These species are P. deltoides (PD), P. balsamifera (PB), and P. angustifolia (PA). This phenomenon of interspecies hybrids is referred to as a hybrid swarm (or hybrid complex). Although poplars are known to hybridize freely in nature (Ronald *et al.* 1973, Ronald and Steele 1974), this hybrid swarm is believed to be unique because pure parent species can still be found amongst the large number of hybrids (Rood and Mahoney, 1991).

The reasons for the existence of this complex are unknown, but it is plausible that the river itself may provide an answer. The Oldman River originates in the Rocky Mountains, and drains eastward and northward into Hudson Bay (Byfield, 1984). As the Oldman River flows downward from the Rockies increasing in size and speed, it changes from a high energy downcutting river to a low energy meandering system. This environmental gradient is believed to have facilitated the development of the hybrid complex where overlapping species range distributions meet. P. balsamifera ranges throughout the boreal forest and extends as far south as

Montana. Alberta marks the limit of its eastern range. P. angustifolia reaches the northern extent of its range in Alberta, south of the Bow River. P. deltoides reaches the western edge of its range in the Lethbridge area. All species combinations of hybrids can be found, although the most common cross is P. balsamifera X P. angustifolia (PX). The range of overlap that has allowed development of the hybrid complex occurs in the area of Lethbridge, Alberta and is shown on the map in Figure 1.1 (Brayshaw, 1965). Given the probability that at least some of these hybrids are fertile, one might expect the species to merge in the area of overlap, creating a poplar complex consisting entirely of hybrids, where some of these hybrids would become species in their own right. The fact that this scenario has not occurred can be explained in several ways. It is possible that none of the hybrids are capable of reproduction and therefore can only exist through parent crosses. Although uncommon, other hybrid swarms have exhibited this characteristic (Keim *et al.*, 1989). It is also possible that the complex is maintained by genetic mechanisms existing in the parent population. Balanced polymorphism is a mechanism of natural selection

Figure 1.1. North American Range of Populus Species Involved in the Study.

RANGES OF *POPULUS* SPECIES
IN
SOUTHERN ALBERTA

P. balsamifera subsp. *balsamifera*

P. balsamifera subsp. *trichocarpa*

P. balsamifera — indeterminates

P. angustifolia

P. deltoides var. *occidentalis*

Hybrid combinations found beyond the range limits of one or more parent species.

Contribution of *P. tremuloides*, which occurs throughout on suitable habitats.

Scale in Miles

Kilometres

Cartography by the Research Branch, Canada Department of Agriculture

where the population favours the expression of two or more heterozygous genotypes not caused by mutations (Russell, 1986). In this situation, hybrids would be maintained in the population amongst parent species. Balanced polymorphism is often evident in plant crops that experience hybrid vigour (Russell, 1986).

Since the existence of this complex is dependent on the environmental gradient created by the Oldman River, any changes to this gradient could have a severe negative impact on the complex, possibly threatening its existence. This possibility is presented by the Oldman River dam, which was built near Brocket by Alberta Public Works and officially opened on July 16, 1992 (FEARP, 1992). As a result, the hybrid complex now functions under artificial flow conditions imposed by the dam. The effects of artificial flow conditions imposed by large-scale alterations on riparian ecosystems are well documented. Altering the natural flow patterns of a river produces a number of effects, the major ones being loss of spring flooding with a concomitant loss of sedimentation deposits and prevention of a gradual recession of flood waters (Bradley and Smith 1986). The net effect on the

ecosystem. Stress is defined here as a state in which an existing equilibrium has been altered by adverse factors or pressure (Woolf, 1981). Because the members of this complex are considered phreatophytes, no distinction has been made between the effects of large-scale alterations on the riparian ecosystem and effects on the hybrid complex. However, the effects could be quite different for the complex. It might thrive under the new conditions, producing no net change; or one or more hybrid species could dominate, changing the characteristics of the complex. A hybrid species is defined here as a new species produced as a result of hybridization between two distinct parent species; the new species is capable of reproducing itself without continued parent crosses. The possible effects of these large-scale alterations on the complex have not received any attention. If the complex cannot survive under the new conditions, a unique scientific opportunity will have been lost.

Predicting the impacts of changing environmental conditions on this *in situ* hybrid poplar complex can be inferred from examining previous research into poplar parent species, hybrid species and clones. With the advent of

silviculture early in this century, research has focussed heavily on genus _Populus_ because of its status as a woody plant that is easy to reproduce, grows quickly, and can be successfully hybridized (Hinckley _et al._ 1989). Much of this research has concentrated on the potential for increased productivity while still maintaining drought tolerance capability. Parent species are quite often excluded from these studies, since it is widely believed in the silviculture industry that hybrids are superior to parents (Hinckley _et al._ 1989). As a result, there is little information about the morphological, anatomical or physiological characteristics of the parent species. Hybrid species are more desirable because their physiological, anatomical or morphological characteristics can be greater than those of their parents, resulting in hybrid vigour (Hinckley _et al._ 1989). Hybrid clones are utilized in studies much more than natural interspecific hybrids due to their genetic similarity. Much of the research centres on stomatal behaviour and conductance under water stress (Schulte _et al._ 1987; Ceulemans _et al._ 1988; Tschaplinski and Blake 1989; Furukawa _et al._ 1990). Other features studied include water

use efficiency, hybrid vigour and growth (Blake *et al.* 1984;
Cain and Ormrod 1984; Dickmann *et al.* 1992). Most current
poplar research is performed in a laboratory or greenhouse
setting rather than in the field, although a small number of
researchers have attempted to combine field and lab research
(Schulte *et al.* 1987; Tschaplinski and Blake 1989). In these
studies, immature trees approximately one to two years old
grown from cuttings were generally used in the field as
opposed to using mature trees existing on site. Ceulemans *et
al.* 1988 is an exception, having used six-year-old trees in
their field study. It is reasonable to state that studies
that measure the physiological behaviour and water status
relations of parent species and their interspecific hybrid in
the laboratory and then compare them with the field responses
are rare, and that very few attempts have been made to
compare the physiological, morphological and anatomical
characteristics of the parent poplar species with those of
the F_1 hybrid. Further study of the parent species in
conjunction with hybrid studies could provide valuable
insight into the origins of hybrid vigour and increased
adaptability and productivity. Such studies would also

increase the understanding of the physiological responses of the species to stress and thus provide insight into possible repercussions arising from large-scale water diversion from the ecosystem.

While combined field and laboratory studies of both the parent species and the hybrid species can provide valuable information, in an ideal situation this ecosystem could be studied under artificial stress conditions similar to those imposed by artificial flow structures. This method of study could certainly be expected to provide a higher standard of information simply because of its inherent similarity to the ecosystem under study. Obviously, such a situation in nature is rare and thus it is necessary to rely on field and lab studies. However, this hybrid complex possesses such a site, in the form of abandoned gravel pits. In these abandoned gravel pit sites adjacent to the Oldman river valley, naturally occurring hybrid species complexes were established during wet years from 1973-1975. Since these trees are not in contact with groundwater, they provide an ideal surrogate for the study of future impacts of water diversion from the riparian ecosystems.

This surrogate drought stress system has been used to determine:

1) physiological responses of the hybrid complex in the

   gravel pit under its imposed water stress conditions and

   if these responses differ according to species

   (Chapter 2)

2) physiological responses of the hybrid complex in the

   laboratory by removing cuttings from trees in the field

   and rooting them for drought stress studies (Chapter 2)

3) species differences with data obtained from studies of

   dieback patterns between the two pure species P.

   angustifolia and P. balsamifera (Chapter 3)

Chapter 2

Physiological Responses to Drought Stress

INTRODUCTION

A plant must exchange gases with the environment in order to grow and survive. Photosynthesis is accomplished by the exchange of CO_2 into the plant and the loss of water via transpiration. This exchange occurs at the surface of the leaf through an epidermal opening called the stoma (Raven *et al.*, 1992). For every CO_2 molecule gained through this process, many more water molecules are lost (Fitter and Hay, 1987). These must be replaced continuously to prevent the leaf from losing so much water that it wilts and dies. The replacement of water molecules in the leaf is accomplished by the establishment of a water potential gradient from soil to leaf. Water flows down this gradient (from high free energy content to low free energy content) and up the tree. The flow of water in this direction opposes gravity; it is accomplished by the hydrogen bonds created between adjacent water molecules which create a cohesion that pulls water molecules upwards (Raven *et al.*, 1992). The flow takes place

in a series of tubes called xylem conduits. The major conducting cells of these conduits are called tracheids and vessel members; since they are dead at maturity the conduit is hollow.

Under ideal conditions (high humidity, moderate temperature, low wind, low water deficit), the flow of water in this manner is uninterrupted. Under non-ideal conditions for gas exchange, such as water stress, mesophytic plants have developed adaptations that allow them to conserve water and survive. These adaptations fall into three broad categories; the acquisition of water, the conservation of use of water, and tolerance of loss of water (Fitter and Hay, 1987). Many plants utilize more than one adaptation.

Water Acquisition

The acquisition of water during water stress is perhaps the most obvious strategy used to avoid water stress; the plant's resources are reallocated so as to allow it to collect the maximum amount of water available to it. This is generally accomplished by alteration of the shoot:root ratio. Since the bulk of a plant's resources are generally directed

towards the shoot, significantly reducing this shoot growth allows the plant to allocate more resources towards root growth in the hopes of locating more water (Turner and Begg, 1981). This strategy also greatly reduces water loss because transpiration is greatly lessened; the only significant source of water loss to the leaf occurs through the cuticle. This loss can be minimized by a thick, waxy layer of cutin (Raven *et al.*, 1992).

Water Conservation

A plant can also respond to water stress by conserving its water supply; since the bulk of water loss occurs through transpiration the most important aspect of water conservation is the minimization of transpiration during photosynthesis (Fitter and Hay, 1987). The first line of defense against water loss is the stomatal response to existing environmental conditions; change in stomatal aperture is the plant's major weapon (Mansfield and Davies, 1985). The amount of stomatal opening can be loosely termed stomatal conductance; a more rigorous definition is provided in the following section. Stomata seem to be highly sensitive

to prevailing environmental conditions (Lange *et al.*, 1982),
particularly high winds and low humidity. When wind speed
increases, CO_2 concentration near the leaf increases due to
the more turbulent boundary layer; when CO_2 concentration
reaches a critical level in the leaf, stomata begin to close
(Mansfield and Davies, 1981). The factors causing closure
under conditions of low humidity are uncertain. Stomata may
close completely or partially, and they may also close for
only short periods at a time. Midday stomatal closure is a
well-known example of the latter; it is common when
saturation deficit is high, and in fact, results in higher
water use efficiency over a day than maintaining stomatal
conductance at a constant value (Mansfield and Davies, 1985).
Water use efficiency (WUE) is defined as a ratio of the grams
of dry matter produced compared with the grams of water lost
through transpiration (Knapp and Smith, 1988). Other
environmental factors that influence stomatal closure to a
lesser degree include light and temperature.

While stomata can close immediately in response to
changing environmental conditions, it is also possible for
stomatal closure to occur in response to lowered leaf water

content (Mansfield and Davies, 1985). This restriction in aperture prevents the overuse of a limited water supply and may also increase the water use efficiency of the plant. Both immediate closure and closure in response to lowered water levels in the plant are strategies widely demonstrated in various plant species (Paleg and Aspinall, 1981).

Changes in endogenous plant hormone concentrations are now also considered possible role-players in a plant's response to water stress, as several hormones have been shown to directly influence the opening and closing of stomata according to their concentrations. Abscisic acid (ABA) is the most well known; ABA accumulation occurs in leaves that develop low water content; stomatal opening is inhibited as a result (Walton, 1980). Auxins and cytokinins are also believed to be involved; cytokinins promote stomatal opening as does IAA (indolylacetic acid) (Hale and Orcutt, 1987). In fact, most plants will respond to environmental stress by changing their hormonal balance, producing more ABA and less cytokinins (Chapin III, 1991).

Research into the hydraulic architecture of plants has also suggested that the water storage capacity of given

tissues could determine the ability of trees to survive water stress, further indicating the importance of water conservation in the plant (Tyree and Ewers, 1991).

Water Stress Tolerance

Both the acquisition of water and the conservation of water are strategies that attempt to avoid the results of water stress; tolerance of water stress is also possible. This involves the development of low osmotic potentials (Morgan, 1984). Osmotic potential is defined as the accumulation of solutes within a plant in response to water stress; it produces a change in the solute potential that is larger than the passive change resulting from water loss (Hinckley *et al.*, 1980). The net effect is the maintenance of turgor potential (pressure potential) which keeps the stomata open even at reduced water potentials. Cell wall elasticity is also an important factor in drought resistance since it slows the rate of loss of turgor as water potential decreases (Hale and Orcutt, 1987). Numerous tree species have been shown to osmoregulate; among them are jack pine, white

spruce, and English oak (Osonubi and Davies 1978; Koppenaal *et al.* 1991).

Poplar Responses to Water Stress

Poplar species show the same responses to water stress as those already discussed.. Poplars can show a shift in their shoot:root ratio during drought (Dickmann *et al.*, 1992), poplar species can osmoregulate, (Tscaplinski and Blake, 1989), and poplars can combine several adaptations as shown by a study of 17 poplar clones and hybrids where water-efficient clones exhibited cuticular hairs, partial stomatal opening early in the morning, and reduced stomatal size and frequency (Blake *et al.*, 1984). Reduced stomatal response has also been observed; in black cottonwood conditions of low soil water potential resulted in reduced stomatal conductance (Pezeshki and Hinckley, 1982). Although unusual, in some cases poplar stomata have remained open under conditions of severe water stress, such as those imposed by Furukawa *et al.*(1990). under controlled laboratory conditions. This stomatal response was also observed in clones of P. trichocarpa; in cut-leaf experiments and whole-plant

experiments the stomata remained open despite leaf water potentials low enough to result in loss of turgor and leaf wilt (Schulte *et al.*, 1987). This was especially significant given that the other poplar species and hybrids involved in the experiments experienced stomatal closure shortly after the onset of water stress.

Rationale for Study

It is clear that the response to water stress varies according to the particular species of Populus under study. Since differences in stomatal frequency, distribution, shape and size all play a role in how quickly and effectively a plant can respond to stress at the leaf level and since there is considerable variation in stomatal morphology and dimensions among poplar clones and hybrids (Ceulemans *et al.*, 1988), one would expect their responses to stress to differ. Thus the response of one species cannot be attributed to another species; each species must be studied individually. The response can be further permutated by unique growth patterns, such as those found in a natural hybrid complex in southern Alberta (see Chapter 1). Knowledge of the

physiological behaviour of this hybrid complex is extremely limited, and few studies have been undertaken for the species of the complex. No ecophysiologically oriented studies have attempted to investigate the water relations of either _P. balsamifera_, _P. angustifolia_ or _P. deltoides_ under conditions of water stress in this complex. Several studies have been conducted on _P. deltoides_ independent of this complex; leaf conductance was examined in the laboratory and in the field (Schulte _et al_. 1987; Hinckley _et al_. 1989). Both studies were conducted using clones exclusively.

A study of this hybrid complex, then, would not only facilitate knowledge of these particular poplar species' responses to water stress, it would also provide valuable information regarding an unusual natural phenomenon, a naturally seeded hybrid complex.

A. Physiological Instrumentation

Physiological measurements

Stomatal conductance (g_m) is the measure of the plant's ability to conduct water vapour through the stomatal openings on a leaf. Transpiration is the net loss of water vapour from the plant; the most important site is the stomata (Raven *et al.*, 1992). Transpiration and diffusive resistance can be calculated from directly measured values of relative humidity, leaf and air temperature and flow rate using a diffusion porometer. g_m cannot be measured directly, but it can be calculated by directly measuring stomatal resistance;

stomatal conductance=1/stomatal resistance

g_m is measured in units of $mol.m^{-2}s^{-1}$; the amount of water vapour in moles per unit surface per unit time. The method used to calculate g_m is shown in Appendix I. A chart on page 137 in Appendix II lists all of the major symbols and acronyms used in this body of work.

There are several conditions that must be adhered to if accurate results are to be obtained. A diffusion porometer must use dry input air to obtain correct values, and relies on silica gel to do this. Silica gel lasts for less than 4 hours with a relative humidity (RH) of 75% and 25°C and because RH values rise slowly when silica gel becomes more saturated, the errors may not be immediately recognized (McDermitt, 1990). Leaf temperature can read falsely high if not controlled during conductance measurements. The best way to do this is to keep the cuvette open and in shade when not measuring. The chamber must be clean; the water absorbed or lost by any dirt in the chamber increases the time required to reach steady-state RH, which lengthens the time required for measurement. Assuming that the measured transpiration rate equals the field transpiration rate can lead to incorrect conclusions about data, since leaf and air temperature and boundary layer conductance often have different values for the porometer than leaves in the field, as do the values of g_m and vapour pressure deficit (VPD) on occasion (McDermitt, 1990).

Water Flux measurements

Water potential (ψ) is a thermodynamic quantity related to the physiological and biochemical processes which control plant growth. ψ is the free energy content of water; its capacity to do work; it indicates water demand (Fitter and Hay, 1987). Values are denoted in bars (1 bar=100 J/kg=100 kPa). Irrigated lands often exhibit a ψ of -5 bars; the permanent wilting point of most plants occurs in the region of -15 bars.

ψ can be measured using a variety of instruments. The most well known method utilizes a pressure chamber (Scholander and Hammel, 1965). The chamber relies on the fact that the water column in xylem is under tension. A plant sample is sealed in a chamber and nitrogen gas is forced into the chamber, causing the water in the plant sample to be forced through the xylem until it appears at the cut end. The pressure indicated on the pressure gauge at this point is the ψ, and it equals (with opposite sign), the tension on the water column in the xylem at the time that the sample was cut (PMS Instruments, Model 1000 Manual). The pressure chamber is

a highly accurate method of measuring ψ, although since ψ changes as the environment changes, alterations in the microclimate can result in falsely low or high readings.

A second method used to measure ψ is the psychrometer. There are several types of psychrometer; soil, leaf-disc, chamber and mount. However, they all rely on the same basic principle. A psychrometer utilizes wet bulb depression while a hygrometer operates in dewpoint mode. For an excellent review see Savage and Cass (1984). Generally, a psychrometer consists of 2 chromel/constantan thermocouples in series; one in the chamber air and the other extending to the sample surface. The former is large and measures the chamber air. The latter is very thin, so that when a current is passed through the two junctions, it cools rapidly by the Peltier effect. The cooling current causes air in the chamber to condense on the smaller thermocouple, forming a drop; the "wet bulb" (Salisbury and Ross, 1978). The difference between the temperatures of these two thermocouples is the water potential of the air in the chamber, and can also be described as the measure of the temperature gradient between the sample and the measuring junction. A microvoltmeter is

used to convert this temperature gradient into a voltage output. ψ in bars is determined by comparing this voltage to voltage outputs of known salt solutions. This method relies heavily on temperature for the determination of ψ, so the accuracy of the method is heavily affected by temperature gradients. This has been a problem in the past (Savage and Cass, 1984), but improvements in instrumentation of both hygrometers and psychrometers have resulted in a more accurate method (Turner *et al.* 1984; Dixon and Tyree 1984).

Microclimate measurements

Air temperature, humidity, sunlight and wind are factors that, along with stomatal opening and closing, affect the rate of transpiration (Raven *et al.* 1992). It is necessary to measure the factors affecting the rate of transpiration because these represent the water demand of the plant (PMS Instruments, Model 1000 Manual). Wind velocity is generally measured by an anemometer; wind speeds are measured in metres per second. The VAISALA humidity probe

measures relative humidity as a percent using a polymer sensor that operates according to the capacitive principle. Air temperature is measured in °C utilizing thermocouples that relay readings to an electronic chart recorder. Light levels are measured by a quantum sensor attached to a chart recorder. The index of measurement used is photon flux density (PFD); the number of photons incident per unit of plane surface per unit of time in the 400-700nm waveband. The number of photons is translated as moles, the unit of plane surface is metres and the unit of time is seconds; the final measurement is μmol.m^{-2}s^{-1} (Pearcy *et al.*, 1991).

B. Field Studies

METHODS AND MATERIALS

A. Study Site

The Oldman River is located in the Interior Plains of

Canada. The plains consist almost entirely of sedimentary

rocks overlain by glacial deposits, such as the

glaciolacustrine tills that dominate the research site

(Hydrological Atlas of Canada, 1978). The specific components

of the sedimentary rocks at the site are sandstone, shale and

concretions (Jackson, 1981). The site itself is located at

49°38'N 112°48'W/O, approximately 25 km east of Granum, in

southern Alberta, and adjacent to the Oldman River. This

abandoned gravel pit ceased production in early 1973, and the

next spring was very wet, as evidenced from meteorological

data from Environment Canada. The bare, sandy soil of the

gravel pit, saturated with water by high spring rainfall,

allowed poplar seeds (released that spring) to establish.

Many of the poplar seedlings established that spring now

reach more than 5 metres in height. This has occurred despite

lack of contact with a permanent water table. The gravel pit

system therefore provides a natural analogue for studying the effects of drought stress in a naturally seeded hybrid poplar complex.

B. Hydrology and groundwater characteristics

Figure 2.1 shows the 30-year normalized values (constant measure average) for the wind velocity, air temperature and precipitation for the measurement station nearest to the site. The data indicate that the Lethbridge area experiences consistently high prevailing westerly winds throughout the year, a maximum daily average summer temperature of 25.9°C, and a pattern of precipitation indicating that May and June traditionally receive more rainfall than any other months. This heavy spring rainfall would coincide with seed release from the trees, allowing seedling establishment.

