University of Alberta

High temperature acclimation of *Xylia xylocarpa* seedlings

by

Suomal Saelim ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**

Department of Renewable Resources

Edmonton, Alberta

Fall, 1997

University of Alberta

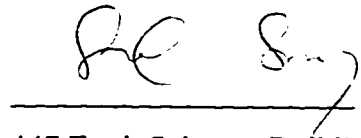
Library Release Form

Name of author: Suomal Saelim

Title of thesis: High temperature acclimation of *Xylia xylocarpa* seedlings

Degree: Master of Science

Year this Degree Granted: 1997



447 Earth Sciences Building
University of Alberta
Edmonton, Alberta, Canada
T6G 2E3

August 7, 1997

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled High temperature acclimation of *Xylia xylocarpa* seedlings submitted by **Suomal Saelim** in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**

Dr. Janusz J. Zwiazek (Supervisor)

Dr. Francis C. Yeh

Dr. Gregory J. Taylor

Dr. Victor J. Lieffers

Date approved: August 1, 1997

ABSTRACT

Xylia xylocarpa is an economically important tree species in Thailand. In the present study, one-month-old *X. xylocarpa* seedlings, from three different seed sources in Thailand, were grown at 25/20°C, 30/20°C, 35/20°C and 40/20°C day/night temperatures for two months and their ability to acclimate to high temperature was evaluated. Results indicate that acclimation involved changes in leaf morphology, gas exchange patterns, protein expression and chloroplast lipid composition. Seeds from Kanchanaburi and Maehongson produced plants with higher overall growth than seed from Nakornratchasima. Seedlings from different seed sources had similar photosynthetic rates, and water relation when measured at their day growth temperatures. When measured at 25°C, seedlings grown at 40/20°C day/night had higher net photosynthetic rates, transpiration rates and stomatal conductance than seedlings from the remaining growth temperatures.

Seedlings grown at 40/20°C had higher net photosynthetic rates at all tested temperatures. At high temperature (50°C), transpiration rates and stomatal conductance increased sharply, suggesting that transpirational cooling was a primary mechanism of heat dissipation in *Xylia xylocarpa* seedlings.

Seedlings from different seed sources grown under a range of different growth temperatures appeared to have different chloroplast lipid compositions, however, unsaturated to saturated fatty acid ratios did not change significantly.

The increase in stability of photosynthesis and thermotolerance of seedlings acclimated to high temperature was correlated with changes in protein expression.

Quantities of several leaf proteins either decreased or increased in plants acclimated to high temperature, including the HSP18.1 low molecular weight heat shock protein.

Acknowledgements

I sincerely thank to my supervisor, Dr. Janusz J. Zwiazek, Associate Professor, Department of Renewable Resources, University of Alberta for his guidance, encouragement, and support throughout my program. I gratefully acknowledge Dr. Zwiazek's advice and comments on my work. I also thank the members of my thesis supervisory committee, Dr. Francis Yeh of Department of Renewable Resources for his helpful comments for data analysis and his suggestions for this thesis. Dr. Gregory G. Taylor of Department of Biological Sciences for his comments, suggestions and critical review of my thesis. Dr. Victor J. Lieffers of the Department of Renewable Resources for his editorial contributions.

I am thankful to Dr. Sylvie Renault, Cameron Lait and my colleagues in Tree Physiology Lab for their encouragement and technical help throughout my program. Chaiyasit Leingsiri for his help with data analysis and slide preparation. Jutitep Bhothipuk and Sununta Khachonsrichon for sending me the materials and information needed for my study. Dr. Elizabeth Vierling for providing HSP 18.1 for this study. I would like to extend thanks to all of my friends here in Canada and also back to Thailand for their support and help throughout my program.

Special thank go to Canadian International Development Agency (CIDA) for financial support through out my program.

My final thanks go to my family for their understanding and support during my study abroad.

Table of Contents

List of Tables

List of Figures

CHAPTER ONE

General Introduction

Temperature is an important climatic factor which should be taken into account when selecting tree species for reforestation programs. High temperature can directly or indirectly influence survival and growth of seedlings in tree nurseries and after planting in the forest (Colombo et al. 1992). Although there are few places in the tropics where temperature extremes limit vegetative growth, plants differ in their temperature requirements and high temperature tolerance (Evans 1992). Destruction of forests, expansion of land use, and other human activities lead to changes in forest ecosystems and may affect the environment. The "greenhouse effect" is a term used to describe the effects of increased atmospheric pollutants resulting in an increase in temperatures worldwide. Based on the concentration increment of CO_2 in the last 100 years, it is projected that an increase in mean global temperature of 4°C might be expected by the year 2100 (Watson et al. 1990). High temperature stress is not limited to tropical and subtropical environments. Larcher (1980) reported that seedlings can experience temperatures exceeding 70°C at mountain sites where the sun's rays fall nearly perpendicularly to exposed areas of dry dark raw-humus soil. At the soil surface of boreal forest sites, temperatures have been reported to exceed 50°C, leading to high mortality of conifer seedlings (Koppenaal and Colombo 1988).