Before the Oldman River dam became operational, flow rates and patterns on the Oldman River upstream and downstream of the Oldman River dam site were nearly identical, as shown by the 1990 discharge patterns in Figure 2.2. The upstream flow pattern was determined by combining

Figure 2.1. 30-Year Normals of the Lethbridge Area (after Environment Canada).
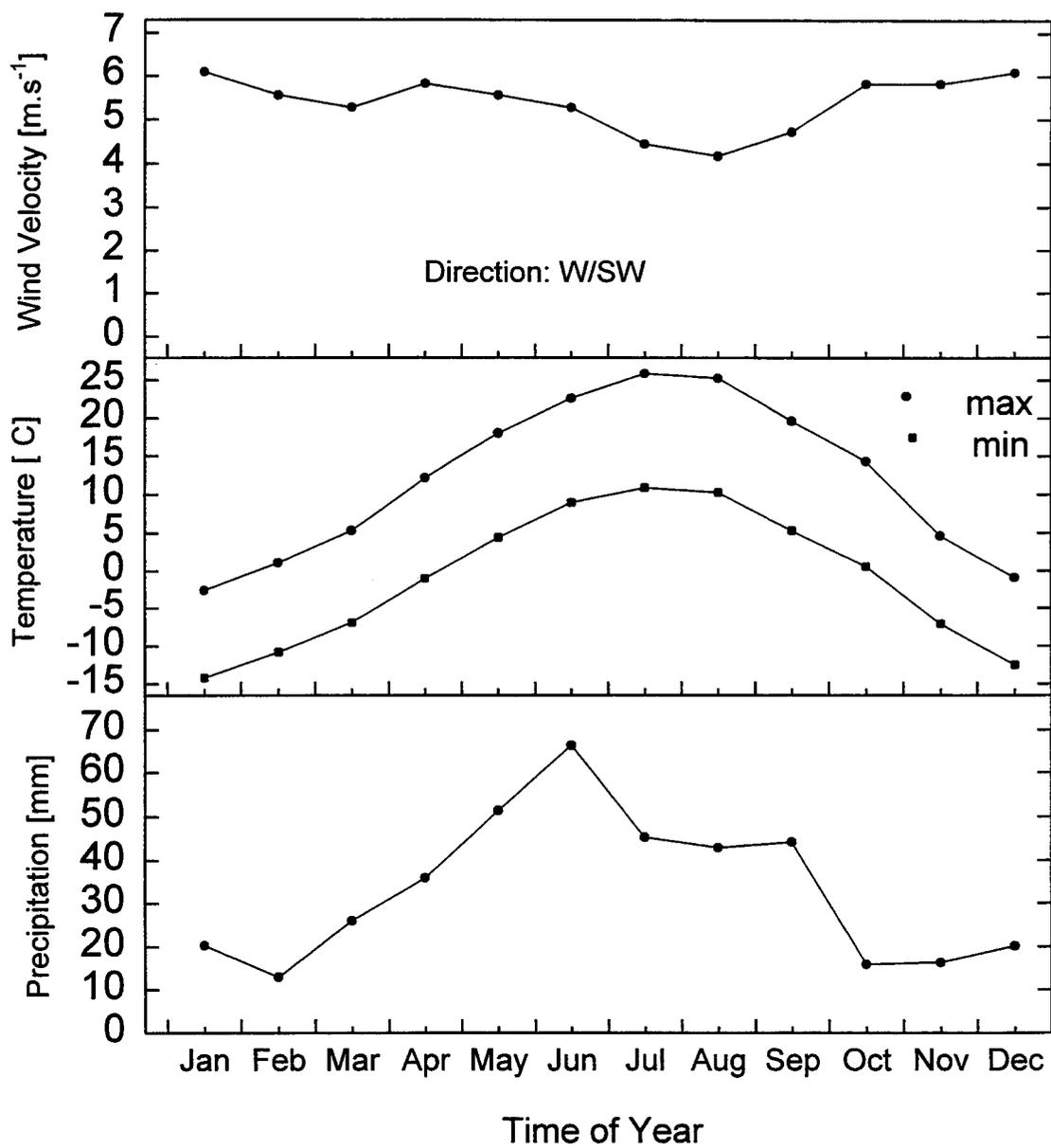
* precipitation=rainfall+snowfall

Figure 2.2. 1990 Discharge Patterns Upstream and Downstream of the Oldman River Dam Site (after Environment Canada).
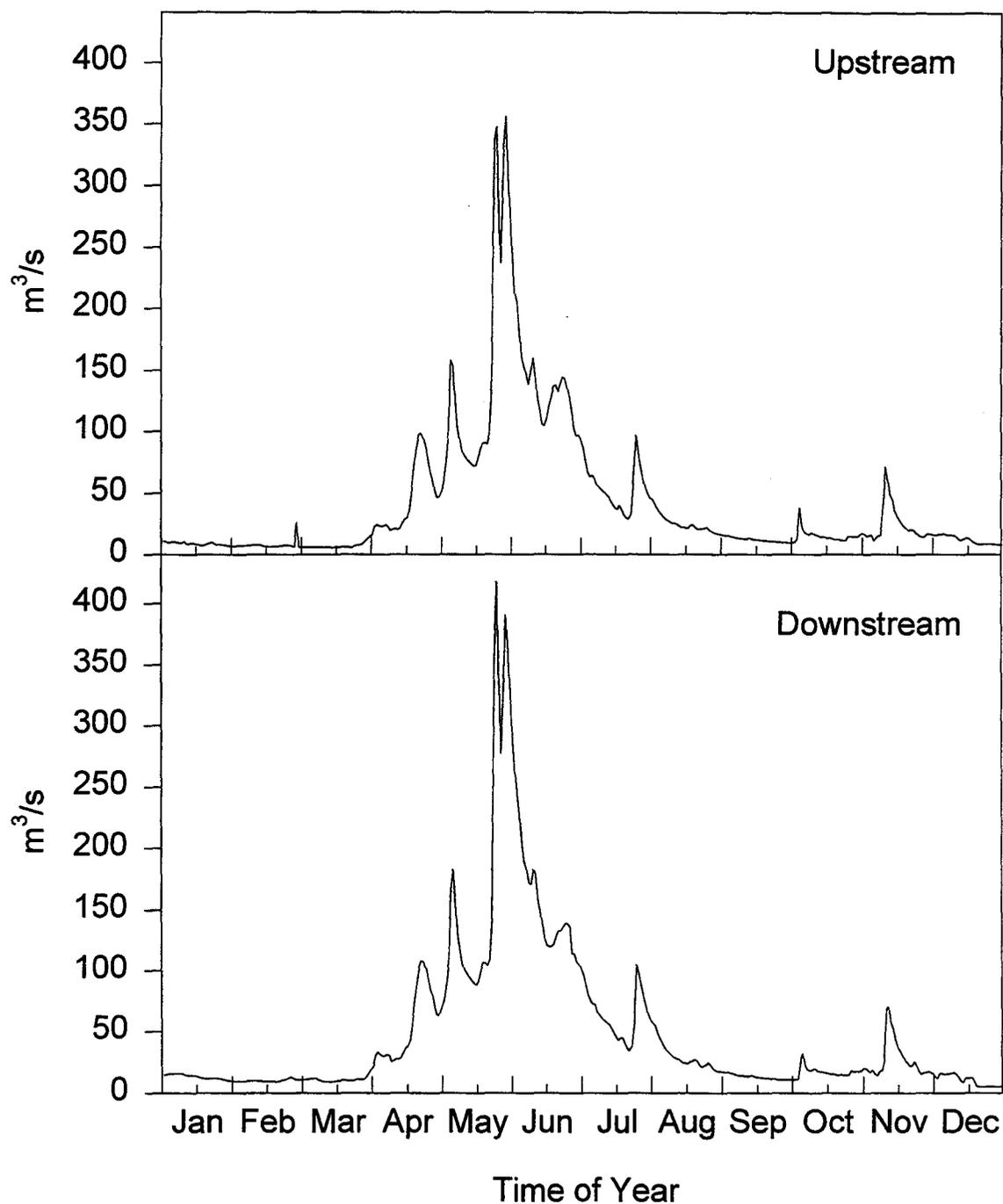
data from three measuring stations located upstream of the dam site, while the downstream values were obtained from a measuring station approximately 5 km downstream from the site. The 1991 and 1992 values were obtained in the same manner. The similarity of the upstream and downstream patterns facilitated seedling establishment that spring. The following year, in 1991, the dam was partially operational, and as a result the spring peak did not occur (Figure 2.3), which prevented widespread seedling establishment (D. Coxson, unpublished). Despite the alteration to the natural pattern of flow, flow rates downstream still peaked because of heavy rains late in June that filled the dam reservoir to capacity and caused overflow, as the spillway gates were not installed (FEARP, 1992). By 1992 the dam was fully operational, and although 1992 was a dry year (Environment Canada), the altered flow pattern is still clearly visible (Figure 2.4). In some months, the maximum discharge value during that month was as much as seven times lower when compared with comparable values from the two previous years. No new seedling establishment was observed in 1992, and the majority from 1990 had died due to lack of water (D. Coxson,

Figure 2.3. 1991 Discharge Patterns Upstream and Downstream of the Oldman River Dam (after Environment Canada).
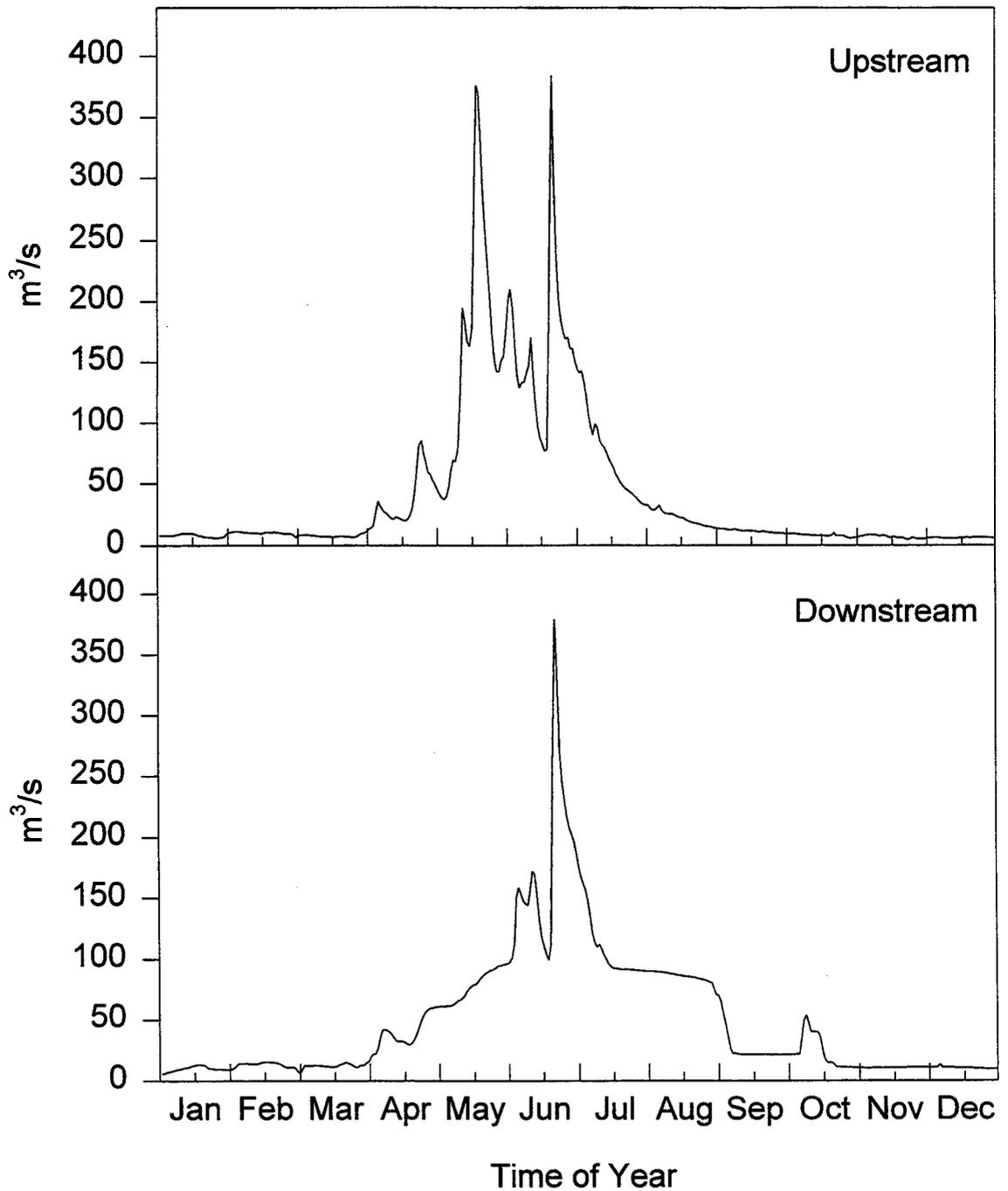
Figure 2.4. 1992 Discharge Patterns Upstream and Downstream of the Oldman River Dam (after Environment Canada).
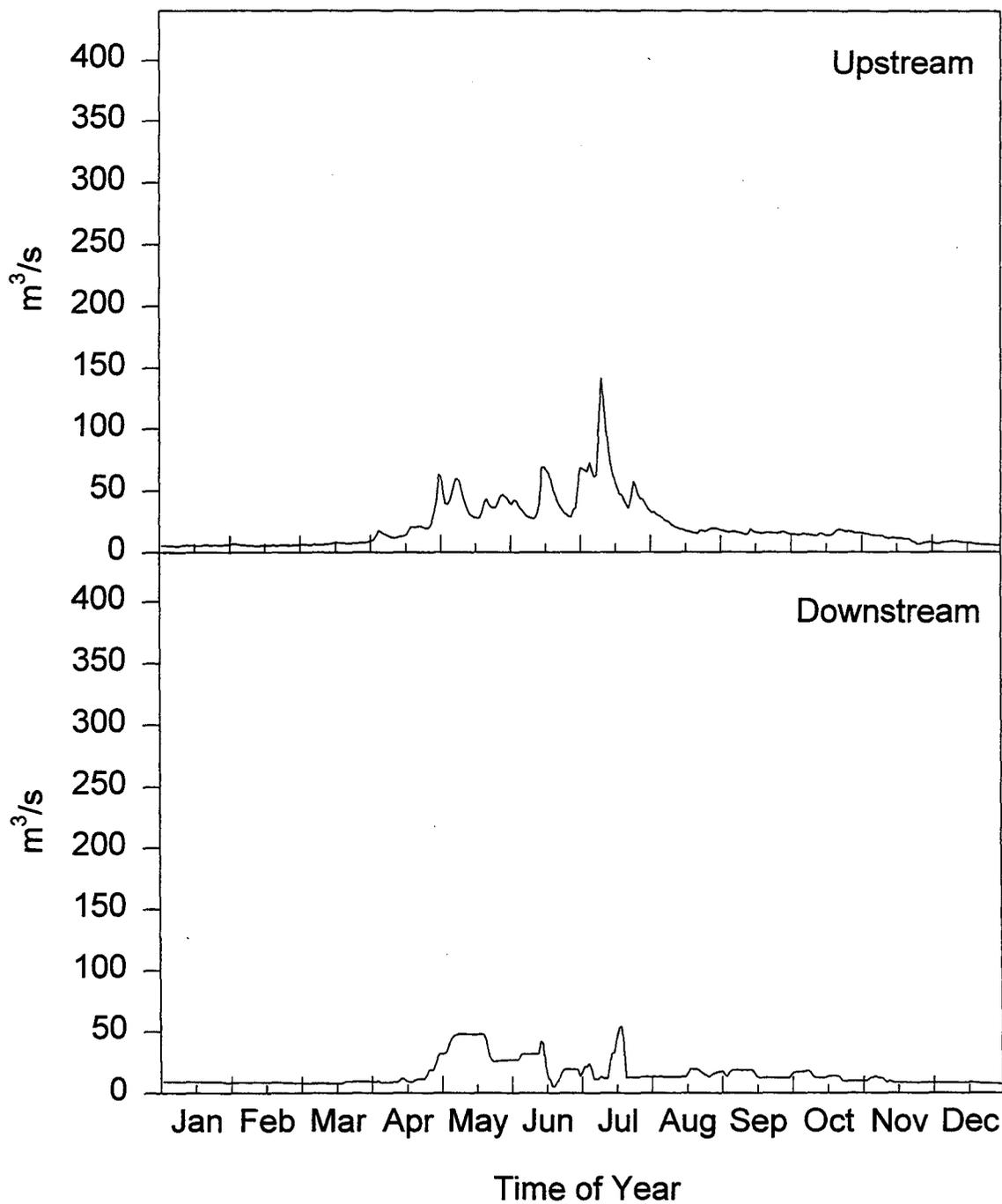
unpublished).

C. Experimental Protocol - Field Studies

Two poplar species, <u>Populus balsamifera</u> (PB) and <u>Populus angustifolia</u> (PA) and their interspecific hybrids were studied in an upland gravel-pit site near Lethbridge on the Oldman River. All trees studied were chosen according to the criteria of height being less than 2 m, a canopy of healthy leaves, side branches at varying levels of the tree, lack of insect infestation and proximity to each other (for ease of instrument use). All of the trees were naturally present; they were not cultivars. Study trees were chosen to represent a given species or hybrid on the basis of foliar characteristics, rather than genetic analysis. Characteristics were taken from Moss (1983). This method has been used in a number of studies (Rood *et al.* 1986; Keim *et al.* 1989; Campbell *et al.* 1993).

<u>1992</u>

Three individual trees of each species and hybrid were studied over a one-week period during a period of good weather in June 1992. In order to facilitate comparison, the

hybrids will henceforth be referred to as a species. Days 1-3 and 5 were used. Each day, three sets of physiological measurements were taken using the LI-COR (Lincoln, NB, U.S.A.) diffusion porometer. Transpiration, diffusive resistance and leaf temperature were among the variables measured. Measurements began at approximately 0800 hours and were performed every three hours with the last set finishing by 1930 hours. These physiological measurements were complemented by water potential measurements. A number of instruments were used to collect this data. The pressure bomb (Model 1000, PMS Instruments, Corvallis, Oregon, U.S.A.) was utilized on Days 1 and 3 for the trees used for collection of the physiological data, as well as trees that fit the general criteria but were not used to collect physiological data. Stem psychrometers (Plant Water Status Instruments, Guelph, CAN) were used daily morning and night as were the SHAS auto-samplers (Lander Control Systems, Guelph, CAN). The auto-samplers were affixed to study trees, the majority of the stem psychrometers were not. These SHAS systems measured PA and PB only; measurements of PA did not extend beyond Day 3 due to unforeseen equipment difficulties. Humidity

measurements were made using a VAISALA humidity probe (Vaisala Oy, Helsinki, Finland) and wind measurements were made using an anemometer (Omega, Stanford, Connecticut).

In order to facilitate the identification of trends, a transform was performed on the X and Y axes of the stomatal conductance data for all species and their individuals. The X-axis transform allowed comparison because it repositioned the solar maximum of each individual at the same X-axis coordinate. The X axis values were simply rated on a 24 hour time scale and plotted accordingly. Transformation of the Y-axis standardized absolute rates of stomatal conductance, thus amplifying the plotting of the X-axis. The Y axis was adjusted to 100%; each individual's maximum value (of the means) was assigned the value of 100 and all other values were ranked accordingly.

1993

This study differed from its predecessor in several respects. Over a one-week period, days 1, 3 and 8 were used as measurement days. The physiological responses were also

measured pre-dawn to achieve a more complete diurnal pattern. Five leaves were measured per individual (the number of individuals was unchanged from 1992). Water potential was measured exclusively by the pressure bomb, three to five measurements were performed over the course of each day, and only the study trees were utilized.

RESULTS

Daily patterns of g_l and ψ

All values are a mean of three individuals; three
repeated measurements were taken for each individual.
Negative error bars were used to indicate standard deviation
of the mean. VPD calculations are shown in Appendix I. Figure
2.5 shows the leaf stomatal conductance (g_l) of the three
species over the four-day experimental period in 1992. The
pattern of g_l within a species differs for each experimental
day as do the patterns between species. Day 2 (June 19) is
the exception; g_l reached an early morning maximum and
declined for the rest of the day. Due to the lack of clear
trends within a species, the individuals of each species were
plotted distinctly; they are shown in Figures 2.6-2.8. Again,
no clear trends are revealed, probably because of large
variation within some of the individuals coupled with
different periods of maximum solar exposure for each branch.
Figures 2.9-2.11 represent the same data shown in the above
figures, but with the X and Y axis transformed. The pattern
of PA is distinct from that of the other two species save on

Figure 2.5 . Stomatal Conductance [mol.m^{-2}s^{-1}] in Field Season of 1992.
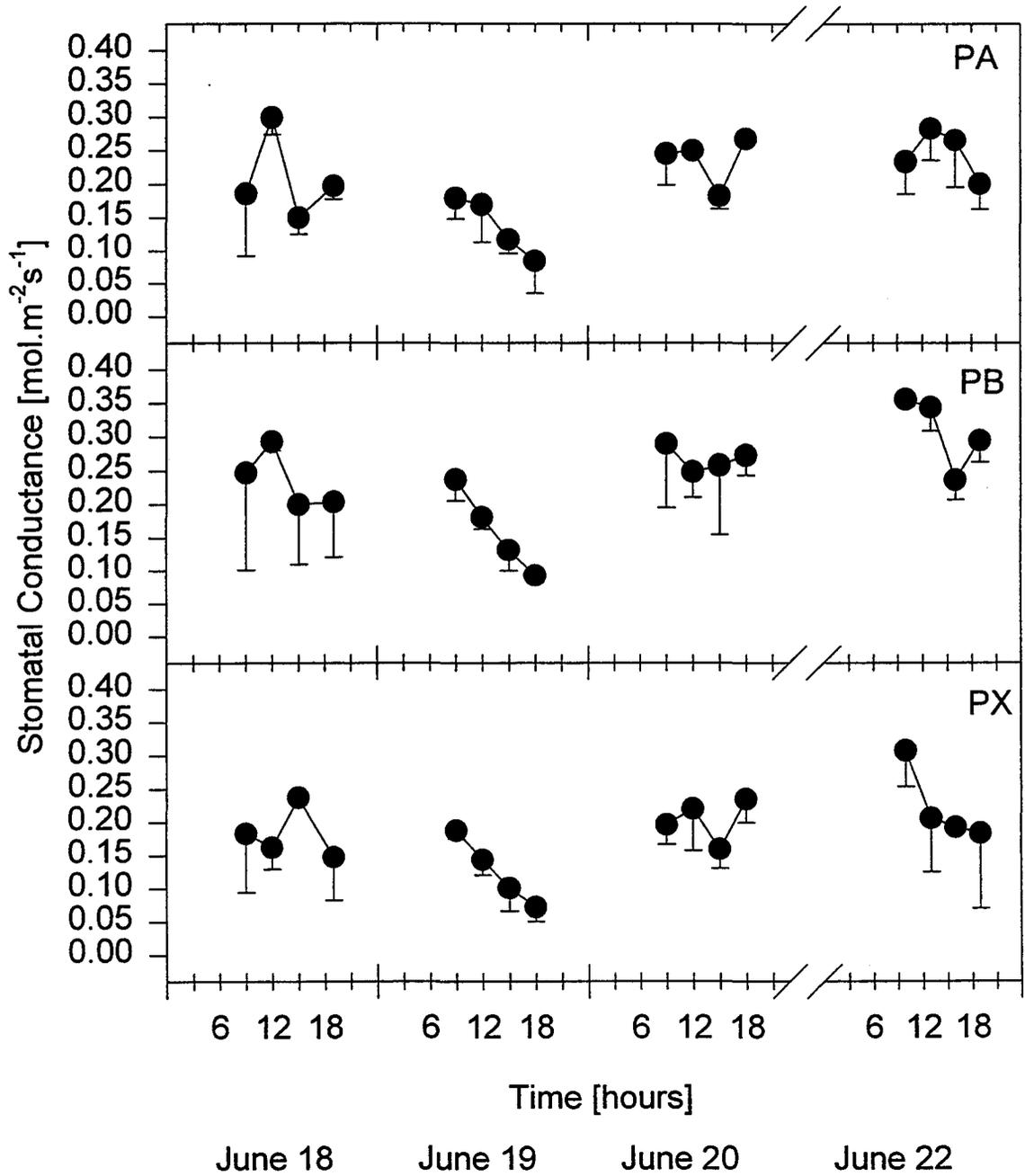
Figure 2.6. Stomatal Conductance [mol.m^{-2}s^{-1}] of PA in the Field Season of 1992.
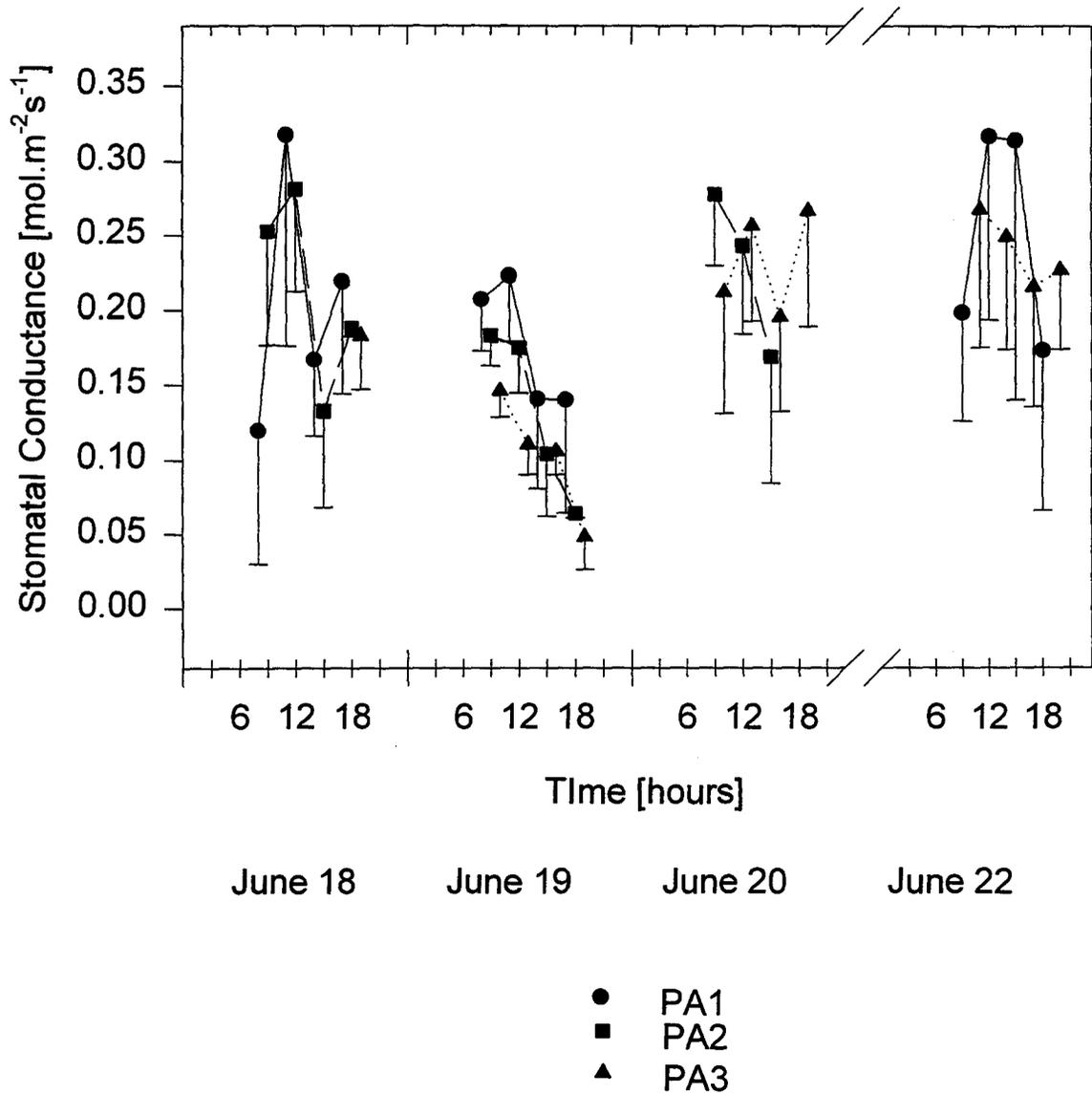
Figure 2.7. Stomatal Conductance [mol.m^{-2}s^{-1}] of PB in the Field Season of 1992.

PB1
PB2
PB3
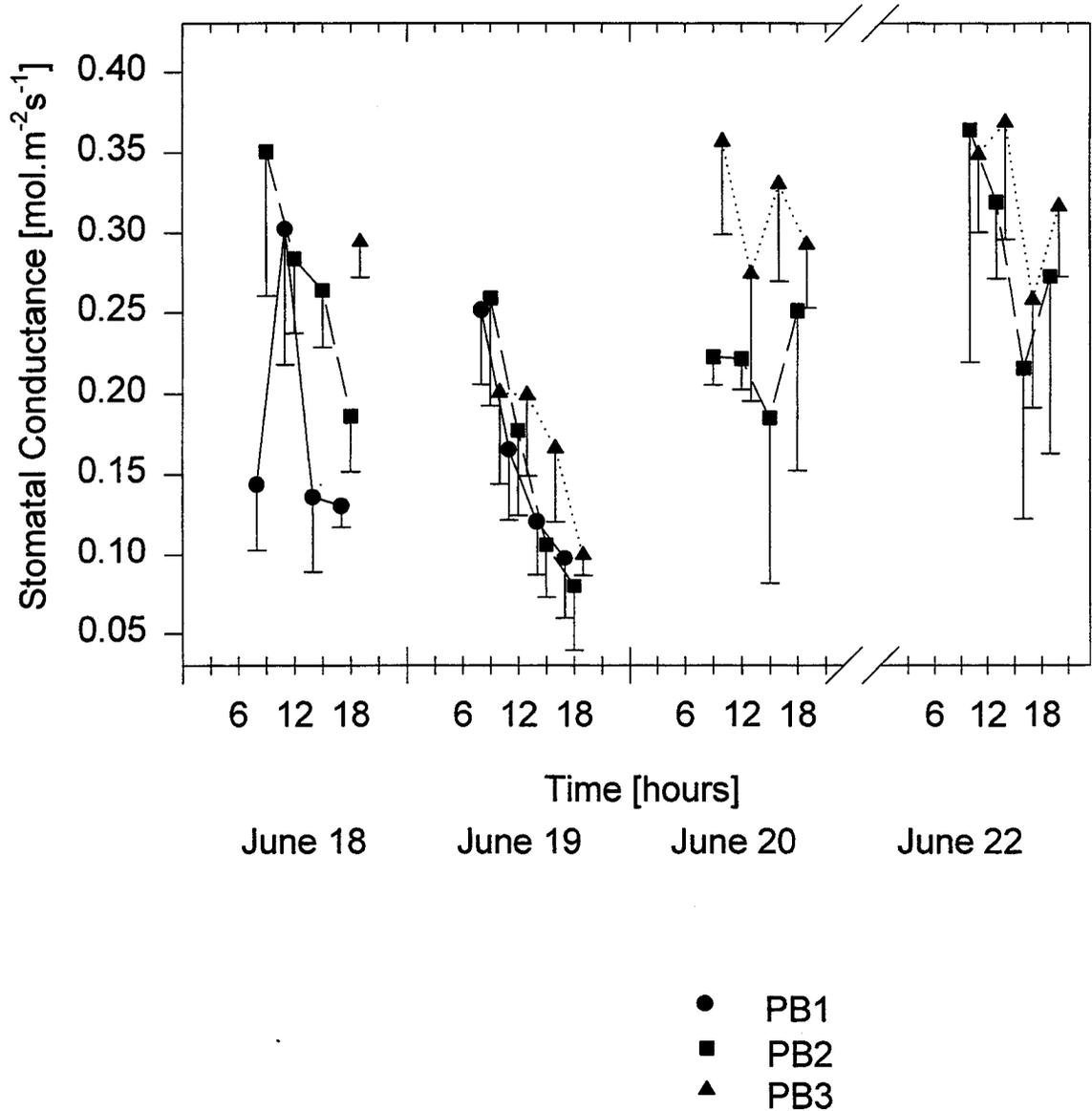
Figure 2.8. Stomatal Conductance [mol.m^{-2}s^{-1}] of PX in the Field Season of 1992.

- PX1
- PX2
- PX3
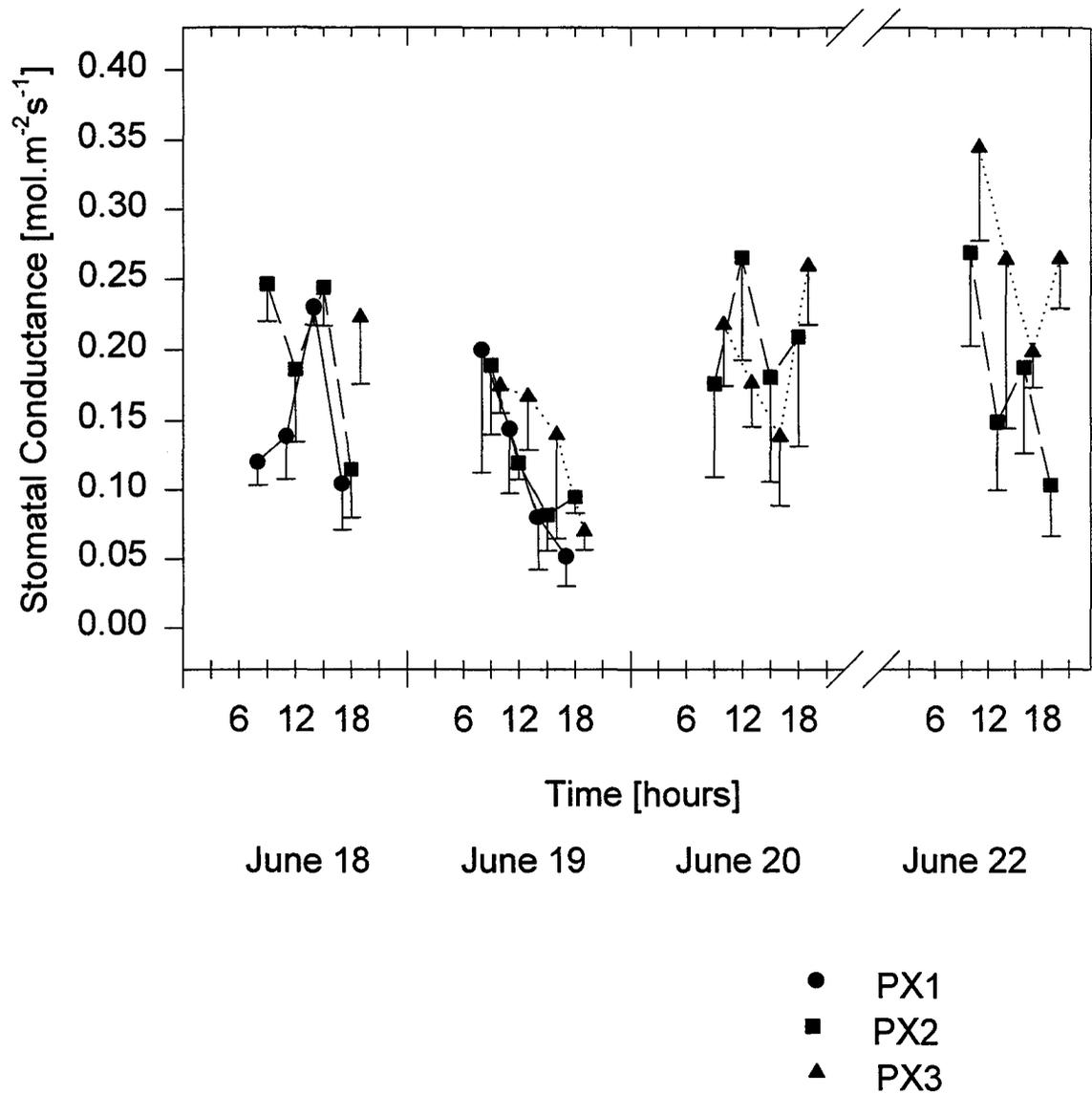
Figure 2.9. Stomatal Conductance Adjustment of PA in the Field Season of 1992.
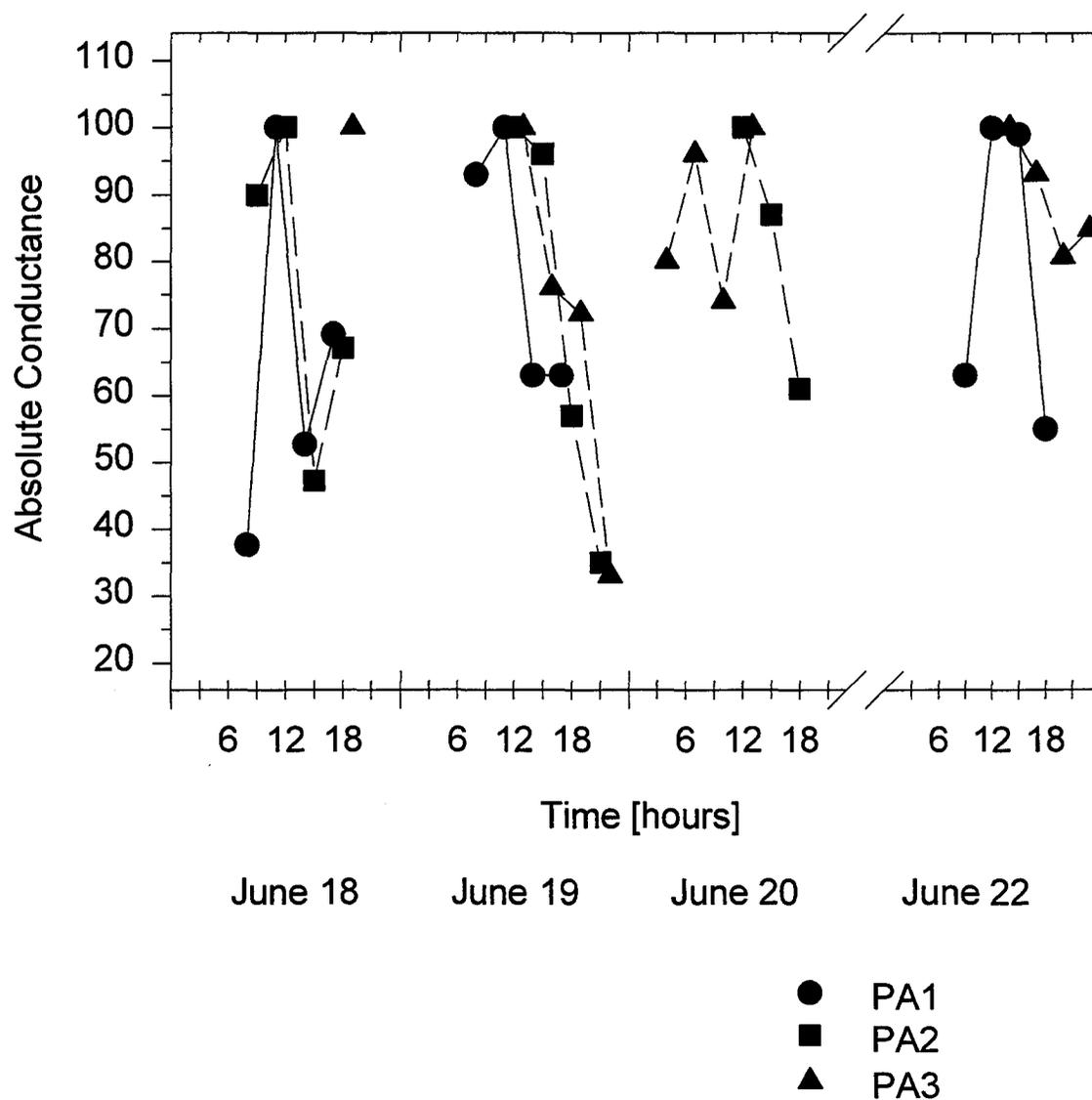
Figure 2.10. Stomatal Conductance Adjustment of PB in the Field Season of 1992.
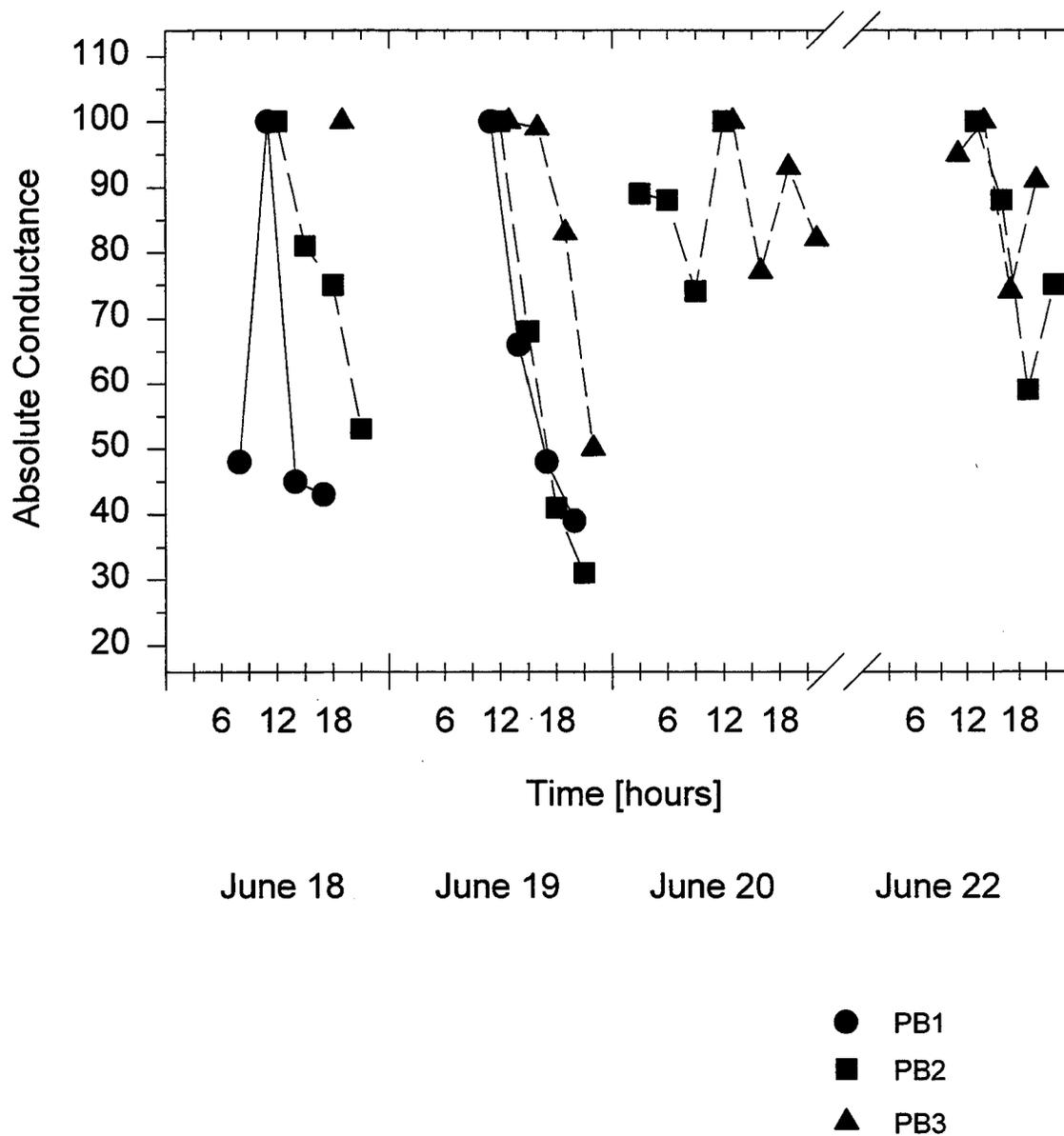
Figure 2.11. Stomatal Conductance Adjustment of PX in the Field Season of 1992.
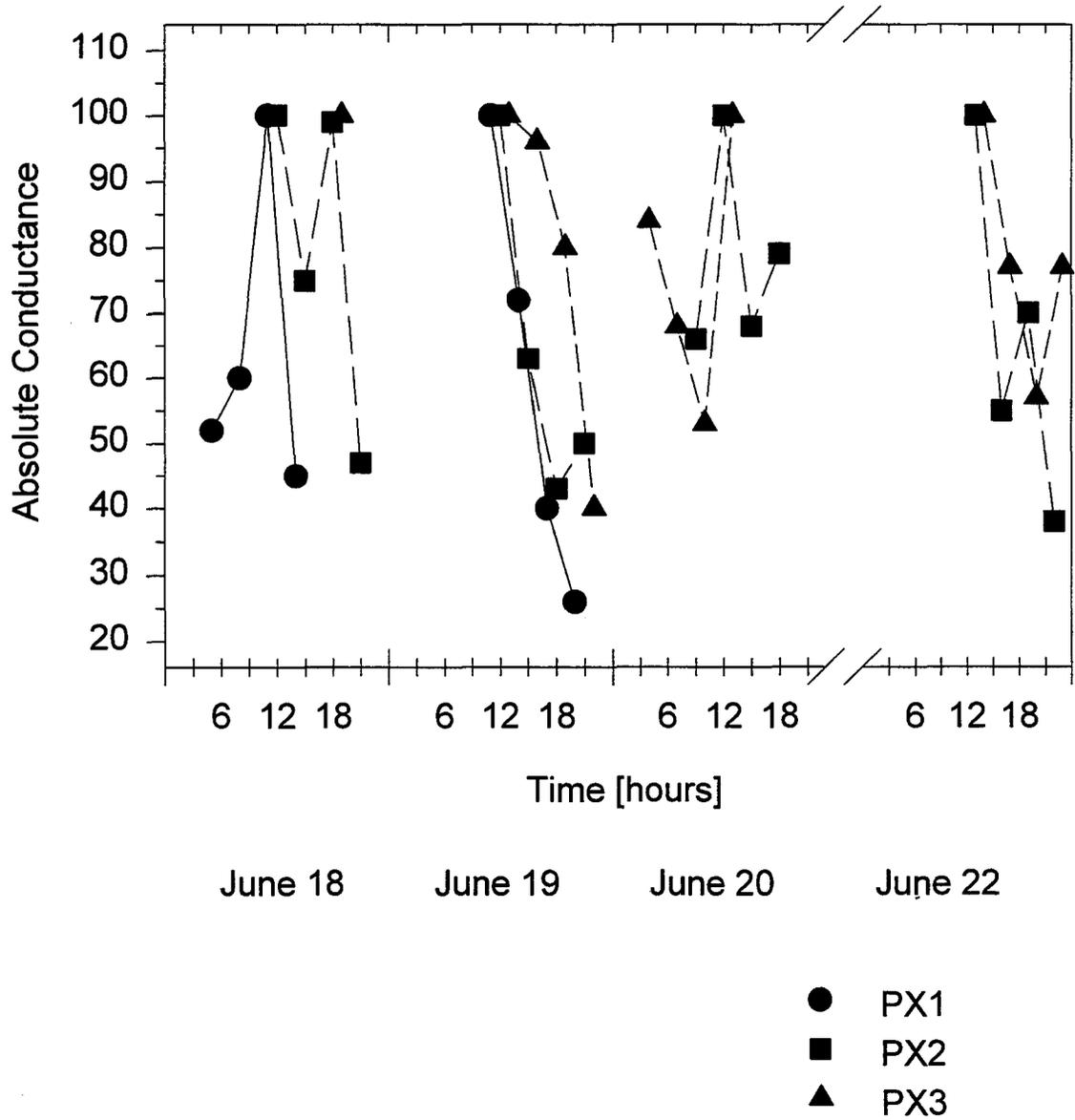
day 2; its pattern is the same as that shown in Figure 2.5.
On days 1 and 3, g_l of PA reached a midday maximum and
exhibited partial afternoon stomatal closure. On day 3, PA
exhibited partial midday stomatal closure and increased to an
early afternoon maximum before falling again. PB and PX
showed similar patterns each day. On day 1, g_l reached a
midday maximum and declined for the remainder of the day. The
day 2 pattern is the same as Figure 2.5. On day 3 g_l decreased
to a mid-morning minimum, increased to a midday maximum and
experienced partial stomatal closure mid-afternoon before
declining again. On day 4, g_l reached a maximum early in the
morning; values declined until late afternoon and then began
to increase again.

The ψ of the species in 1992 are shown in Figure
2.12. All species were measured using the pressure chamber on
days 1 and 3; PA and PB were also measured daily using
psychrometery while PX was not. Based on the pressure chamber
data, the pattern of water loss between species is
consistent, with pre-dawn values never dropping below -0.3
MPa. The psychrometer data indicates that the general pattern
of ψ is a decrease throughout the day to an evening minimum

Figure 2.12. Water Potential [MPa] in the Field Season of 1992.
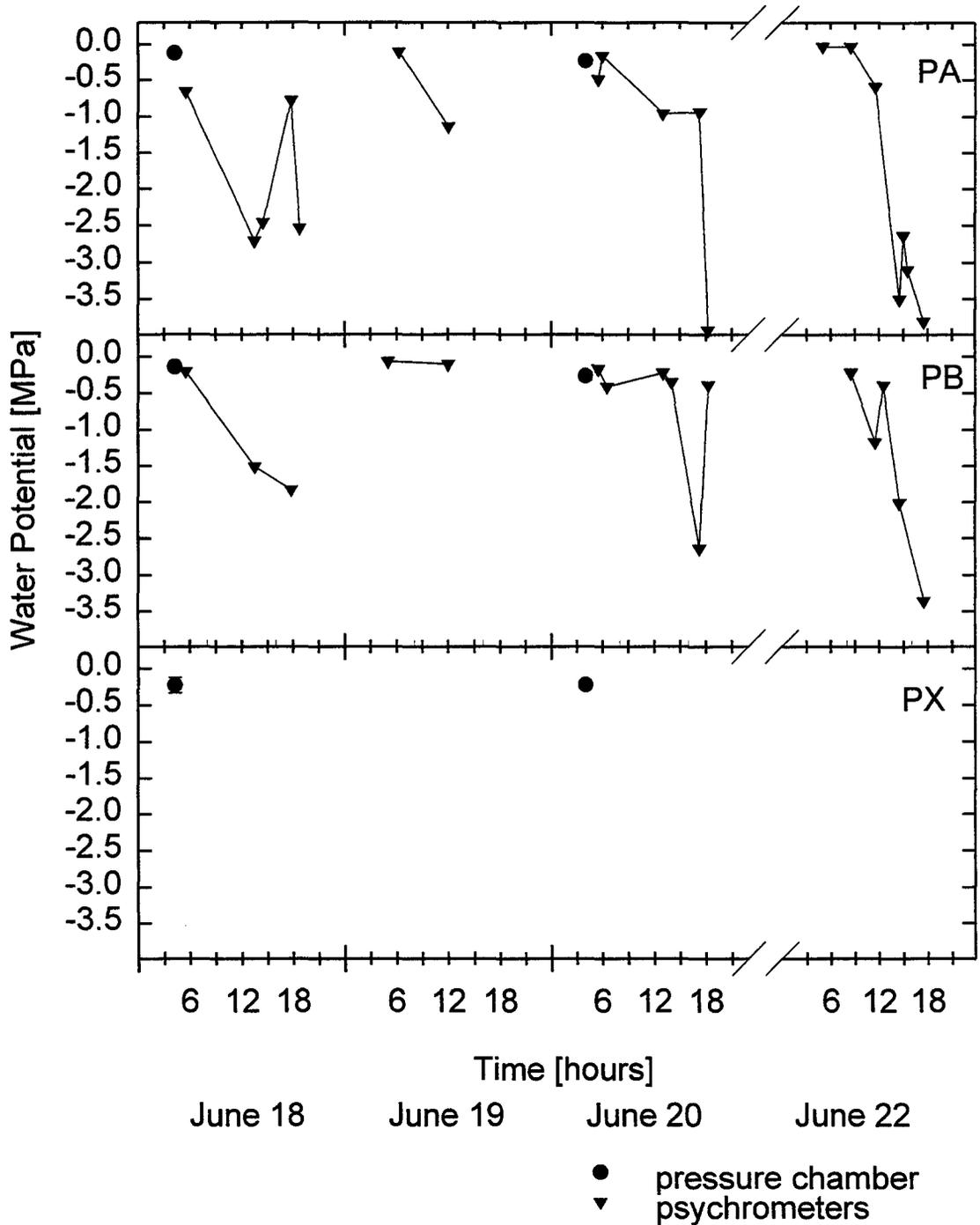
with several minor exceptions. On day 1, ψ in PA decreased at midday and increased mid-afternoon before dropping again; on day 2, PB showed little change in ψ, remaining near 0 MPa at midday and on day 3, PB recovered to greater than -0.5 MPa at the end of the day. Figure 2.13 shows the continuous ψ measurements of PA and PB taken in 1992 by the automated hygrometer sampling system. PB exhibited lower values than PA throughout the entire experiment; values fluctuated between -0.5 MPa and -2.0 MPa for PB and 0 MPa and -0.8 MPa for PA. The pattern for both species is cyclic; pre-dawn values were high and dropped throughout the day to a mid-afternoon minimum before recovering to pre-dawn values near midnight. The changes in the pattern occurred at the same time of the day regardless of species.