In Thailand, most species of tree seedlings are raised in the nursery and are exposed to full sunlight and drought before transplanting into the field. This hardening method is believed to improve drought and high temperature tolerance and lead to

increased survival after planting. A new problem has recently emerged with the introduction of exotic tree species to Thailand. These exotic species were intended to replace the native tree species lost through logging practices and were selected according to their tolerance to both drought and high temperature. These species include the rapidly growing *Eucalyptus spp.* and *Acacia spp.* The practice of introducing non-native species is controversial in terms of environmental impact, biodiversity and ecosystem management issues. In order to promote the native tree species for use in reforestation, it is important to know more about their heat tolerance, and understand more about the mechanisms of heat tolerance and acclimation methods. It would be beneficial to select a native species or genotype which is adapted to the local climatic extremes of a particular area, instead of planting exotic species whose long-term adaptation is unknown.

Xylia xylocarpa was reported by Phukittayacamee et al. (1993) to grow well in dry areas where the maximum temperature can reach 39°C. As a result of its adaptability to high temperature, this species might be a potential candidate for planting programs aimed at environmental conservation, agroforestry, and watershed protection in high temperature environments. However, the effects of high temperature on this species and its ability to acclimate are largely unknown. This information is important due to the relatively high sensitivity of young seedlings to heat stress. The objectives of this study were 1) to examine responses to high temperature stress of *Xylia xylocarpa* seedlings from different seed sources, and 2) to study the mechanisms of high temperature acclimation in seedlings by a) measuring changes in photosynthesis and water relations,

b) examining protein expression, and c) examining changes in chloroplast membrane lipids.

1.1 References

Colombo, S. J., Colclough, M. L., Timmer, V. R., and Blumwald, E. 1992. Clonal variation in heat tolerance and heat shock protein expression in black spruce. Silvae Genetica **41** (1-5):234-239.

Evans, J. 1992. Plantation forestry in the tropics. 2nd edition. Clarendon Press, Oxford. 115 p.

Koppenaal, R. S., and Colombo, S. J. 1988. Heat shock protein synthesis and thermal tolerance in wheat. Plant Physiology **90**:140-145.

Larcher, W. 1980. Physiological plant Ecology. 2nd edition. Spinger-Verlag, Berlin. Germany. p. 20.

Phukittayacamee, P., Manglkarat, J., Pong-anan, K. and Nongnueng, S. 1993. Forest tree planting: The manual for forestry officers, social forest project 1991. Royal Forest Department, Bangkok, Thailand. pp.136-143.

Watson, R. T., Rodhe, H., Oesdneger, H., and Siegenthaler, U. 1990. Greenhouse gases and aerosols. In climate change, the IPCC Scientific Assessment. *Edited by* Hougtor J.T., G.J. Jenkins and J.J. Ephraums. Cambridge University Press, Cambridge. p 140.

CHAPTER TWO

Literature Review

2.1 Biology of *Xylia xylocarpa* Taub. var. kerrii Nielsen

A common name of iron wood, *Xylia xylocarpa*, is Daeng in Thai. This tree species belongs to the Mimosaceae family. It is an economically important tree species distributed throughout northern, western, and northeastern Thailand in mixed deciduous and dry dipterocarp forests. These forests cover land from sea level to 500 m with an annual rainfall of about 1000-1500 mm and sandy loam, sandy clay loam and clay loam soils (Boonyavetchevin 1982).

Xylia xylocarpa are deciduous trees, 20-37 m in height at maturity and 30-120 cm in diameter at breast height (Keating and Boza 1982), usually with a straight clear bole (>12 m). The bark is grey to red with small lenticels. Leaves are compound, bipinnate and paripinnate and are spirally arranged. The leaflet is ovate with an acute tip and oblique leaf base. The tree flowers during March and April at the time of leaf emergence, producing small, yellow, compound flowers. Seeds mature from February to March (Phukittayacamee 1993) in pods that are red brown colored, 3.5-6 cm wide, 12-17 cm long, hard and smooth. Each pod normally contains 7-10 seeds. The seed is flat, brown colored, about 1-cm wide and 1.2-cm long (Smitinand and Larsen 1985).