Figure 2.14 shows g_l of the three species over the three-day experimental period in 1993. Pre-dawn g_l values were discarded because of dew contamination on the porometer. The general pattern of g_l was as follows; maximum g_l occurred early morning and values decreased throughout the day to a late afternoon minimum. This pattern was most pronounced on day 1, when the initial conductance was as high as 0.8 $mol.m^{-2}s^{-1}$;

Figure 2.13. Continuous Water Potential Measurements using SHAS instruments in the Field Season of 1992.
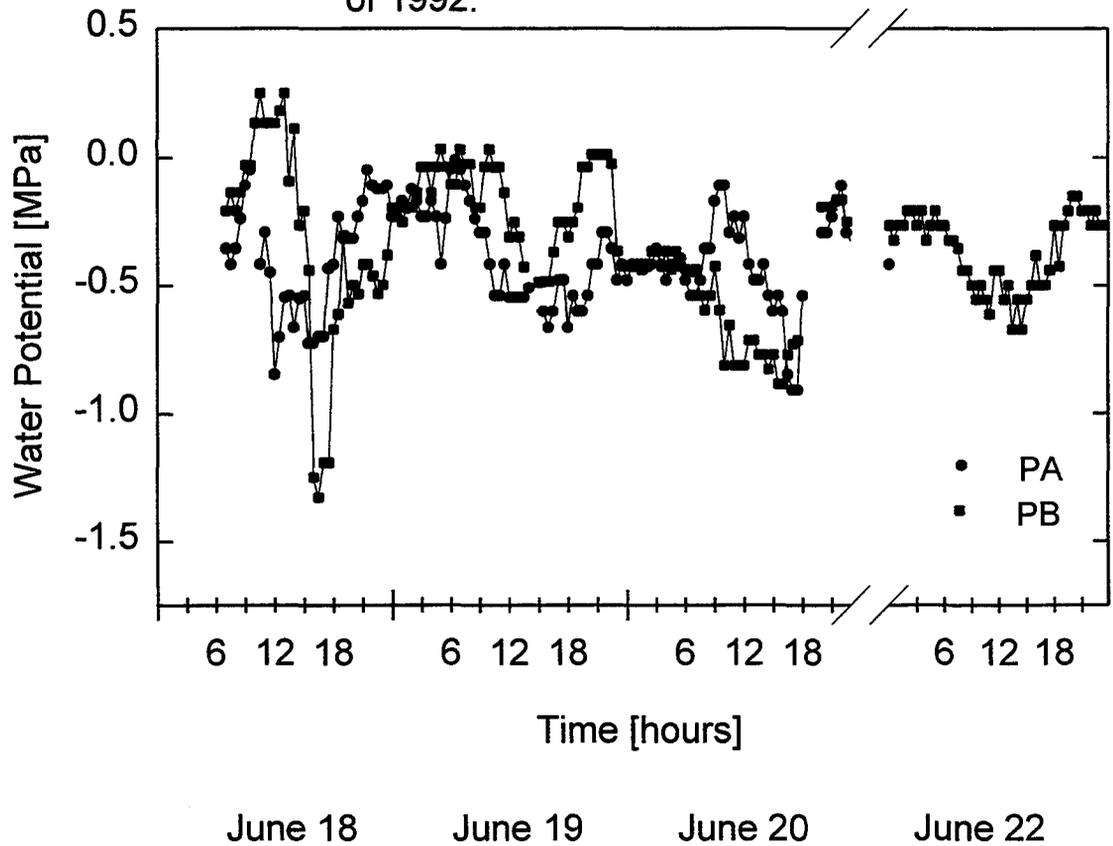
Figure 2.14. Stomatal Conductance [mol.m^{-2}s^{-1}] in Field Season of 1993.
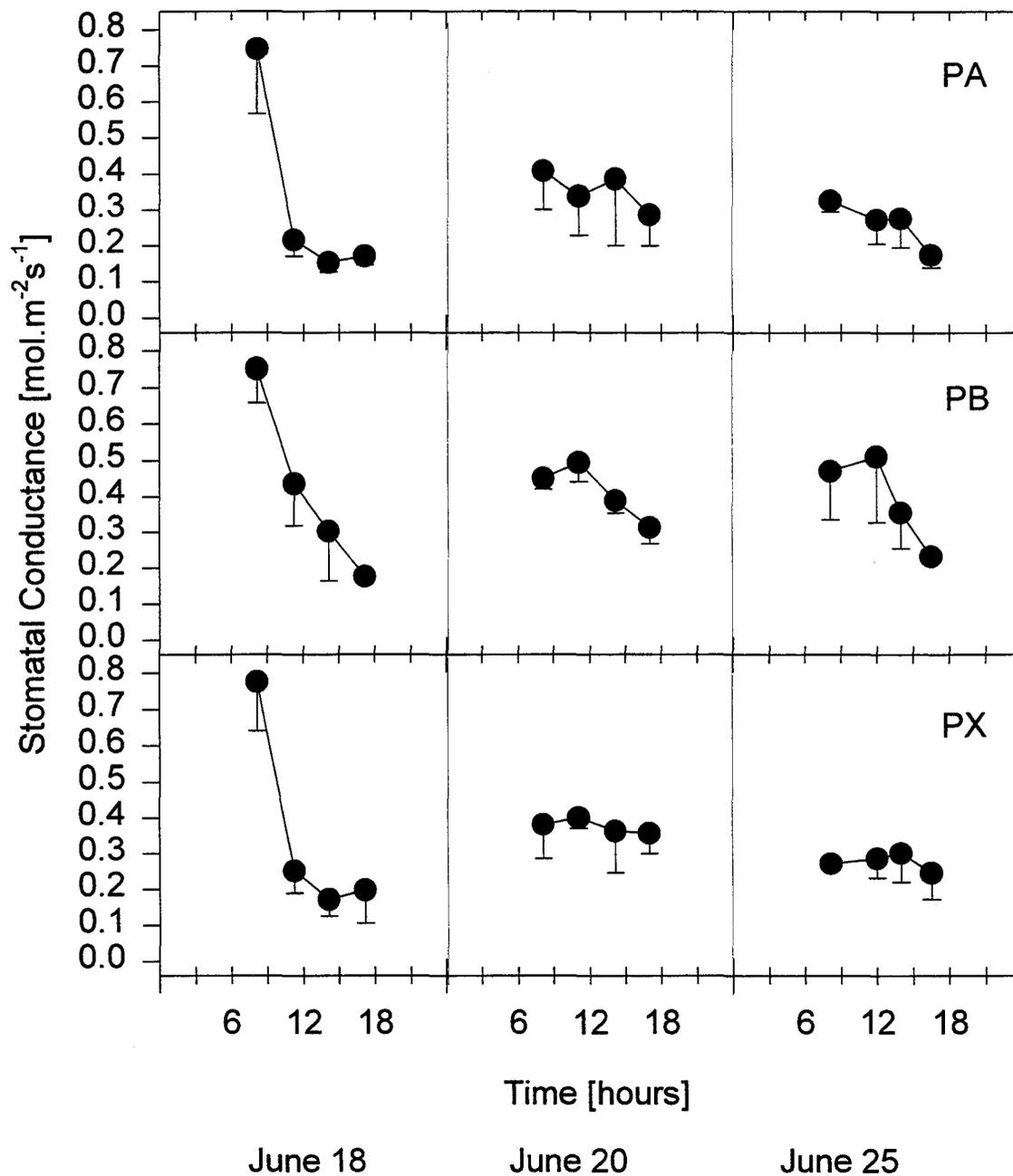
patterns between the species were virtually identical. Patterns differed between species on days 2 and 3. PA exhibited different patterns both days; on day 2 values fluctuated within a narrow range and on day 3 the general pattern was observed. In PB, g_l increased to a late morning maximum before dropping the rest of the day. g_l values of PX exhibited little change regardless of time; values remained within 0.1 mol.m^{-2}s^{-1} the entire day.

The 1993 ψ values are shown in Figure 2.15. The pattern of water loss between species is consistent, with pre-dawn values never dropping below -0.2 MPa. On all days for all species, ψ declined from its pre-dawn value to a midday minimum, followed by a late afternoon increase. There were two minor exceptions; day 2 values showed only a slight late afternoon increase, and the day 3 values of PX showed virtually no recovery.

Microclimate Variables

The leaf temperatures of the study leaves used during the 1992 field season are shown in Figure 2.16. The temperature showed great diurnal variation but varied little

Figure 2.15 . Water Potential (±SD) in Field Season of 1993.
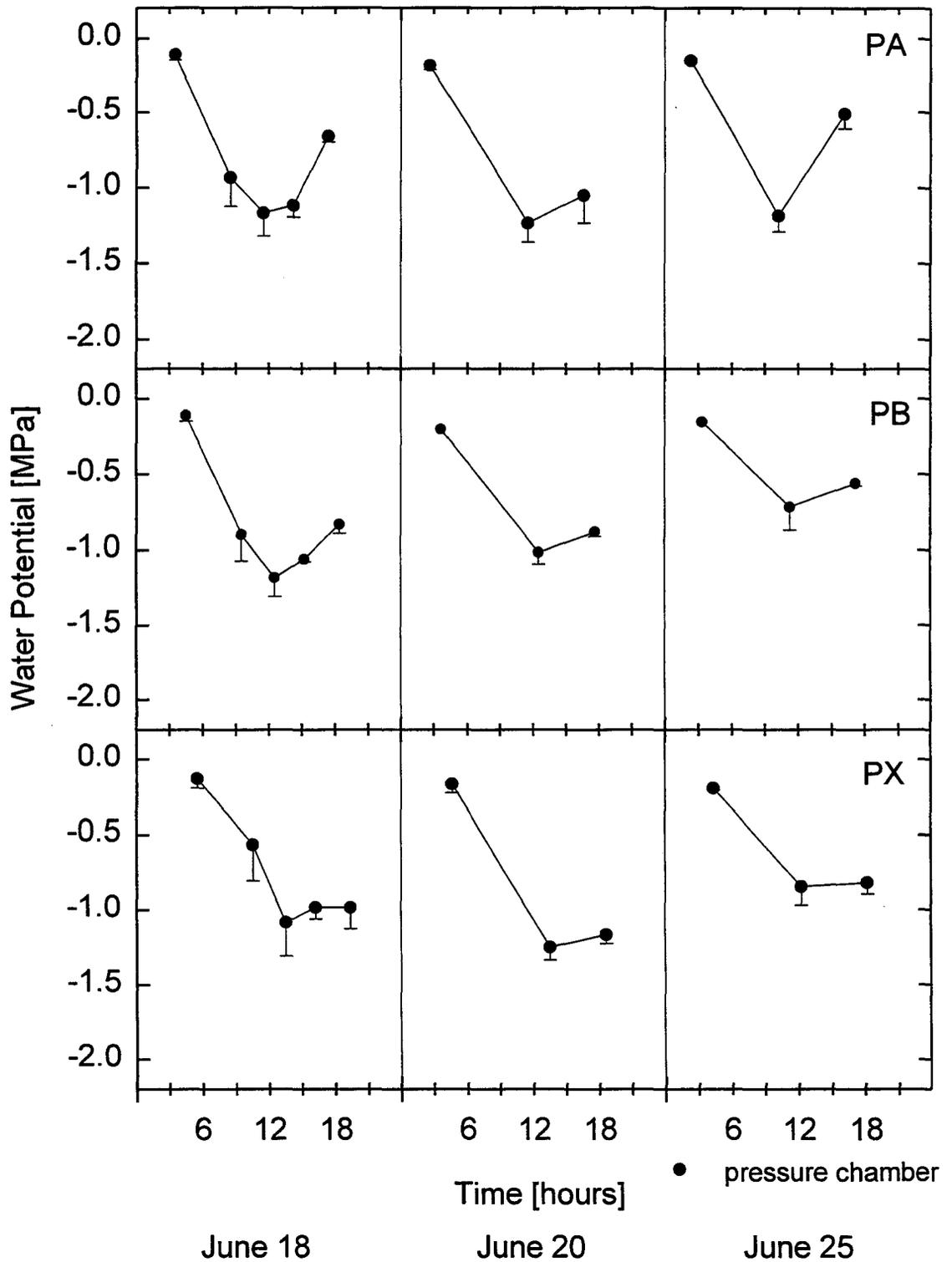
Figure 2.16 . Leaf Temperature (±SD) in Field Season of 1992.
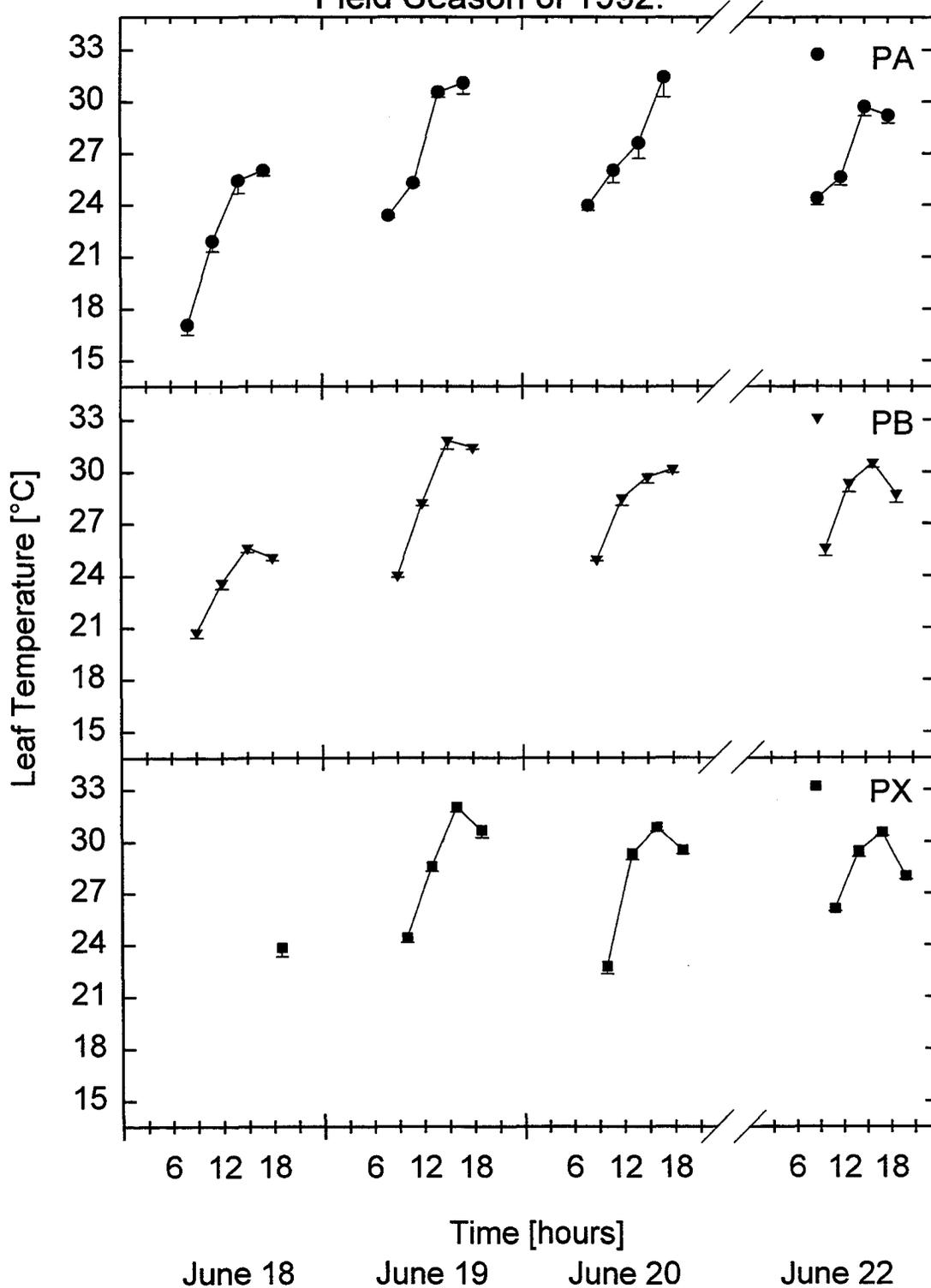
either between the species nor from day to day. The pre-dawn temperature was >18°C and rose to a midday maximum no higher than 33°C.

Figure 2.17 describes the field microclimate during 1992. Light intensity was measured in $\mu mol.m^{-2}s^{-1}$; early morning values were as low as 275 $\mu mol.m^{-2}s^{-1}$ and rose to a midday maximum not exceeding 2200 $\mu mol.m^{-2}s^{-1}$ before falling again to pre-dawn values. The high early morning PPFD values on days 2 and 4 may be explained by a complete lack of cloud cover as compared with the other two days. Wind velocity varied each day, but was generally higher in the afternoon than in the morning. This situation was reversed on days 3 and 4; these were windy days with speeds reaching as high as 7 $m.s^{-1}$. Air temperature (T_a) was measured in °C and maintained a fairly constant pattern during the experiment, with minimum early morning values rising to an end-of-day maximum not exceeding 30°C. RH fluctuated in a cyclic manner over the four-day period, decreasing each day to a mid-afternoon minimum of less than 10% and then recovering during the remainder of the day to complete saturation by midnight. Part of day 1 and most of day 3 is missing as a result of

Figure 2.17. Microclimate Data of 1992 Field Season.

Time [hours]

June 18   June 19   June 20   June 22

● Field Site
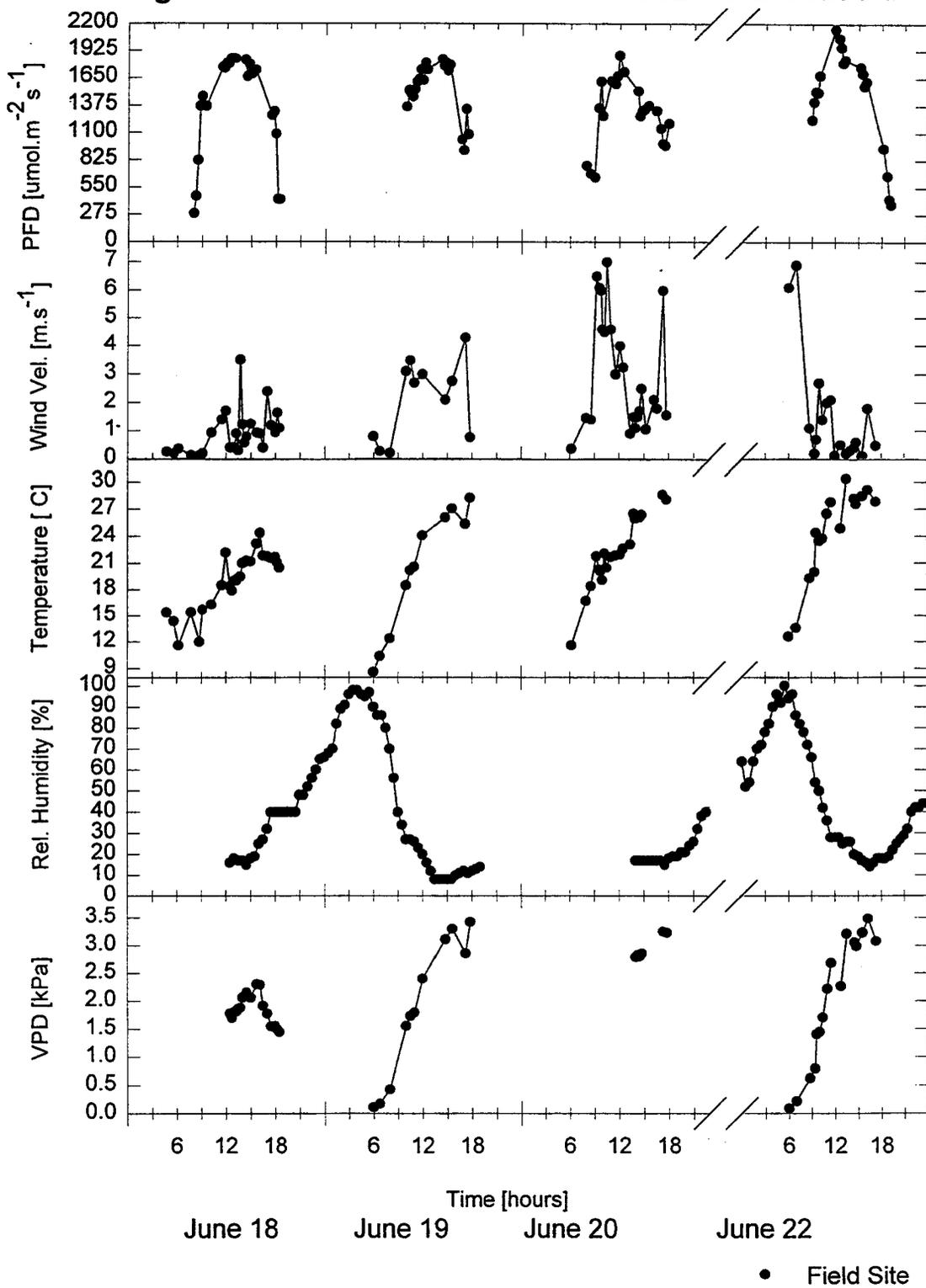
equipment breakdown. Vapour pressure deficit values are incomplete, but from the values plotted, it is clear that VPD closely follows T_a, indicating that VPD is directly related to T_a.

The leaf temperatures of the study leaves used during the field season of 1993 are shown in Figure 2.18. The temperature showed little variation, either between the species nor from day to day. The temperature within each species however, indicated large diel variation; the pre-dawn minimum of approximately 10°C increased throughout the day to a late-afternoon maximum not exceeding 30°C.

Figure 2.19 describes the field microclimate during the study period in June 1993. Light intensity was measured in $umol.m^{-2}s^{-1}$; it decreased to 0 $umol.m^{-2}s^{-1}$ each night and rose to a midday maximum not exceeding 1950 $umol.m^{-2}s^{-1}$ before falling again to pre-dawn values. Wind velocity varied each day, but was generally higher in the afternoon than in the morning; speeds did not exceed 5.5 $m.s^{-1}$ at any point during the experiment. Temperature was measured in °C and maintained a fairly constant pattern during the experiment, with minimum early morning values rising to an end-of-day maximum not

Figure 2.18 . Leaf Temperature (±SD) in Field Season of 1993.
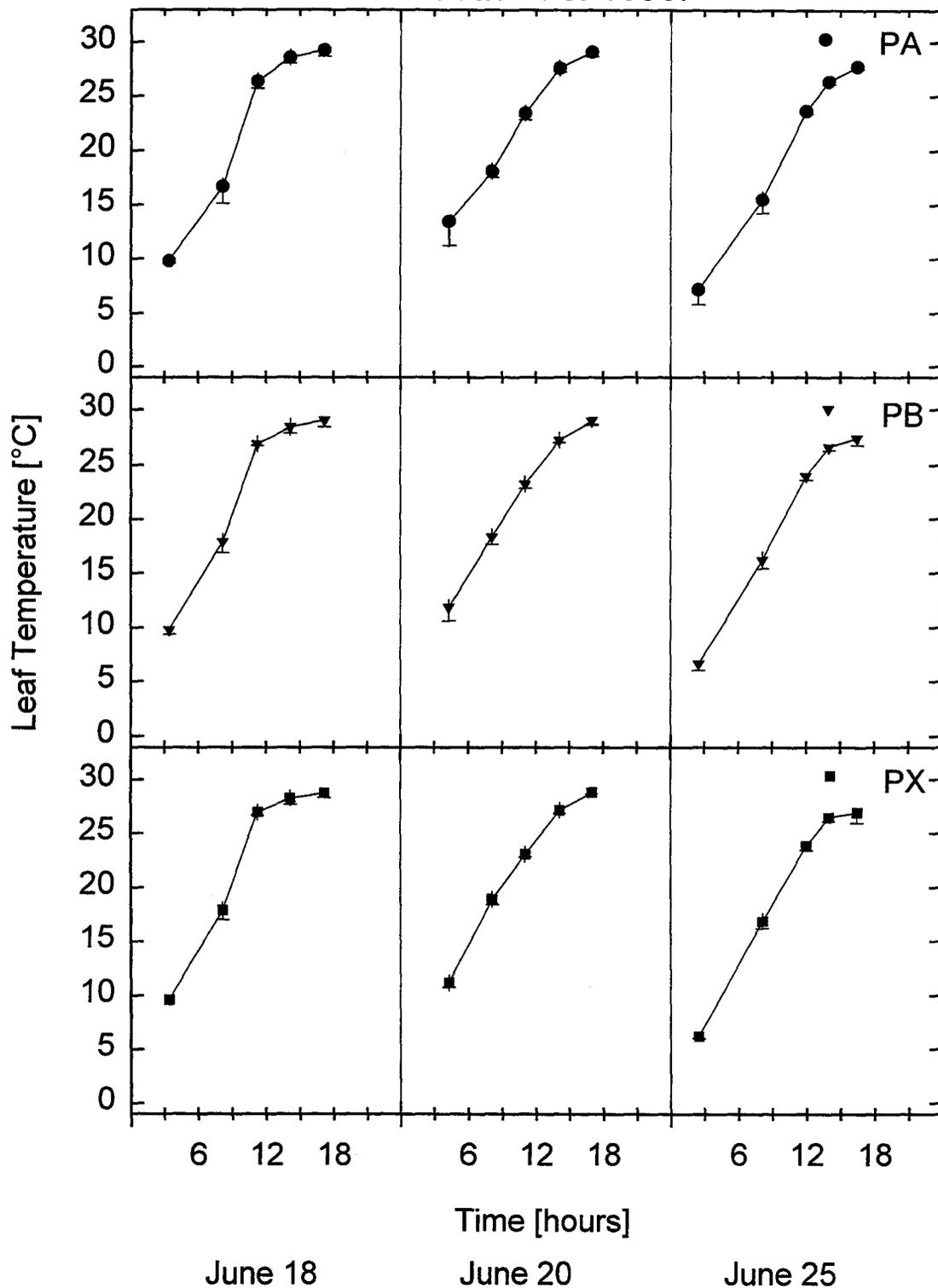
Figure 2.19. Microclimate Data of 1993 Field Season.
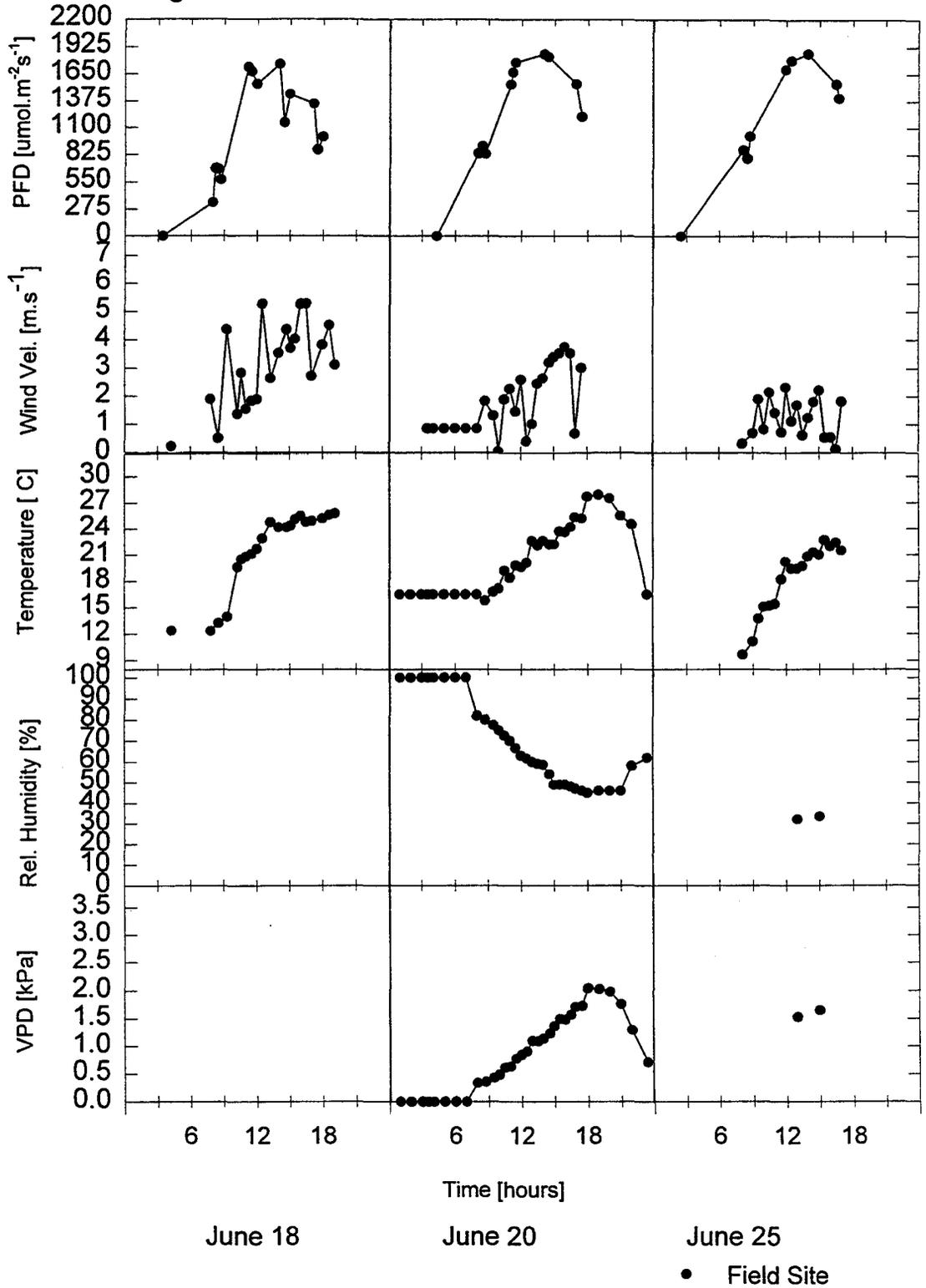
exceeding 28°C before falling again to pre-dawn values. The RH and VPD data are incomplete due to equipment difficulties; from the data available it is apparent that on day 2 RH decreased to a mid-afternoon low and reached complete saturation by midnight. VPD closely followed T_a, indicating that VPD is directly related to T_a; it fell when RH values increased, suggesting that VPD is inversely proportional to RH.

DISCUSSION

All of the species used in this research were identified using foliar characteristics, a technique that seems crude but provides identical results to those produced by more advanced methods. Brayshaw (1965) was one of the first authors to suggest that different species could be identified in this manner, and used a bivariate plot technique to analyse his data and validate the hypothesis. His research was conducted at no less than 50 sites in southern Alberta, and his results suggest that southern Alberta is host to a natural, trispecific hybrid swarm. Depending on the specific geographical location, all of the species and hybrids may be present, but often a reduced number is seen because each species reaches the limit of its eastern or northern range here. With respect to the research site under investigation herein, Brayshaw's results suggest that the site is indeed populated by P. balsamifera, P. angustifolia, and a hybrid of the two. Other authors have independently verified Brayshaw's conclusions. Rood *et al.* (1986) thought that Brayshaw's treatment of his data was inadequate, and analyzed their data using multivariate PCA

(principal components analysis). Their findings were identical to those of Brayshaw. Clones from that study were further analyzed by gas chromatography-mass spectrometry (GC-MS) to determine bud exudate profiles (Greenaway *et al.*, 1991). Results showed a clear positive correlation between the leaves characteristic of a pure species and the bud exudate of that species, indicating that identification based on foliar morphology is an accurate method of taxonomic analysis.

The g_l patterns in 1992 bear little resemblance to one another, save one unifying feature. With the exception of PA on day 3, all species on all days demonstrated midday g_{lmax} values. These midday maximums would seem to indicate that the species was not experiencing water stress, with the possible exception of PA on day 3. Even in the case of PA, the closure was partial, representing a loss of only 30% of the maximum response that day (see Figure 2.9). The strategy of midday closure is common to plants grown in hot, arid environments, where evaporative demand is offset by at least partial closure of stomata during the midday heat (Roessler and Monson, 1985). Although midday closure was not commonly

observed, mid-afternoon closure was more common. Closure during this portion of the day risks leaf dessication due to heat injury; it does, however, conserve valuable water resources in the face of increased evaporative demand. Reduced midafternoon g_l has been observed in black cottonwood under similar field conditions (Pezeshki and Hinckley, 1988); it resulted from increased VPD.

The day 2 pattern is a result of greatly increased evaporative demand through the course of a day. Although PFD, T_a and wind velocity are similar to the other study days, RH is decreased and VPD is increased. This increased demand, combined with the high T_a and PFD values caused a downward trend in g_l for the day.

ψ pre-dawn pressure-bomb values are also suggestive of mild stress; they were greater than -0.25 MPa, regardless of species. The psychrometer data of PA and PB do not show the generally accepted diurnal curve of a high pre-dawn ψ value that drops to a midday minimum and then slowly increases the remainder of the day until the original pre-dawn value is attained. All curves save one, however, indicated a decline of ψ which generally occurred midday.

Several curves showed a sharp decrease in ψ later in the day from which recovery did not occur during the period of measurement. This low level of water stress suggests that it is unlikely that ψ values would have dropped even as low as -2.0 MPa in the course of a day, although this magnitude of diurnal oscillation has been known to occur in field-grown stressed plants (Hanson and Hitz, 1982).

The SHAS results indicate different degrees of stress for PA and PB on day 3; the former being less stressed than the latter due to its much greater minimum daily Ψ value. It is unlikely that it was experiencing less stress due to a greater source of water as both species were in close geographical proximity. However, PB was part of a complex of trees while the PA tree was relatively isolated from other trees, with at least 4 m in any direction free of another tree. Thus, it did not have to compete with other large vascular plants for its water supply. The increased stress of PB over PA then, is indicative of resource competition rather than differences in evaporative demand or plant water status. The Ψ values above 0 MPa on days 1 and 2 are false readings;

possibly a result of field deviation from calibrated values. These false values should be ignored.

The 1993 study shares numerous similarities with its predecessor, the most crucial being that it also experienced mild stress. The first day of the study, evaporative demand was extremely high due to wind velocities that peaked at 5.5 $m.s^{-1}$. Unfortunately, VPD values are unavailable for day 1, but based on day 2 values, VPD would have contributed to an increased evaporative demand. This demand could have resulted in the general downward trend seen on day 1 that resulted in almost complete afternoon stomatal closure for all species. A similar pattern was observed in the 1992 study on day 2.

PX exhibited the lowest g_l values of the three species studied but its pattern on days 2 and 3 indicated that its stomata remained open to the same extent regardless of the time of day. This lack of diurnal pattern is a pattern in itself, and suggests that the stomata are relatively insensitive to stress, remaining open in all but the most severe conditions. This lack of stomatal sensitivity has been observed before in P. trichocarpa (Pezeshki and Hinckley 1982, 1988; Hinkley et al. 1989), as well as in a poplar

hybrid (Furukawa *et al.*, 1990). However, both of the above responses were only observed under severe stress conditions, which PX did not seem to be experiencing.

PA appeared to adopt a pattern of water conservation as indicated by partial stomatal closure at or before midday on all study days; afternoon recovery was demonstrated only on day 2. This could be given to indicate that PA was under stress at the time of the study, but given the high Ψ values, only extreme microclimate conditions could result in greater than mild stress. Since g_{lmax} is approximately 0.4 mol.m^{-2}s^{-1}, similar to other 1993 values, as well as 1992 values, it is doubtful that it was being subjected to more than mild stress. The magnitude of closure was admittedly slight, since g_l still remained above 0.2 mol.m^{-2}s^{-1}.

PB appeared to adopt a different strategy, since it reached a mid-day maximum of g_l before declining for the rest of the day. This is reminiscent of the 1992 study; again the most influential factor would appear to be microclimate changes; in this instance PFD.

The commonly observed ψ pattern in mesophytic plants is a gradual decline to a midday or early afternoon minimum,

followed by a steady recovery throughout the rest of the day (Hanson and Hitz, 1982). The 1993 ψ responses of all species reflect this pattern. Given the pre-dawn values hovering near 0 MPa each successive day throughout the study period; it is unlikely that the species was experiencing significant stress.

Both field seasons experienced relatively mild and wet conditions, making their use as predictors of drought response pattern somewhat restricted. Pre-dawn Ψ values for both field seasons are indicative of mild stress, with 1993 being slightly wetter than 1992. Given the microclimate background during the study and the fact that this study site had no access to groundwater, these values seem somewhat high. However, both the spring of 1992 and 1993 experienced heavy rains (Environment Canada), which would have created a temporary water table due to the highly saturated soil. This information implies that it is probable that g_l would remain relatively constant over the course of the experiment, until Ψ began to decrease. This is indicated for all species in both studies; stomata remained at least partially open throughout the day on each day of the experiment. This has

been seen in field studies of black cottonwood and red alder, where g_l did not decrease until Ψ dropped below -1.0 MPa (Pezeshki and Hinckley, 1982).

The pattern of g_l between 1992 and 1993 field seasons was different for each species. While 1992 saw a general pattern of midday maximum g_l values followed by decline and afternoon recovery, 1993 values indicated a midmorning maximum and then a decline through the rest of the day. This may be a result of the possible difference in soil water availability mentioned earlier. Also, g_l values in 1993 were higher as compared with 1992 values; this could be due to a difference in soil water status; the week preceding the 1993 study was wetter than the comparable period of 1992, having received 28mm more precipitation (Environment Canada). The result was an increased soil water content in 1993 over that in 1992 which allowed for greater stomatal opening with a concomitant increase of g_l. A similar finding was reported in a 1988 study by Pezeshki and Hinckley for black cottonwood, where g_l values were higher for 1981 and 1982 than 1980 even though pre-dawn Ψ values did not differ between years.

Patterns however, did differ between years, indicating that factors other than Ψ and soil water status were influencing the trends.

Although differences were noted between 1992 and 1993 and also between the species themselves, these differences are relatively minor and therefore cannot support the conclusions stated in the abstract. To solve this problem, it is necessary to consider these results in association with those of controlled experiments, which are discussed in the following section. Through this association, a more definite pattern of response to drought will be ascertained and discussed.

C. Laboratory and Greenhouse Studies

METHODS AND MATERIALS

A. Experimental Protocol - Growth Chamber Studies

<u>Plant Material</u>

Cuttings of three poplar species <u>Populus</u> <u>balsamifera</u> (PB), <u>Populus</u> <u>angustifolia</u>(PA), and <u>Populus</u> <u>deltoides</u>(PD) and one hybrid (PA X PB) were obtained from the field location on the Oldman River near Lethbridge, Alberta. The plants were cultivated in the greenhouse at McMaster University in plastic pots (6"diameter, 10"deep) filled with an equal mixture of sand and potting soil. Potted plants were watered daily and fertilized every week with 50 grams of 20-20-20 fertilizer. Extra light to maintain a long growing day was provided by fluorescent light banks. After three months the cuttings were well rooted; they stood approximately one foot high. At this point they were moved from the greenhouse to a growth chamber. This was done to allow the plants to acclimate to experimental conditions. The chamber was on a 14 hour light/10 hour dark cycle at 27°C in the greenhouse at McMaster University. Air flow was supplied to the chamber by

an air hose on continuous flow. Several preconditioning drying runs were completed on the plants before the onset of the experiment. Three to five days was required for each drying run, in which the plants were dried until their water stress levels were near wilting. This level was determined using WESCOR C-52 chamber (Logan,Utah, U.S.A.) thermocouple psychrometers.

Experiment

Three plants of each species and the hybrid were studied over a period of four days. No measurements were made on the second day, as preliminary data had suggested that little change in water status takes place on the second day. The plants were well watered the day prior to the experiment and then not again until the end of the study. The physiological responses of the plants to the successive period of drought were measured using a LI-COR (Lincoln, NB, U.S.A.) 1600 Diffusion Porometer. Transpiration, diffusive resistance and leaf temperature were the major parameters recorded. Three leaves were measured per individual. Water

potential was measured using a pressure bomb (Model 1000, PMS Instruments, Corvallis, Oregon, U.S.A.), WESCOR C-52 chamber psychrometers and WESCOR leaf psychrometers; one measurement was taken for each individual. Microclimate data of air temperature and incident light were recorded continuously; the growth chamber recorded temperature while a LI-COR quantum sensor attached to an electronic chart recorder measured light intensity levels at the canopy level of the plants.

B. Experimental Protocol - Greenhouse Studies

Plant Material

The plants used in the growth chamber experiment were allowed to recover in the growth chamber and were moved to the greenhouse one month later. After being allowed to remain undisturbed for nearly two weeks in order to acclimatize to the new light/dark cycle in the greenhouse, the experiment was conducted as described above; all psychrometer data was later discarded due to faulty readings. The pots were placed on tables and watered every other day. Artificial light was

provided by several large high-intensity sodium vapour lights and two banks of fluorescent lights. Natural light was augmented by these artificial lights, which operated on a 14 hour light/10 hour dark cycle and produced additional light levels of 35 $umol.m^{-2}s^{-1}$ as measured by a LI-COR quantum sensor. Continuous air temperature readings were measured by thermocouple. Temperature control was maintained by slats in the greenhouse that opened and closed in a negative feedback response to the temperature setting of the room. The experiment occurred over a three day period, and the second day was used as an experimental day because of the speed at which the drying run was occurring.

RESULTS

Daily patterns of g_l and Ψ

Figure 2.20 shows g_l of the four species over the three-day experimental period in Experiment #1. On each day, the light period began at 1000 hours and extended until 2400 hours, as shown by lines on the figure below the X axis. The pattern of g_l within a species differed for each experimental day; patterns between species were dissimilar the first two days of the experiment. Pre-dawn values of g_l ranged between 0.02 $mol.m^{-2}s^{-1}$ and 0.1 $mol.m^{-2}s^{-1}$ throughout the experiment except on day 1, when PA and PB values reached approximately 0.15 $mol.m^{-2}s^{-1}$, indicating that the stomata were already open. On day 1, PA reached maximum g_1 midmorning and declined throughout the remainder of the day; PD experienced partial midday stomatal closure and reached g_{lmax} in the afternoon before late afternoon decline. These respective patterns were maintained by these two species on day 2 with several minor differences. The magnitude of afternoon recovery of g_l for PD was reduced by 75%, and PA showed overall reduced g_l, its

Figure 2.20 . Stomatal Conductance [mol.m^{-2}s^{-1}] in Experiment #1.
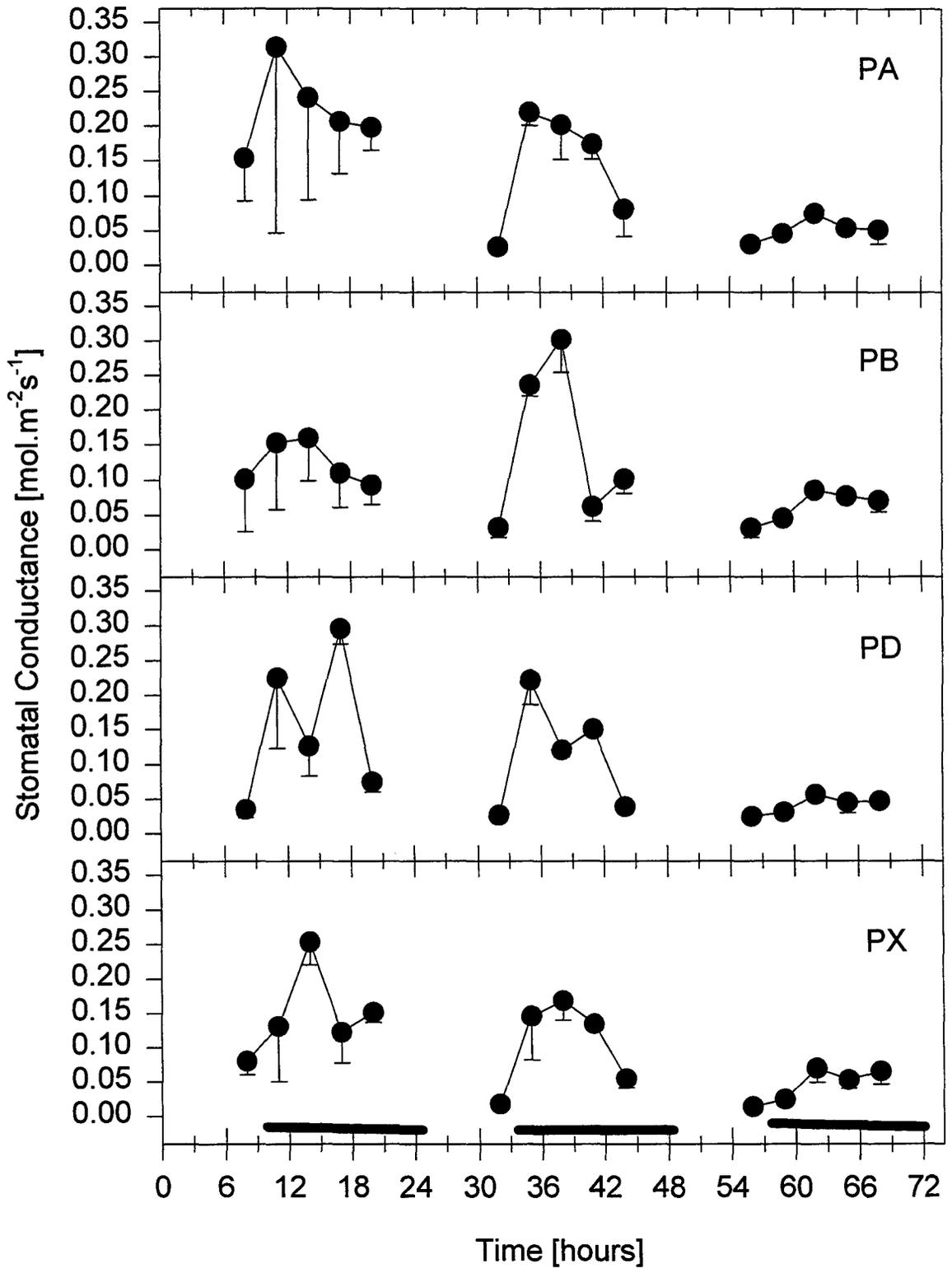
pre-dawn value being much lower than day 1. On day 1, PB and PX reached maximum g_l midday; PB declined throughout the remainder of the day while PX experienced partial mid-afternoon stomatal closure and late afternoon recovery. The patterns were reversed for day 2; PX showed a steady decline after reaching a midday maximum, PB showed partial mid-afternoon closure. On day 1, the maximum g_l of PB was the lowest value of the four species; on day 2 it was the highest. PB's day 2 maximum was also twice as high as its day 1 maximum. All end-of-day g_l values were higher than pre-dawn values; generally there was less than 0.05 $mol.m^{-2}s^{-1}$ difference between the two. By the third day, the patterns for each species were virtually identical. g_l was below 0.10 $mol.m^{-2}s^{-1}$ for every species and remained low for the duration of the day.

The Ψ of the species in Experiment #1 are shown in Figure 2.21. Based on the pressure chamber data, the pattern of water loss between species is fairly consistent. The initial value is approximately -0.5 MPa with the exception of PA, which has an initial value of -0.8 MPa. Ψ declined throughout the experiment to a level of less than -1.0 MPa.

Figure 2.21. Water Potential[MPa] in Experiment #1.
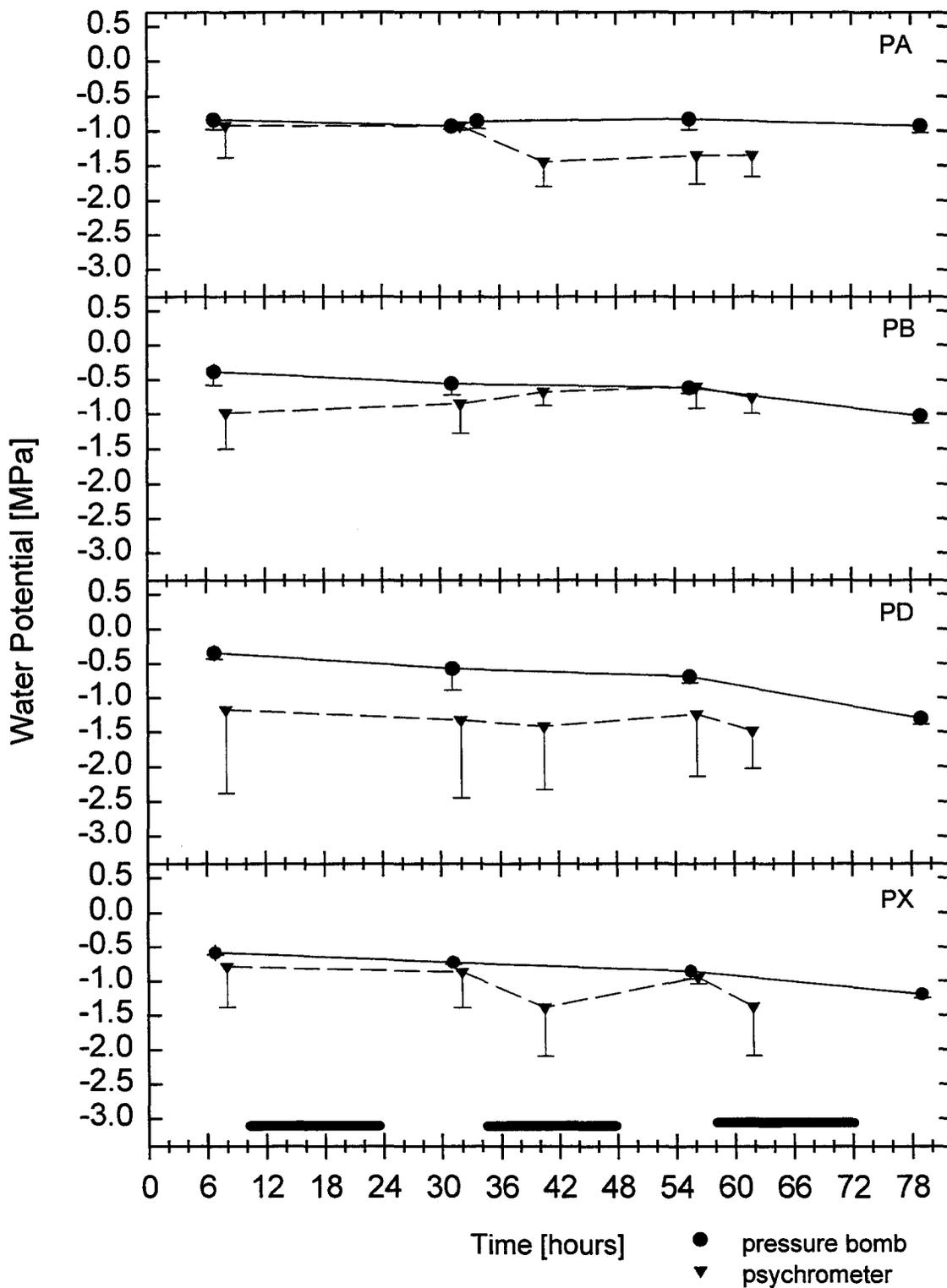
A decline in Ψ is indicated in all species; by the end of the experiment all species were experiencing moderate to severe stress, as shown by values at or below -1.0 MPa. Leaf wilt was also clearly visible in all individuals. Daily variation in Ψ is indicated by the psychrometer readings, which, depending on the day, reached a near minimum value of -1.5 MPa.

Figure 2.22 shows g_l of the four species over the three-day experimental period in Experiment #2. Stomata in each species are fully open only on day 1. On days 2 and 3, they opened slightly if at all; the highest g_l is less than 0.1 $mol.m^{-2}s^{-1}$. On day 1, three out of the four species exhibited a diurnal pattern of g_l where it increased to a mid-afternoon maximum before dropping later in the afternoon to a level that exceeded the pre-dawn value by at least 0.05 $mol.m^{-2}s^{-1}$. PB was the exception. g_l at midday and mid-afternoon were virtually identical, as were the late afternoon and pre-dawn values. However, its pattern is still quite similar to that of the other three species.

Predawn Ψ measurements of the species from Experiment #2 are shown in Figure 2.23. Based on the pressure

Figure 2.22 . Stomatal Conductance [mol.m^{-2}s^{-1}] in Experiment #2.
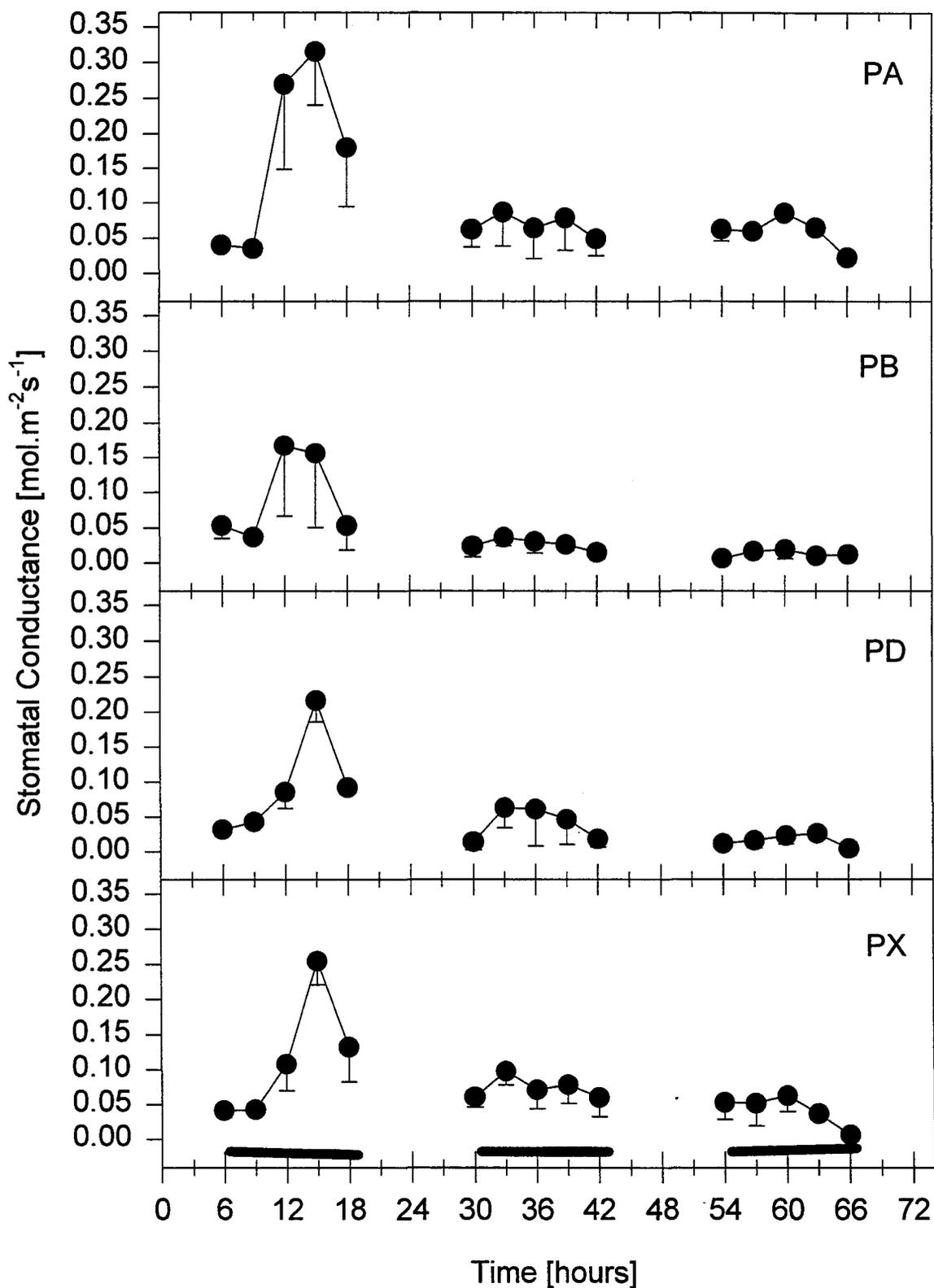
Figure 2.23. Water Potential[MPa] in Experiment #2.
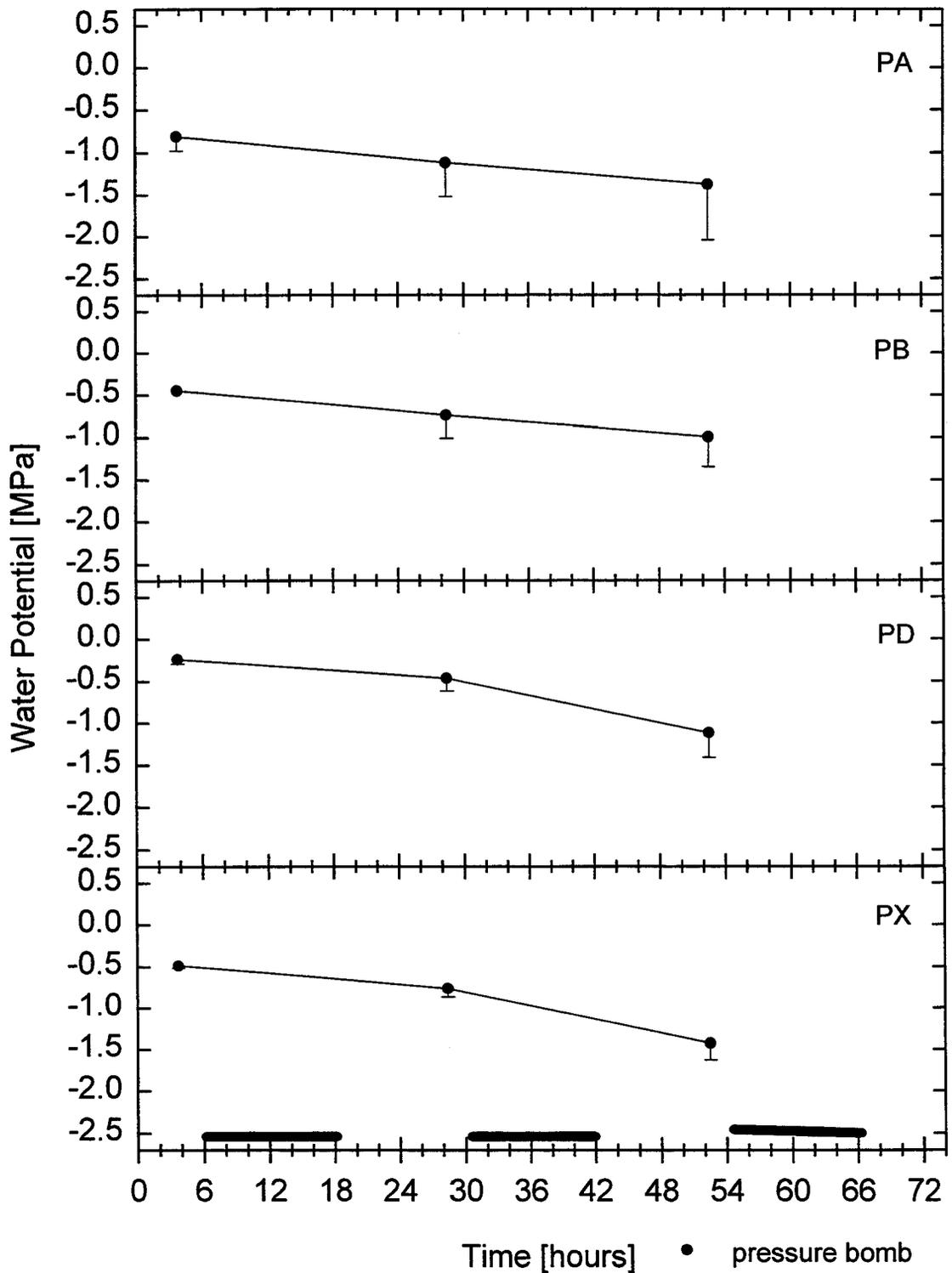
chamber data, the pattern of water loss between species is fairly consistent. The initial value is less than -0.5 MPa with the exception of PA, which has an initial value of -0.8 MPa. Predawn Ψ declines throughout the experiment to a level of approximately -1.0 MPa.

Microclimate Variables

Leaf temperature (T_l) of the study leaves used during Experiment #1 are shown in Figure 2.24. T_l showed little variation, either between the species or from day to day. The pre-dawn temperature was approximately 25°C and rose quickly to approximately 27.5°C. For the rest of the day, it fluctuated no more than 1.5°C above that value, and did not decrease below it.

Figure 2.25 describes the growth chamber microclimate during Experiment #1. Light intensity was measured in $\mu mol.m^{-2}s^{-1}$; it decreased to 0 $\mu mol.m^{-2}s^{-1}$ each night and reached a maximum of 280 $\mu mol.m^{-2}s^{-1}$ during the day. Temperature was measured in °C by the growth chamber's tempmerature recorder and maintained a fairly constant pattern during the experiment. The night-time low was 16°C at the onset of the

Figure 2.24 . Leaf Temperature (±SD) in Experiment #1.
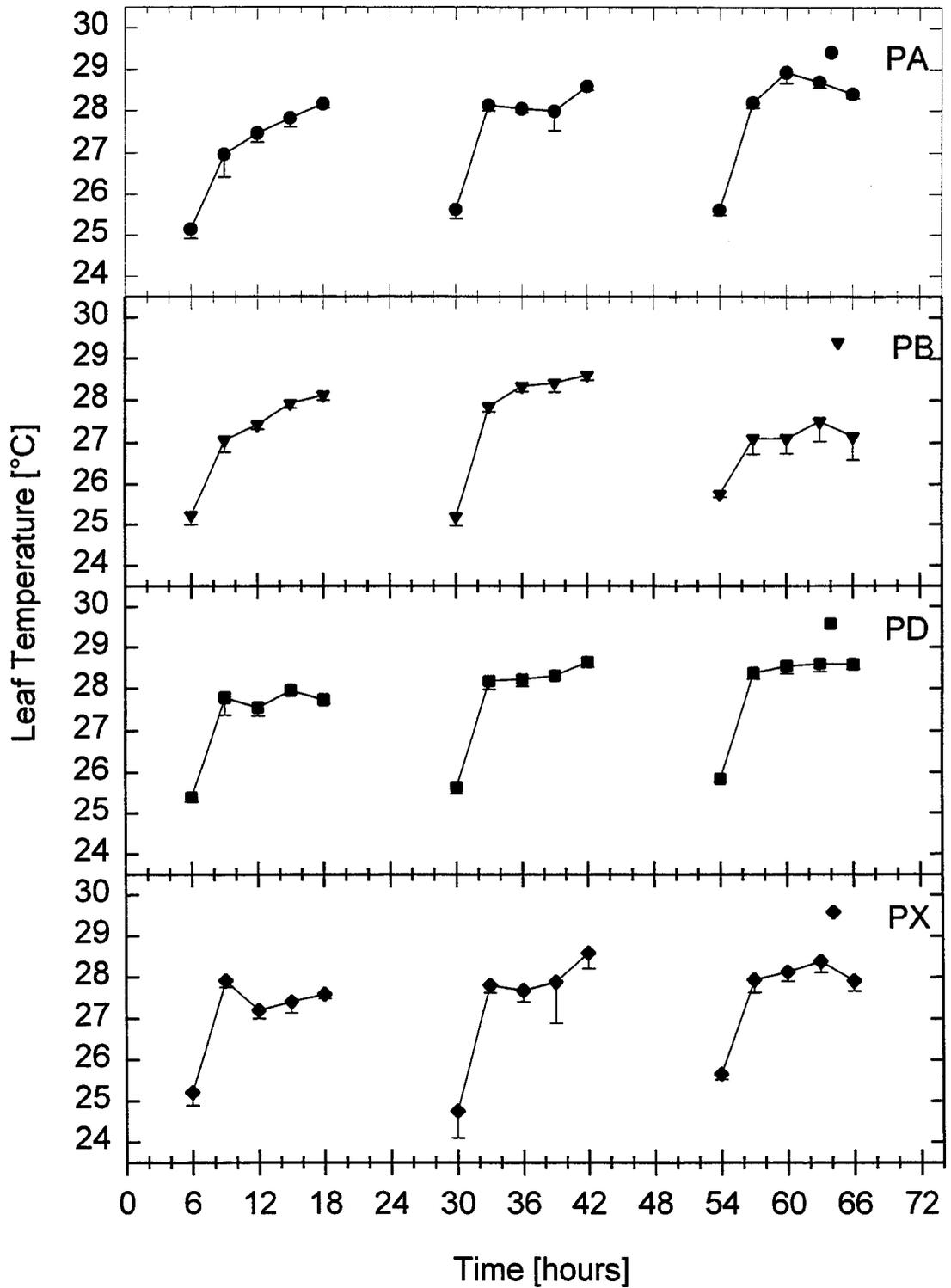
Figure 2.25. Microclimate Data of Experiment #1.
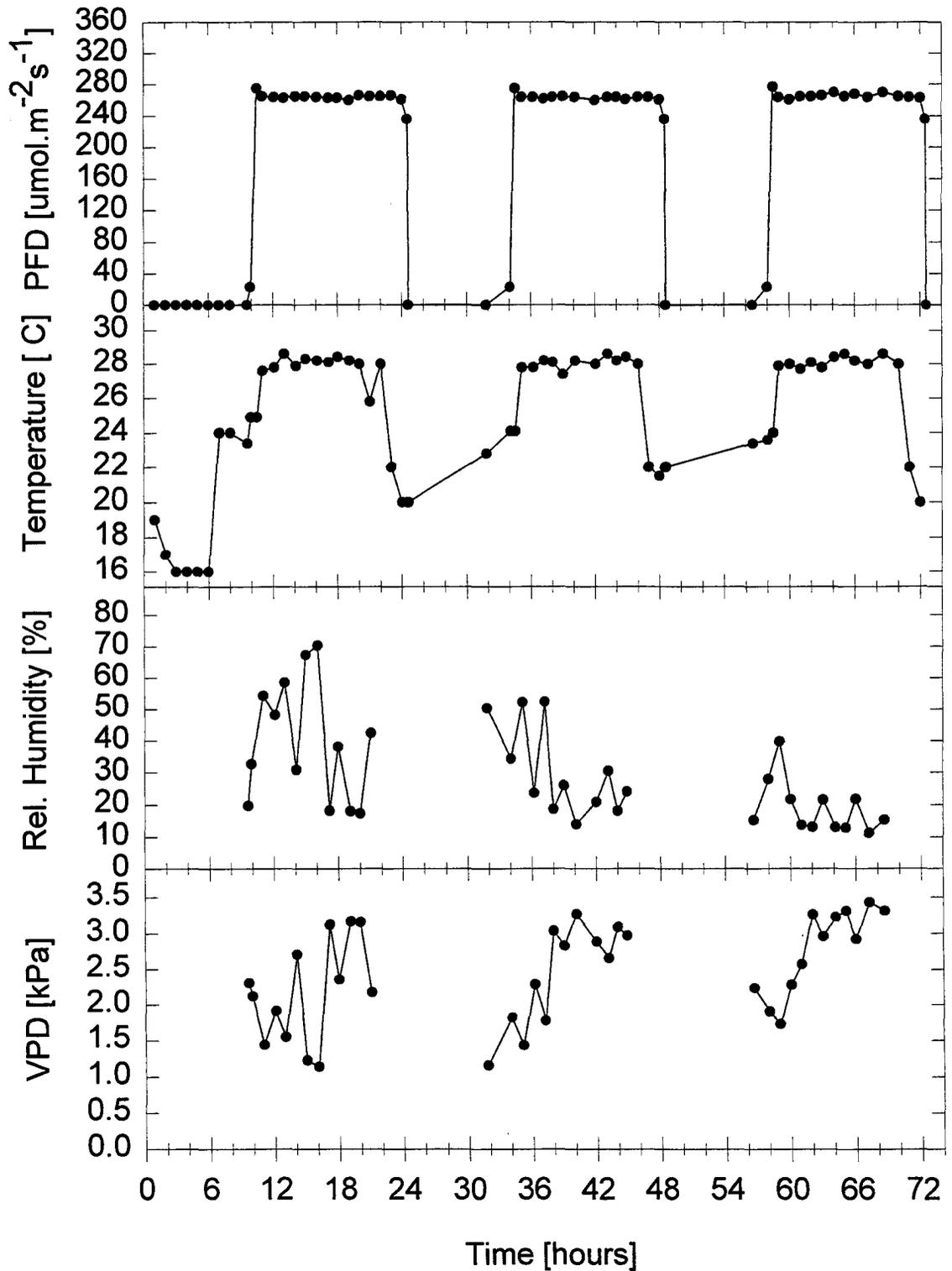
experiment; it was adjusted to approximately 20°C for the remainder of the experiment. Daytime temperatures reached upwards of 29°C. The pattern on the first day increased to 24°C early in the morning and plateaued there briefly before increasing again to 29°C. RH fluctuated over the three-day period, reaching a high of 71% on day 1. On day 2, the humidity did not reach values above 52% and on day 3, the maximum value reached was 40%. VPD values fluctuated throughout the experiment, reaching their highest values when RH was at its lowest values.

T_1 of the study leaves used during Experiment #2 are shown in Figure 2.26. T_1 showed little variation between the species; the pattern for day 1 differed from the pattern exhibited on days 2 and 3. On day 1, the pre-dawn temperature was 19°C; the temperature rose slowly to a mid-afternoon maximum of 26°C. The remaining days the pre-dawn value was approximately 22°C; the temperature dropped mid-morning to as low as 16°C before reaching a mid-afternoon maximum that varied between 22°C and 26.5°C.

Figure 2.27 describes the greenhouse microclimate of Experiment #2. Light intensity was measured in $\mu mol.m^{-2}s^{-1}$; it

Figure 2.26 . Leaf Temperature (±SD) in Experiment #2.
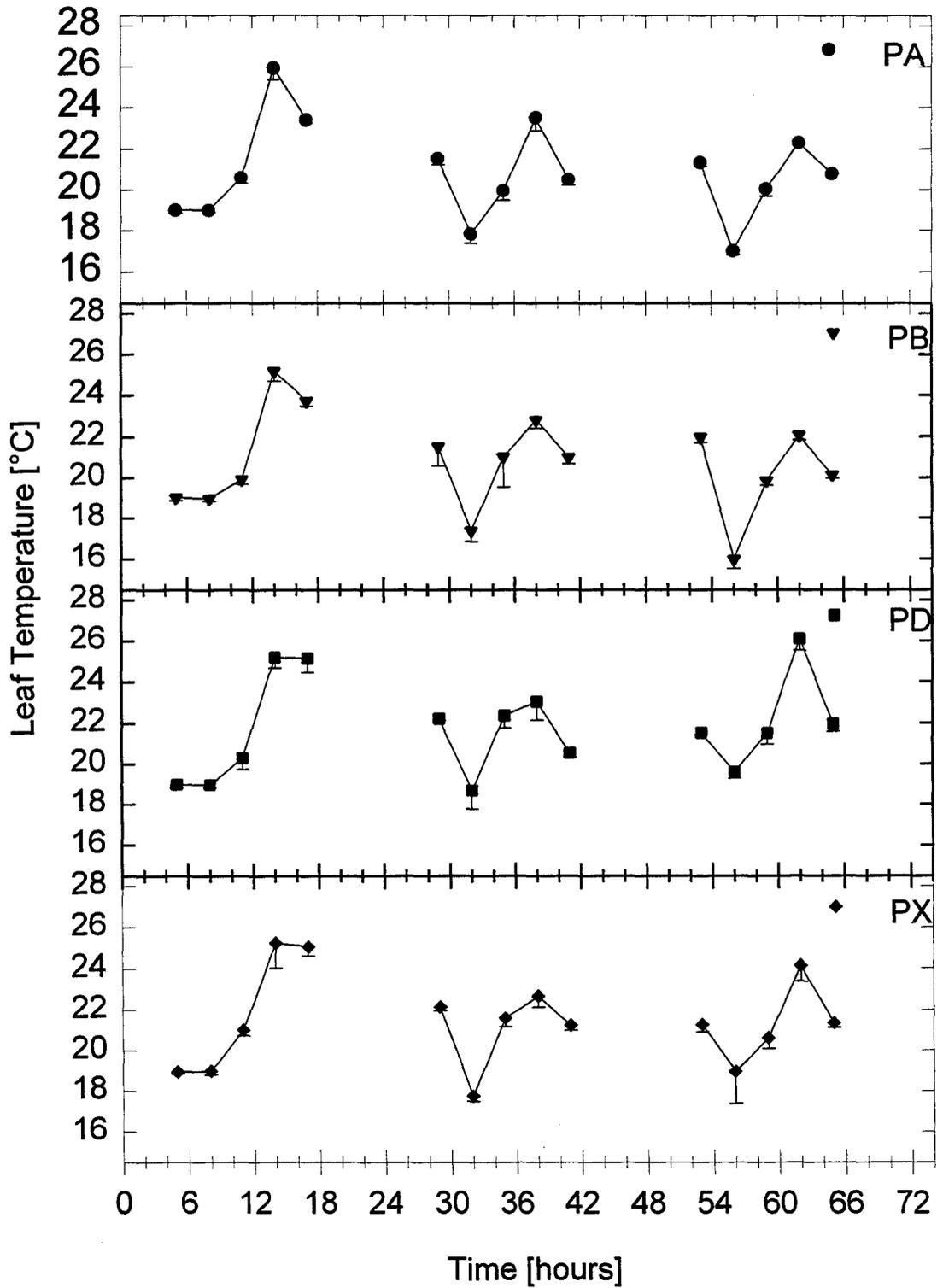
Figure 2.27. Microclimate Data of Experiment #2.
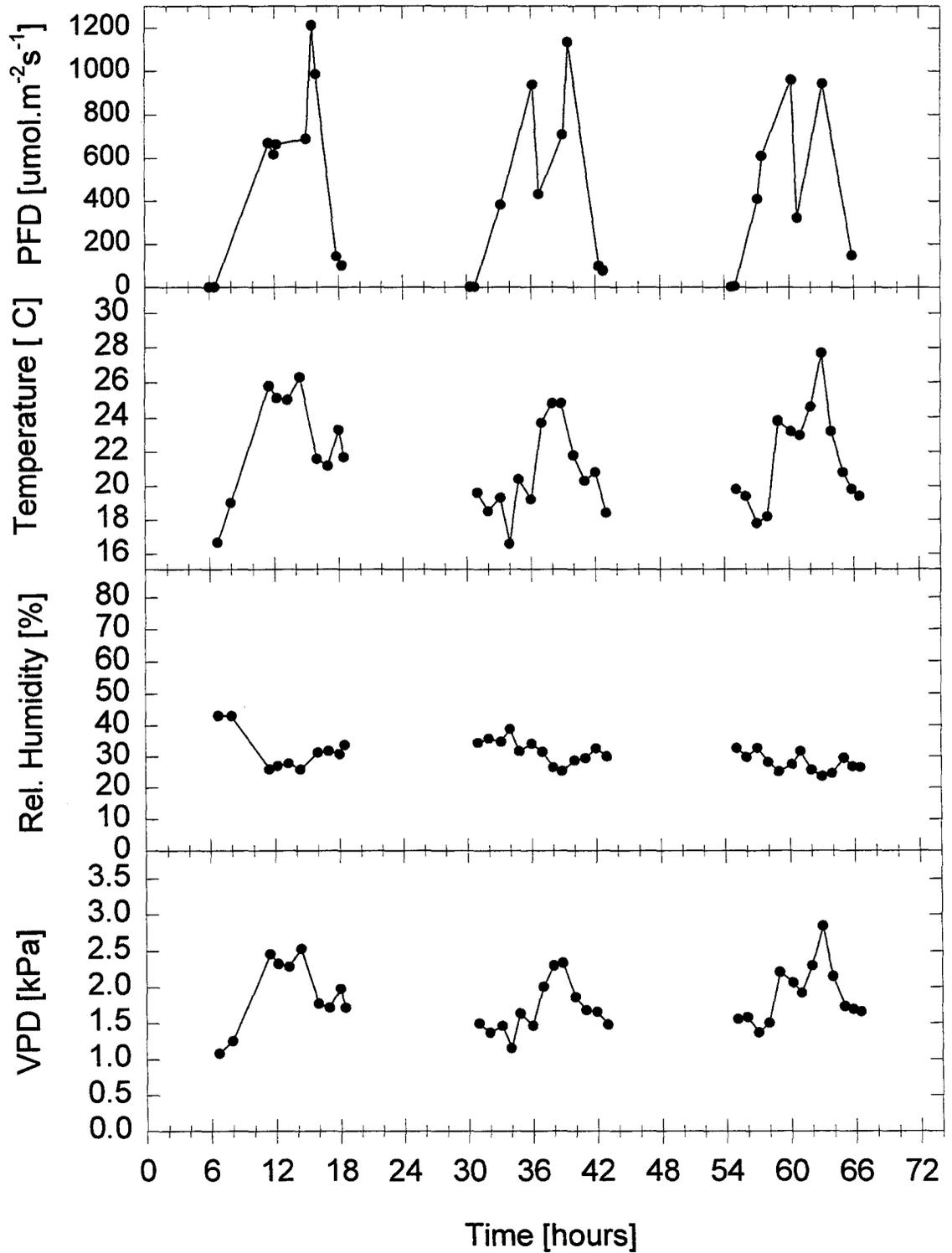
decreased to 0 $\mu mol.m^{-2}s^{-1}$ each night and reached a maximum of 1200 $\mu mol.m^{-2}s^{-1}$ during the day. Levels began increasing at approximately 0600 hours each morning and rose to a mid-morning value close to 1000 $\mu mol.m^{-2}s^{-1}$, dropped at midday and rose again to a mid-afternoon peak before falling at the end of the day. The midday drop is most probably a result of increased cloud cover during that period. On day 1, an extended plateau occurred; it extended from mid-morning to early afternoon before giving way to the daily maximum. T_a was measured in °C; the pattern differed each day. The measured evening low dropped as low as 16°C with daytime temperatures reaching upwards of 27°C. The daily pattern variation may be due to the opening and closing of the greenhouse slats, which could have created brief but steep changes in temperature. RH fluctuated marginally over the three-day period; a high of 42% was reached on day 1, thereafter it was maintained between 27% and 37%. VPD values fluctuated throughout the experiment; the patterns follow those exhibited by T_a, indicating that as temperature drops, so does VPD.

DISCUSSION

Preconditioning the plants to water stress before running experiment #1 seems to have had an effect, based on ψ values. The stomata in every species were closed throughout day 3, even though ψ at that point had only reached -1.0 MPa. This generally indicates moderate stress to plants (Hsiao, 1973), although given the loss of leaves and stomatal closure, a ψ value of -1.0 MPa may indicate severe stress to a poplar. In P. trichocarpa, preconditioning resulted in stomatal closure when the guard cells lost turgor, before cell plasmolysis could occur (Schulte and Hinckley, 1987). Stomatal preconditioning has been shown to be an important component of drought resistance (Tschaplinski and Blake, 1989). This is clearly shown in some poplar species, where stomata in non-preconditioned plants remained open even under severe stress but closed in subsequent experiments after preconditioning had occurred (Schulte *et al.*, 1987). It is unlikely that osmoregulation was occurring in experiment #1, since osmotic adjustment requires days or weeks of slowly developing stress to be expressed (Hanson and

Hitz, 1982, Hale and Orcutt, 1987). In some instances osmoregualtion can occur quickly, but these occurrences are rare and have never been observed in phreatophytes (Jones and Rawson 1979; Koppenaal *et al.* 1991). Stomatal closure by day 3 could also be an indication that these species are less tolerant of water stress than other species studied; their turgor loss point is reportedly -1.5 MPa (Smith and Gatherum, 1974). However, given the nature of stomatal response under field conditions in the 1992 and 1993 studies, it is probable that the early closure is a result of preconditioning.

The general decrease in relative humidity from day 1 to day 3 is most probably a result of the enclosed conditions of the growth chamber. As the soil dried out and the plant lost moisture through its leaves, this was not replaced and was reflected in the gradually declining RH values.

While day 3 indicated a uniform response regardless of species, during the first two days of experiment #1 the species' responses were not necessarily matched. The g_l values of PB and PX indicated different patterns for days 1 and 2, a reflection of decreased soil moisture content over

the two-day period. ψ values indicate that this water loss was marginal, which would explain why the stomata opened on day 2, albeit with reduced g_l. A shift in evaporative demand did not occur; microclimate data was similar for the two days, the only noticeable difference being a lower maximum RH value on day 2 combined with overall decreased RH during the afternoon. T_1 was uniformly high throughout experiment #1, regardless of the day, remaining above 25°C. This temperature range is higher than the optimum reported for both PA and PD (Regehr *et al.* 1975; Foster and Smith 1991). The reasons for the shift in g_l pattern are not certain; it is possible that PB and PX are utilizing different strategies of water stress response. On day 1, PX experienced partial stomatal closure when evaporative demand was at its highest. In contrast, on day 2 PX seemed to be tracking the environmental parameters, increasing as VPD and RH decreased. This suggests that PX is unable to exert significant stomatal control during stress beyond a certain threshold of Ψ, and increased transpirational cooling with a concomitant loss of water supply results. The only evidence of stomatal control was the

overall reduced magnitude of g_l. This is analagous to other studies of hybrid poplars, which have also indicated that hybrid response, rather than being intermediate between the parents' response, is greater than either parent response (Stettler *et al.* 1988; Hinckley *et al.* 1989). The shift in PB is especially puzzling; one would expect g_l values to decrease as stress increased rather than the reverse. Closer scrutiny reveals that the diel response pattern is similar for both days; only the magnitude differs. The increased magnitude of g_l on day 2 over that seen on day 1 is most probably due to differences in microclimate, and more specifically, differences in RH. RH values were at their highest on day 1 and may have resulted in reduced transpiration due to a reduced gradient driving water loss. This loss of transpiration is common in cases of high humidity (Grubb, 1977) and it follows that if transpiration decreases, a concomitant decrease in g_l would occur. The pattern of g_l recovered on day 2 after humidity levels had dropped. The pattern of g_l for PD is more expected. The day 2 pattern is identical save for a reduced mid-afternoon peak, indicating that PD was conserving resources when evaporative

demand was at its highest for the day. Unlike the other species though, PD is considered a mesophyte incapable of tolerating water stress (Tyree and Ewers, 1991). Thus its g_l response is more conservative than that of the other species. PA is similar to PD in that its day 2 g_l pattern resembles the day 1 pattern, with minor exceptions. The day 1 g_l pattern of PA would seem to indicate that even under moderate stress, its stomata open shortly before dawn and remain open throughout the day. The mid-morning maximum of g_l indicates that PA accomplished the bulk of its gas exchange while evaporative demand was low, but that its stomata remained open unless water stress became more severe. This maintenance of stomatal opening also allowed for transpirational cooling, a desirable goal under hot, dry conditions. The day 2 pattern was identical except that stomata remained closed until after dawn, stayed open the entire day at reduced g_l and closed after dark (at 2200 hours). The pattern of closure could be indicative of a threshold for stomatal closure. Pezeshki and Hinckley (1982) commented on this threshold, noting that when soil water potential reached a level of mild stress at -0.13 MPa, the threshold was raised from -1.0 MPa to -0.5 MPa.

Given stomatal response, the initial threshold would seem to approximate the value reported above. However, its g_l pattern indicates that generally speaking, PA is more adapted to water stress than the other species, and so water is not conserved until it becomes necessary.

An entirely different pattern of stress response is indicated in experiment #2. Given the nature of the diurnal stomatal response and the low daily maximum values of g_l throughout, it would appear that the system was already under stress at the onset of experiment #2. This is further suggested by microclimate response. RH remained below 50% each day, and the minimum value of VPD stayed above 1.0kPa. High PFD values also contributed to the creation of high evaporative demand. This demand, coupled with a fixed soil water supply (due to the fact that the plants were in pots) resulted in the accelerated rate of drying evident in Figure 2.22. Pre-dawn ψ values, which ranged from -0.25 MPa to -0.7 MPa on day 1, confirm the hypothesis of the presence of water stress at the onset; mild stress is indicated by slightly lowered values down to -0.5 MPa (Hsiao, 1973). At a value of -0.7 MPa, PA was experiencing moderate stress.

Given the stress these plants were under, it is surprising that no mid-morning maximum of g_l occurred on day 1, especially since evaporative demand is lower in the morning than in the afternoon. g_l instead reached its day 1 maximum mid-afternoon, when evaporative demand was highest (VPD>2.0 kPa, RH<30%, T_a =24°C). However, T_l was approximately 26°C at that time, and so the increase in g_l would have facilitated increased evaporative cooling, thereby dissipating some of the radiation load (Hanson and Hitz, 1982). Even at its maximum, however, g_l did not exceed 0.32 mol.m^{-2}s^{-1}, much lower than the reported g_{lmax} for poplars of 0.6 mol.m^{-2}s^{-1}. This is yet another indication of stress, but is also a reflection of the preconditioning these plants received due to their use in experiment #1. Also, PFD was approximately 700 μmol.m^{-2}s^{-1} when maximum g_l occurred. When it rose to 1200 μmol.m^{-2}s^{-1}, greatly increasing the evaporative demand, g_l plunged sharply for all species, reaching near closure in all species save PA, whose stomata remained partially open at 0.18 mol.m^{-2}s^{-1}.

The pattern of g_l in Experiment #2 is completely different from that in Experiment #1. The reasons for this are uncertain but may be a result of the differing conditions under which the two experiments were conducted. Since Experiment #2 was conducted in the greenhouse rather than in the growth chamber, the area would have been exposed to more possible changes in microclimate due to the opening of slats, watering of other plants in the same room, and much higher PFD values. Thus the area was less controlled.

Chapter 3

Dieback Processes

INTRODUCTION

Dieback, or death, of an entire plant or portions of its branches, has traditionally been viewed as a biological process resulting from secondary conditions. To use a relevant example; water stress predisposes the plant to dieback, but the actual death results from a secondary mechanism such as insect attack or the onset of various fungal diseases (Food and Agriculture Organization of the United Nations 1980; Tao *et al.* 1984). Under the circumstances of decreased vigour, either of these can spread quickly, reducing and often destroying the integrity of the plant. In this scenario, water stress could be viewed as the catalyst leading to the plant's eventual demise; it is not a causal factor.

Although this theory of the cause of dieback has generally been accepted, there is evidence to suggest that water stress is often a primary mechanism in the process of dieback. This evidence has accumulated in the form of

acoustic emissions studies on vascular plants (Tyree and Dixon 1983, Dixon *et al.* 1984). Acoustic emissions (AE) studies are conducted in the ultrasonic frequency range; emissions from plants have been detected in this range and seem to originate from the sapwood. These emissions can be measured and counted; they increase in number as ψ decreases or as the net rate of water loss increases. These measurements provide direct evidence of xylem cavitation events in plants under water stress. Cavitation is the phase change from water to water vapour in the xylem conduits; it is generally violent and impairs, if not renders impossible, the ability of the conduit to transport water (Taiz and Zeiger, 1991). The threshold at which cavitation is initiated differs for different species, but it generally occurs when water stress levels reach -1.0 MPa. For most tree species, this level represents the onset of moderate stress (Fitter and Hay, 1987). As water stress exceeds this threshold so many conduits embolize (fill with air) that water often cannot be efficiently rerouted around the site of injury (Taiz and Zeiger, 1991). As a result, the cavitation in xylem conduits can result in the death of the plant beyond the

point of cavitation, due to the plant's inability to transport water to areas requiring it. Tyree *et al.* (1994) showed that drought-induced cavitation led to a 50% loss of hydraulic conductivity. Under these extreme physical conditions, it is probable that some portion of the tree will die as a result of reduced water availability. Since water stress can cause cavitation and cavitation can result in dieback, it is logical to state that water stress can result in dieback.

Geographical areas that experience periodic low rainfall and dry summers are obviously likely candidates for drought. As plants in these areas strive to conserve water, it is reasonable to expect that dieback of portions of these plants will occur. Southern Alberta is considered a semi-arid climate due to its long, hot, dry summers and low incidence of rainfall (Hydrological Atlas of Canada, 1978). As a result, end-of-summer dieback is often observed in the poplar trees native to the area, especially if the trees have been planted in rows along a property line and hence, have no access to groundwater. There are three major poplar species native to this area; <u>Populus balsamifera</u> (PB), <u>Populus</u>

angustifolia (PA), and Populus deltoides (PD). All three of these species experience dieback, but the pattern of dieback appears to differ noticeably between the first two species (the third species was not considered here because of the limitations of its range). The two distinct patterns are shown in Figure 3.1a,b. Figure 3.1a indicates the typical pattern of PB while that of PA is shown in Figure 3.1b. The striking differences in the patterns of dieback led to the hypothesis that the pattern of dieback is a visual representation of the physiological manner in which the individual species responds to water stress. This hypothesis arose as a result of informal observations made of the two pure species at a site on the Oldman River located near Lethbridge, Alberta. This site was chosen for the present study because it is an abandoned gravel pit site where the trees have no contact with groundwater. This creates an on-going situation of drought, thereby increasing the likelihood of dieback. If differences between the two species' patterns do exist, they should be more clearly evident in such an extreme environment.

The objectives of this preliminary study were to quantitatively measure the amounts of dieback occurring for each of the study species, to determine the nature of the pattern of dieback for each species, and to determine if these patterns differed significantly between the two species. Possible links to physiological processes will be discussed. This study considered a number of variables thought to be important in determining dieback patterns. Among them are the degree of branching, the height of the branch above ground and the orientation of the branch (based on a 360° circle).

Figure 3.1. Dieback Pattern of P. balsamifera.

Figure 3.1b. Dieback Pattern of <u>P</u>. <u>angustifoliia</u>.

METHODS

<u>1992</u>

Six trees were chosen for the dieback study in the spring of 1992; three of P. <u>balsamifera</u> and three of P. <u>angustifolia</u>. The trees were located on the gravel pit site at the Oldman River near Lethbridge, southern Alberta. All trees had healthy leaves and branches and were young, sexually mature trees reaching less than 2 m in height. The main trunk of each tree was labelled with a metal tag and designated as the primary or first degree branch. Any major branch arising from the main trunk was designated in its entirety as a second degree branch, meaning that all of the smaller branches associated with it were considered part of that branch. Any major branch arising from a second degree branch was designated as a third degree branch. Again, all of the smaller branches were considered part of that branch. This designation process continued until the level of fifth degree branch was obtained. A sample tree with designated branches is shown in Figure 3.2. Clearly, a second degree branch contains all of the smaller branch designations, from third degree to fifth degree. Therefore, if dieback occurred

Figure 3.2. Sample Tree Showing Branch Designation.

5th

4th

3rd

2nd
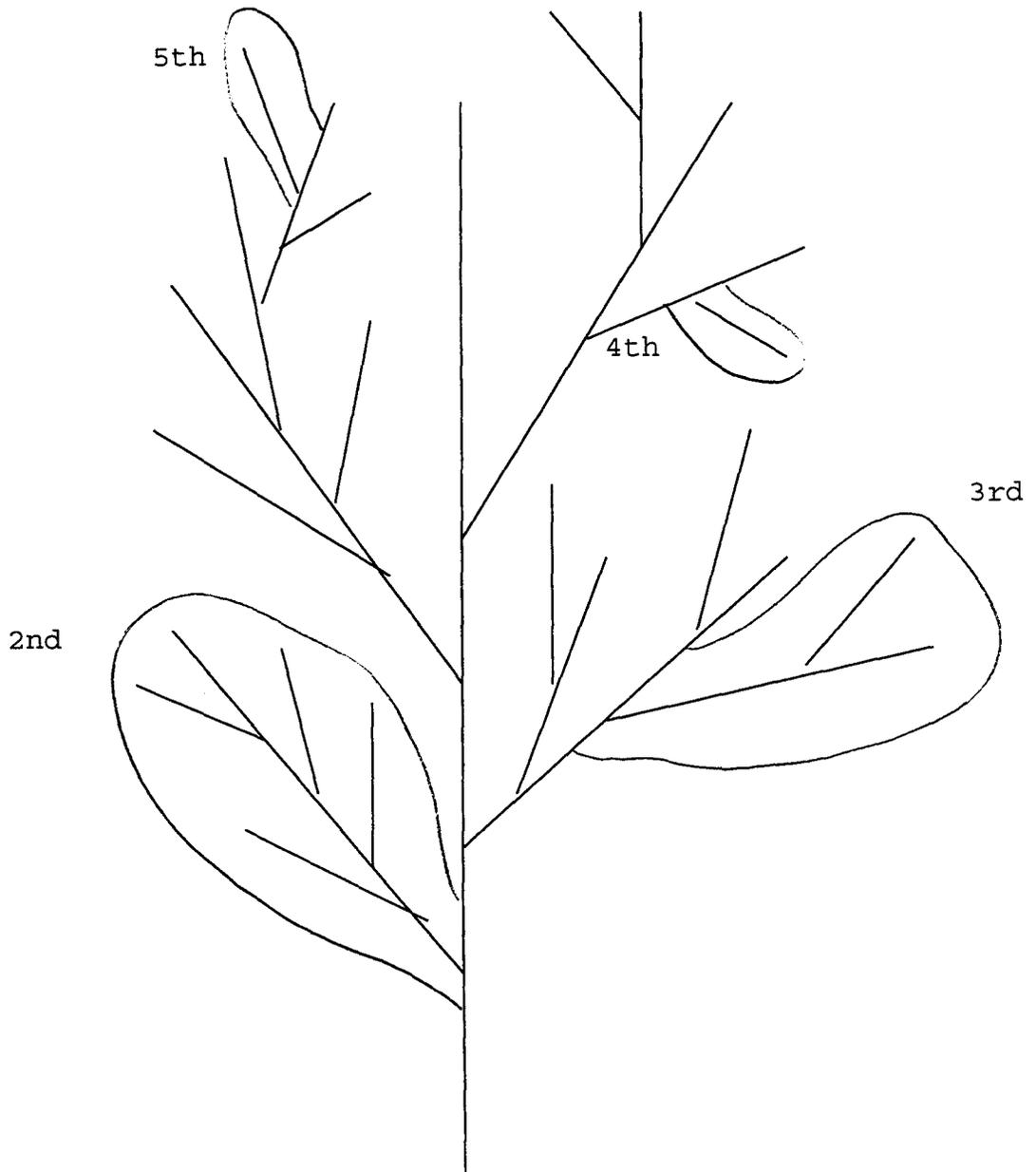
LEGEND: Circled areas were used to indicate degree of branching;
they were labelled according to the branch designation
being represented. In this situation, a first-degree branch
would be represented by the entire tree.

on a third degree branch, the second degree branch from which the third degree branch arose has experienced dieback. All major branches on each tree were tagged. In order for a branch to be regarded as a major branch, it had to have other branches arising from it. As a result, suckers were not labelled with a tag.

Each tagged branch was scored for dieback in May, when new growth was occurring. The number of shoots on each branch was counted, including any other labelled branches existing on that branch. This enumeration process was repeated in August, by which time the majority of tree dieback would already have occurred. The amount of dieback was determined by comparison of each branch at the two points in time. Dieback was presumed to have occurred in those cases where the branch had a lower live shoot count in August than in May. Once the amount of dieback for the entire tree was determined, it was further analysed to determine which side of the tree the dieback occurred on, which degree of branch, whether it occurred in the upper or lower half of the tree, and whether new growth that year was associated with the dieback.

In the Results section, dieback is expressed as a percent for third degree or greater branches as well as for second degree branches. Obviously, if a third degree branch dies, the second degree branch from which it arises has experienced dieback. However, Table 3.1 shows third degree dieback in cases where no second degree dieback occurred. This is because in order for a second degree branch to be counted as having dieback, at least 40% of its smaller branches must be dead. Thus, if less than 40% of the branches comprising a second degree branch died, the existing dieback is referred to as third degree or greater. This method of tabulation gives a clearer indication of pattern differences. To illustrate; if a high percentage of dieback occurs on third degree or greater branches, the dieback has occurred over the entire tree, with a large percent of second degree branches experiencing a small amount of dieback. Thus the dieback is recorded as third degree or greater. Conversely, a high percentage of dieback on second degree branches indicates that dieback occurred selectively on a small number of second degree branches, where at least 40% of each branch died. In this instance, the dieback is recorded as second

degree. A sample calculation of tree dieback is shown on pages 135-136 in Appendix I.

<u>1994</u>

Due to the wet year of 1992 according to meteorological data from Environment Canada, another study of the area was conducted in 1994 during the second week of September. This study, unlike that of 1992, was not comparative in nature. Rather than enumerating individuals at the beginning and end of summer and forming a picture of dieback pattern based on the ratio of the two periods, individuals were enumerated only once. This was possible because dieback was at the stage where dead and live branches were still easily discerned, but fall leaf drop had not yet occurred. The method of counting was identical save that large branches were not tagged; six individuals of each species were counted rather than only three in the 1992 study. Only two out of the six 1992 individuals were used in the 1994 study; the other four trees had either experienced early leaf fall or dieback was not clearly present. All other aspects were identical to the 1992 study.

RESULTS

Table 3.1 gives the results for the two species of Populus studied in 1992. Several trends are evident. The first trend is a lack of correlation between the amount of dieback and the type of species. P. angustifolia experienced a different percent dieback for each tree, and the amount of dieback for two of the three repeats was less than 10%. PA-987 experienced the highest amount of dieback at 20%, while PA-558 exhibited only 7% dieback and PA-963 virtually none; 1%. P. balsamifera exhibited even less dieback with all three repeats below 10%. All of the dieback in PA occurred on third degree or greater branches, while 93-100% of dieback in PB occurred on third degree or greater branches. The second trend is concerned with the side of the tree that experiences more dieback. Dieback occurred on both the eastern and western portions of the tree for the two species studied. The side with a higher percent dieback varied for each tree and also for each species, indicating that there appears to be no correlation between the species and the side of the tree with greater dieback. However, several trends are plausible. Two out of three PA repeats had a ratio of

Table 3.1. 1992 Dieback Results for <u>Populus</u> <u>angustifolia</u> (PA) and <u>Populus</u> <u>balsamifera</u> (PB).

TREE	% of Tree with Dieback	% of Dieback in Upper Half: Lower Half of Tree	% of Dieback on Third Degree or Greater Branches versus Second Degree Branches	% of Dieback on Eastern Half: Western Half of Tree
PA #987	20	100:0	100:0	67:33
PA #558	7	99:1	100:0	42:58
PA #963	1	50:50	100:0	56:44
PB #082	8	44:56	93:7	73:27
PB #689	5	62:38	96:4	24:76
PB #657	1	0:100	100:0	0:100

LEGEND:

% of tree with dieback is defined as the sum amount of dieback that occurred on the tree; all branches are included, regardless of their designation. A sample calculation is shown in Appendix I.

Dieback is said to occur on a second degree branch when 40% of the subtending shoots have died. Otherwise, the dieback is counted as occurring on branches third degree or greater.

approximately 60:40, which suggests that dieback occurred all the way around the tree regardless of branch orientation. Two out of three PB repeats had a ratio of approximately 75:25, suggesting that dieback occurred more strongly on one side of the tree than the other. This is further confirmed by the third repeat, whose dieback occurred entirely on the western side of the tree.

The pattern of dieback between species also differed according to height. In P. angustifolia, all branch dieback occurred in the upper half of the tree with the exception of PA-963, which exhibited only 50% dieback in the upper half. This is due to the fact that only two small branches died back on this tree; a larger amount of dieback may have indicated more dieback in the upper portion of the tree. In P. balsamifera a maximum of 60% occurred in the upper half of the tree.

All of the trees experienced considerable new shoot growth between the May and August enumeration periods (result not shown in Table 3.1), and in three of the trees, the new growth occurred on some of the branches that experienced

dieback. This growth equalled or exceeded the amount of dieback that took place.

The 1994 study yielded somewhat different results. Strong trends were revealed for both species. Table 3.2a lists the findings for P. angustifolia. Several PA trees experienced less than 10% dieback for the entire tree. Dieback occurred evenly on the eastern and western sides of the tree; the average ratio was 60:40 E:W. With the exception of PA#1, a greater percent of dieback occurred in the lower half of the tree than in the upper half of the tree. In all cases, over 66% of this dieback occurred on branches that were third degree or greater, indicating that the outer and terminal portions of branches were those that died back.

The findings for P. balsamifera are shown in Table 3.2b. Two trees experienced less than 10% dieback; it varied as high as 28%. Four out of the six individuals showed a strong trend of dieback occurrence on the eastern half of the tree; all of the dieback on these trees occurred on the eastern half. The other two trees demonstrated a strong trend towards dieback on the western half of the tree; at least 94% of the dieback occurred on the western half for both trees.

Table 3.2a. 1994 Dieback Results for <u>Populus</u> <u>angustifolia</u>
(PA)

TREE	% of Tree with Dieback	% of Dieback in Upper Half: Lower Half of Tree	% of Dieback on Third Degree or Greater Branches versus Second Degree Branches	% of Dieback on Eastern Half: Western Half of Tree
PA#1 (#558)	37	100:0	89:11	n/a
PA#2	13	19:81	100:0	n/a
PA#3	22	41:59	99:1	61:39
PA#4	9	8:92	66:34	59:41
PA#5	8	50:50	100:0	66:34
PA#6	14	39:61	85:15	41:59

n/a: not available

Table 3.2b. 1994 Dieback Results for _Populus balsamifera_ (PB)

TREE	% of Tree with Dieback	% of Dieback in Upper half: Lower half of tree	% of Dieback on Third Degree or Greater Branches: Second Degree Branches	% of Dieback on Eastern half: Western half of tree
PB#1	28	0:100	0:100* (85)	100:0
PB#2	14	0:100	6:94* (50)	6:94
PB#3	15	0:100	0:100* (46)	0:100
PB#4	9	0:100	12:88* (40)	100:0
PB#5	5	0:100	0:100* (75)	100:0
PB#6	14	0:100	0:100* (100)	100:0

LEGEND:
The * indicates that the dieback occurred on one major second degree branch; the number in parentheses before the star indicates the percent of this major branch that died.

For all six individuals, 100% of the dieback occurred in the lower half of the tree. In all trees a minimum of 88% of the dieback occurred on one major second degree branch, where at least 40% of this major branch died back.

DISCUSSION

It is generally believed that poplars cannot regain vigour after a lengthy period of drought, and that most, in fact, die. According to Tyree *et al.* (1994), the two North American species of poplar used in this study are among the most vulnerable to drought-induced cavitation. This makes them ideal subjects for dieback studies.

The rate of dieback within any species is dependent on a number of environmental factors such as soil, water, wind, and humidity. It is also influenced by a number of biotic factors related to the tree including species type, location of the tree, its age, its relative health, and the amount of cavitation. Differences in dieback rate were indicated by both the 1992 and 1994 results; the actual amount of dieback differed for each tree, even within the species.

The prevailing winds of an area expose the plants of that region to a harsh environmental variable. Strong winds can lower boundary layer resistance with the result that stomatal conductance is initially increased. However, continued strong winds eventually result in decreased

stomatal conductance, decreased photosynthesis and an overall reduced growth rate (Fitter and Hay, 1987). The side of the plant facing into the wind is obviously more susceptible to the physiological stresses resulting from strong winds, and so would be expected to exhibit a proportionately higher amount of dieback when compared with the rest of the tree. In southern Alberta, the prevailing winds are westerly and consistently high according to meteorological data from Environment Canada, thus the western side of the tree would be more exposed to this strong wind. It follows that these portions of the tree would be expected to exhibit higher relative amounts of dieback. However, wind direction and speed do not appear to be contributing factors to dieback. According to the 1992 results, the side of the tree on which dieback predominates appears to be dependent on the individual tree under study rather than the species, since there is no correlation within a species as to which side of the tree experiences more dieback. Dieback would therefore seem to occur independently of the secondary branch's orientation. This is further confirmed by the 1994 PA individuals. However, a high percentage of dieback in the

1994 PB individuals occurred either on the eastern half or the western half of the tree, depending on the individual. The reasons for this disparity are unknown, and may be associated with the microclimate of the trees rather than any actual species differences.

Figure 3.1 indicates that the terminal portion of a PA tree dies back first while larger, mature branches closer to the base of a PB tree are the major locations of its dieback. In circumstances of drought, it would seem logical that the newest areas of growth would be the first to dieback, due to the vulnerability of the young shoot. Drought-induced cavitations would occur below this new growth in conduits in other parts of the tree. If these conduits embolize, it becomes much more difficult for the plant to successfully reroute the water to the areas of the plant furthest from the roots (Taiz and Zeiger, 1991). Figure 3.1 suggests that this did indeed occur in PA, as the terminal portion of the tree with its smaller branches and recent growth is the area where dieback was concentrated. PB, however, did not follow this pattern. The physiological basis for the difference is unknown, but it is possible that

cavitations occur in a different manner that restricts the water supply from only one or two branches. The 1992 and 1994 data supports the pattern differences denoted by Figure 3.1; in 1992 all PA dieback occurred in the upper half of the tree, while only 60% of PB dieback occurred there and in 1994, all PB dieback occurred in lower half of the trees. The only discrepancy is the 1994 PA data, which showed that the majority of dieback occurred in the lower half of the tree, rather than in the upper half. This disparity is most easily explained by noting that in numerous cases the base of the second degree branch was in the lower 50% of the tree while the smaller branch that actually died back was in the upper 50% of the tree.

The hypothesis suggests that PA trees will experience a higher percent dieback of smaller branches, while PB trees will experience a large amount of dieback on one or two major branches. This trend did in fact occur. In 1994, a minimum of 66% of PA dieback occurred on third degree or greater branches while only 0 to 12% dieback occurred at the same branch level in PB. The 1992 pattern is less clear. All of the dieback in PA occurred on third degree or greater

branches, as did a minimum of 93% of the dieback in PB. The higher value of third degree dieback in the 1992 PB trees may result from the unusually wet conditions of that year. After the initial period of drought, a period of good rainfall occurred which may have restored soil moisture levels sufficiently to allow the tree to effectively reroute its water supply around damaged conduits. Thus dieback would have been initiated, but would not have proceeded far enough for the true pattern to emerge. The crown dieback seen in PA trees may be due to the hydraulic architecture of the tree; certain branches obtain their water only from certain roots. This theory is referred to as the unit-pipe model of a tree (Tyree and Ewers 1991). According to the model, branches that obtain their water from shallow roots would be more likely to dieback in a drought situation than those that obtain their water from deep roots (Tyree et al. 1994). A difference of hydraulic architecture between the two species could account for the observed differences in the pattern of dieback.

The explanation for the differential responses of the two trees to drought is as yet unknown, but current research suggests that the differences may be physiological

in nature. When drought occurs, the general response of a plant is reduced growth and disruption of physiological activity, including metabolic processes and g_l (Fitter and Hay, 1987). However, under repeated exposure to drought, the physiological responses of different species may vary. Two distinct poplar clones exposed to an initial period of water stress, rewatered and stressed again showed similar results during the initial period, but very different results during subsequent periods of stress (Mazzoleni and Dickmann, 1988). One clone continued to grow after the initial stress period showing new leaf growth, but many leaves died during the second period of stress. The other clone exhibited less new growth but did not show signs of dieback. The difference was attributed to different physiological strategies; the allocation of energy to root growth versus shoot growth. The clone that allocated more energy to root growth was the clone that showed no signs of dieback. It has also been suggested that the loss of hydraulic conductance experienced as a result of cavitation can greatly reduce the daily amounts of stomatal conductance, which in turn reduces the assimilation and accumulation of essential carbohydrate reserves (Tyree et

al. 1994). Carbohydrate reserves are important factors in the maintenance of cellular processes (Taiz and Zeiger, 1991). It is also possible that the physiological differences between the species exist because the number of cavitations required to initiate dieback differs, or that the location of cavitation sites differs (ie. lower in the tree for PB than for PA), or the manner in which water is redirected around the cavitation site. Obviously, there is more than one major physiological difference that could account for the differences in patterns.

The exact cause of the dieback itself still remains in dispute. It is clear that drought-induced cavitation can result in dieback, but the role of insect infestation and fungal disease is uncertain. Other studies also indicate that the foliar nutrient concentrations may play an important role in the causation of dieback (Bernier and Brazeau, 1988), and so the differential uptake of necessary trace elements could also be important. More study is required to determine the precise role of each of these factors. It is probable from this study though, that the pattern of dieback is dependent on species and that this difference is probably a result of

inherently different physical responses. Further study will reveal the exact nature of these responses.

The sample populations of these studies are necessarily small. However, their size prevents the author from reaching any definite conclusions, since the results of such a study would be too limited in scope to allow the extrapolation of results to the general population. Further studies using a larger sample size would be better able to reveal strong trends and possess greater ability to draw conclusions from the data.

Chapter 4

General Discussion of Key Findings

Out of all of the studies done herein, Experiment #1 displayed perhaps the most realistic, full-range picture of the species' physiological response to water stress. This is not surprising since it took place in a growth chamber, the most controlled environment available for this type of study. Others have commented on the usefulness of growth chambers for this purpose (Lange *et al.*, 1982). Due to these highly controlled conditions, species can be studied under the full range of stresses. By comparison, conditions in the field are often milder. Results of this experiment clearly indicated that stomata of all species, including the hybrid, could respond to decreases in soil moisture as well as increases in evaporative demand.

Given the low values of g_l throughout the studies, whether in the field or the laboratory, it is entirely possible that these species exhibit low g_l values as a norm, even when not under stress. Poplars as a genus are generally considered plants that avoid stress by closing stomata early

to reduce water loss and by losing their leaves (Kozlowski *et al.*, 1991). Other studies of poplars have suggested that an approximate g_{lmax} value for poplars is 0.6 mol.m^{-2}s^{-1} (Pezeshki and Hinckley 1982, 1988; Schulte *et al.* 1987; Ceulemans *et al.* 1988), although this will vary between species. The species studied herein revealed lower values than cited above, and may indicate a reduced tolerance of drought conditions. All species studied herein generally responded in a similar manner to decreases in ψ, dropping g_l and opening stomata later in the morning and closing them earlier at night. With the exception of the 1993 g_l values which ranged from 0.3 to 0.5 mol.m^{-2}s^{-1}, g_{lmax} experiment values were almost identical to field values at approximately 0.3 mol.m^{-2}s^{-1}. Lower than the 0.6 mol.m^{-2}s^{-1} reported, these values could indicate a modified response to long-term stress. It has been implied before that long-term water stress may modify stomatal response and perhaps influence midday stomatal closure (Lange et al., 1982).

Preconditioning may impart a competitive advantage to plants that have undergone it; the 1992 field study and both experiments indicated early stomatal closing. This advantage

was also suggested by a study of jack pine and white spruce (Koppenaal *et al.*, 1991). Another competitive edge is conferred by the ability of preconditioning to affect the degree of osmoregulation (Morgan, 1984). However, not all plants exhibit osmoregulation and the extent that preconditioning is able to modify plant stress response is dependent on leaf maturity at the time the stress was originally induced (Hinckley *et al.*, 1989).

The general pattern of g_l for each species varies; responses would seem to indicate that PD and PB avoid water stress through immediate stomatal closure as soon as Ψ values begin to decrease, while PA's stomata remain open until the wilting point is reached. PX appears to be the most highly adapted species, tracking environmental parameters and altering its stomatal response accordingly. This suggests that the hybrid is highly adaptable and thus its ability to ameliorate water stress may exceed that of both of its parents. Both field and laboratory data indicate that the general pattern of g_l for each species is as follows. PA's stomata remained open throughout the day, even under moderate water stress, as long as microclimate conditions were not

severe. PB reached a midday maximum before declining the remainder of the day. PD conserved its resources through partial midday stomatal closure and afternoon recovery. PX showed virtually no change in diurnal response in the face of mild water stress; as the stress increased, it altered its pattern according to environmental demand. This ability to adapt to changing conditions may confer a greater survival advantage for the hybrid, since natural selection favours species that can adapt to changing conditions. The hybrid could therefore greatly impact on the continued survival of the hybrid complex of which it is a part, given the current water restrictions imposed by the Oldman river dam. Knowledge of these species' stomatal response provides the first source of physiological data concerning the hybrid complex, and sets the stage for further studies to elucidate the mechanism(s) of its continued existence.

The differences in the water stress response pattern of PA and PB are echoed by the dieback results, which also show differing patterns for the two species. Specifically, PA experienced the majority of its dieback on small, terminal branches throughout the tree while PB dieback occurred

largely on one major branch. These patterns are also suggestive of differing responses to drought stress, according to the location of cavitation sites within the plant and the vulnerability of the species itself to cavitation. It is possible that a relationship exists between physiological response and dieback response, in that the pattern of dieback is a reflection of the physiological response. PA, for example utilized less stomatal control than PB and so when drought became severe it had no cache of water. Thus dieback occurred throughout the tree on small branches furthest from the roots. PB, which utilized a first line of defense by closing its stomata even before drought became severe, presumably would have greater soil water availability. Thus when drought became more severe, PB experienced the majority of its dieback on one or two main branches, an indication that cavitation had occurred at those sites, but water could be rerouted around them. This is still conjecture, but the fact that a relationship may exist between the two processes is encouraging. Further physiological, dieback and especially, cavitation studies are necessary to elucidate the mechanism of this interaction,

which could prove to be of extreme importance to the understanding of drought as it relates to silviculture, agriculture, and the forestry industry

One must be cautious when comparing results of field studies directly with those of laboratory experiments, as leaf anatomical differences were seen between poplars grown in growth chambers versus those grown outside (Pallardy and Kozlowski, 1980). Cuticle development in the former was much greater than in the latter. This aspect was not considered herein. It has also been suggested that growth in the field is less sensitive to changes in leaf water potential than growth in the greenhouse or growth chamber, meaning that greater changes in photosynthesis and therefore transpiration and conductance could be observed in the artificial system (Bunce, 1977). Age differences between field and laboratory plants could exert influence; growth chamber experiments were necessarily conducted on seedlings less than one year old while field experiments utilized adult trees. This age separation could have yielded different results for a number of reasons; as stomata increase in age they become less responsive to light and tend to remain open (Benecke *et al.*,

1981). This could be mistakenly interpreted as the plant exhibiting less control on the stomata, and therefore less water conservation. Although plants studied in both field seasons exhibited open stomata for the majority of the study, this is probably a result of the plant water status, favourable environmental conditions, possible field adaptations due to long-term stress, or a combination of the above and is not due to stomatal insensitivity. Leaf age itself plays a role; mature leaves and expanding leaves of other poplar species differ in their water stress response (Hinckley *et al.*, 1989).

Future research could follow a number of avenues, as there are numerous opinions of the mechanism(s) responsible for the cause of and response to stress. One theory suggests that all plants have a centralized system for handling stress, regardless of the nature of that stress, and therefore, research should focus not on the study of the effects of individual stressors, but instead strive towards the creation of an integrated system of stress response (Chapin III, 1991). In this system, the basic response of all plants is a decrease in growth rate and in the rate at which

resources are acquired. This response is hormonally mediated but involves integrated changes in nutrient, water, carbon, and hormonal balances of plants. It has also been suggested that perhaps the most important factor in determining drought resistance in a plant is to measure the resistance of xylem to cavitation events (Tyree and Ewers, 1991). Since hydraulic conductivity decreases when cavitation occurs, the authors feel that knowledge of this parameter in combination with those properties already measured (root:shoot ratios, low solute potentials, differences in cell wall elasticity, osmoregulation) would provide a better estimate of a species' drought resistance capacity. Inclusion of this measurement in future studies has another implication; it could confirm and subsequently describe the link between physiological responses to water stress and the dieback process.

Field studies of the same species conducted on the banks' of the Oldman River would complete the picture of drought stress response already formed by the controlled experiments and the 1992 and 1993 field studies. Completion of this picture would allow evaluation of the most tolerant

species to drought stress, and this information could then be used to mitigate forest decline through directed plantings.

APPENDIX I

CALCULATING STOMATAL CONDUCTANCE AND VAPOUR PRESSURE DEFICIT

A. Stomatal Conductance

Diffusive leaf resistance, r_l, was measured in s cm^{-1} using a steady-state porometer (LI-1600, LI-COR, Lincoln, Nebraska, USA). r_l was measured on the lower side of a leaf by enclosing the leaf in a cuvette aperture. The ambient relative humidity is maintained while a sample is held in the cuvette by a continuous flow of dry air that is pumped into the cuvette. The porometer then calculates the resistance based on several equations and asssumptions, all of which are detailed in the LI-COR LI-1600 manual. r_l values can be used to determine leaf stomatal conductance, g_l, in velocity units (cm s^{-1}). Following LI-COR (1981), stomatal conductance can be calculated as

$$g_l = \frac{1}{r_l}$$

so that stomatal conductance is simply the inverse of diffusive resistance. This value is in units of velocity, cm s^{-1}. However, in most cases, molar units are more desirable

because conductance in mole units is less dependent on environmental conditions such as temperature and pressure than conductance in velocity units (McDermitt 1990). As a result, values in mole units facilitate ease of comparison due to their more general nature.

The equation used to convert velocity units (g_v) to molar units (g_m) is expressed as

$$g_m = \frac{P}{R(T_l + 273)} g_v$$

where P is the barometric pressure at the elevation where the measurements were taken, R is the ideal gas constant (8.314 Pa m^{-3} mol^{-1} K^{-1}) and T_l is the leaf temperature (McDermitt 1990).

B. Vapour Pressure Deficit

Total atmospheric pressure is a representation of the sum of partial pressures exerted by gases contained in the

atmosphere. The vapour pressure, $e_{(T)}$, respresents the partial pressure exerted by water vapour in the atmosphere; it is much smaller than the other partial pressures, accounting for only several percent of the air mixture, or about 4 kPa (Oke 1978; Trewartha and Horn 1980). Saturation vapour pressure, $e_{s(T)}$, is the maximum amount of air that can exist in the vapour phase at a given temperature. According to Buck (1981), $e_{s(T)}$ can be calculated from temperature using the following equation

$$e_{s(T)} = 0.6112 \exp(\frac{a \times T}{b \times T})$$

where T is the ambient temperature in °C, and a and b are coefficients given by the author. $e_{(T)}$ can be calculated from the dewpoint temperature or estimated using the equation

$$e_{(T)} = e_{s(T)} \times RH$$

where RH is the relative humidity as a percent divided by 100 (Salisbury and Ross 1978; Pearcy et al. 1991). The vapour pressure deficit, VPD, is simply the difference between the

ambient vapour pressure and the saturation vapour pressure (Oke, 1978), and is represented by the equation

$$PD = e_{s(T)} - e_{(T)}$$

VPD is generally expressed in kPa rather than mb to facilitate comparison with other climatological parameters, all of which are now expressed in pascals or kilopascals.

SAMPLE CALCULATION OF TREE DIEBACK

Branch#	Degree of Branch	% Dieback	Change in Branch Number
941	2nd	10	10→9
266	3rd	0	2→2
942	4th	0	8→12
943	4th	0	6→7
944	3rd	100	2→0

Note: all branches are weighted according to the standard that each branch has an arbitrary value of 25

Branch 941: 20/200

> (each branch equals 25; counted only eight since two 3rd degree branches were large enough to be counted directly

Branch 266: accounted for by its only two branches, both of which were counted individually (942&943)

Branch 942: 0/200

Branch 943: 0/150

Branch 944: 75/75

20/200 + 0/200 + 0/150 + 75/75 = 95/625 = 15.2% dieback of the branch

All other branches on the tree are analysed in similar fashion. Values are totalled to determine % tree dieback, as shown on the following page.

PA Tree #1: Summary

Dieback Fraction	Branch	% Dieback
100/525	960	19
25/600	239	4
0/650	954	0
62/1300	940	5
95/625	941	15
131/3000	161	4
500/1200	251	42
0/875	933	0
8/662	962	1

TOTAL: 921/9437 = 9.7% of the tree died

The value of dieback that occurred, in this case 921, is
given the value of 100%. This is done because, of the dieback
that occurred, I wanted to know what % took place on second
degree branches as opposed to those third degree or greater.

500/921 = 54% of dieback occurred on second degree branches

421/921 = 46% of dieback occurred on third degree or greater
            branches

The process for determining orientation and height ratios was
identical.

APPENDIX II

SYMBOLS

AE	acoustic emissions
Ψ	water potential (MPa)
$e_{s(T)}$	saturation vapour pressure (kPa)
$e_{(T)}$	ambient vapour pressure (kPa)
F_1 hybrid	first generation hybrid
g_l	leaf stomatal conductance (mol m^{-2} s^{-1})
g_{lmax}	maximum stomatal conductance (mol m^{-2} s^{-1})
g_m	stomatal conductance in molar units (mol m^{-2} s^{-1})
r_l	leaf diffusive resistance (s cm^{-1})
PA	<u>Populus</u> <u>angustifolia</u>
PB	<u>Populus</u> <u>balsamifera</u>
PD	<u>Populus</u> <u>deltoides</u>
PFD	photon flux density (μmol m^{-2} s^{-1})
PX	<u>Populus</u> <u>angustifolia</u> X <u>Populus</u> <u>balsamifera</u>
R	ideal gas constant (8.314 Pa m^{-3} mol^{-1} K^{-1})
RH	relative humidity (%)
SHAS	stem hygrometer auto sampler

T_a air temperature (°C)

T_l leaf temperature (°C)

VPD   vapour pressure deficit (kPa)

WUE   water use efficiency

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