Iron wood is extremely hard and dense. It is used in construction of bridges, house flooring and structural posts, boats, railway ties, and agricultural implements (Smitinand and Larsen 1985). Bark is used as a red dye for fabrics_and the leaves are used for medicinal purposes (Santisuk and Niyomthamma 1983).

As a result of its slow growth, there are no large scale reforestation programs using *Xylia xylocarpa* in Thailand. However, it has been reported that in Sabah, Malaysia, experimental trials using *Xylia xylocarpa* exhibited high growth rates in the first year following planting. Phukittayacamee et al. (1993) also suggested that *X. xylocarpa* is a potentially suitable species for planting in dry areas with high temperature.

2.2 High temperature stress and high temperature acclimation in plants

2.2.1 Introduction

Plants growing in nature or in plantations are frequently exposed to various environmental stress factors, which limit their growth and distribution (Hale and Orcutt, 1987, Lång 1993). Biotic and physicochemical stresses are especially detrimental to young seedlings. Biotic stresses such as herbivory, can result in a pronounced reduction in growth rates (Treshow 1970). Physicochemical factors, including extremes of temperature and availability of water, constitute the most serious limitation for optimal growth of both crop plants and forest trees (Kramer 1980, Boyer 1982). There is evidence that temperature is one of the most important factors limiting plant distribution and it may affect to plant evolution. Daubenmire (1974) showed that rates of mutations increased with increased temperature under experimental conditions. Woodward (1983) suggested that differences in specific leaf area between upland and lowland species may be due to differences in air temperature. Plants are sensitive to changes in ambient temperatures and they cannot escape stressful temperatures (Nitch 1963). Therefore, plants must have mechanisms to prevent damage from undesirable temperature by either avoiding or tolerating stress.

Plants dissipate heat to the surrounding atmosphere by radiation, conduction, and convection (Larcher et al. 1973). However, transpiration is considered to be the most efficient mechanism for heat dissipation from plants. Plants which are adapted to different habitats have different mechanisms to control leaf temperature under high temperature conditions. Desert species, like cacti and agave (*Agave shawii*), close stomata during the day to reduce water loss through transpiration (Ehrler 1975). In addition, leaf temperature is controlled by air circulation around the leaf. Transpiration rates in many plants, including maize (*Zea mays* L.) and mesquite (*Prosopis chilensis* Stuntz) are high when these plants are exposed to high temperatures (Ehrler 1975). It is difficult to provide an adequate quantitative estimate of heat stress in plants since this response depends on a number of factors including the thermal adaptation of the particular species to their habitat, genetic make-up among different species and within species (Krishnan et al. 1989), the duration of the exposure to high temperature, and the activity or stage of growth of the exposed tissue (Levitt 1972, Larcher et al. 1973, McWilliam 1980, Landis et al. 1992). For example, Koppenaal and Colombo (1988) reported that the current year's shoots of *Picea mariana* were more sensitive to heat stress than older shoots. Clonal variation with respect to heat tolerance of black spruce root cuttings, has been observed (Colombo et al. 1992). Higher plants from thermally contrasting habitats show considerable differences in photosynthesis, membrane fluidity, water relations, stomatal responses, floral fertility, and protein synthesis (Berry and Björman 1980, Cherry et al. 1987).

Heat stress affects many plant processes and can alter the permeability of cellular membranes (Berry and Björman 1980). In addition, high temperature alters enzyme

stability (Burke et al. 1988, Burke 1990), photosynthesis, and respiration (Björman et al. 1980). Even slight increases above the optimum temperature, may affect the physiological and biochemical processes of plants (Singla et al. 1997). Growth and yield of plants are often reduced (Burke 1988, Harding et al. 1990).

2.2.2 Photosynthesis and growth

Temperature plays a significant role in controlling metabolic activity, chemical reactions, gas solubility, mineral absorption, and water uptake (Treshow 1970). Photosynthesis is among the most sensitive mechanisms in plant cells to high temperature (Havaux 1993). However, the mechanism of plant resistance to high temperature and the process of photosynthetic inhibition by high temperatures have not been thoroughly studied. One reason for the scarcity of research data is that photosynthesis is a complex process in the plant cell. Hypotheses explaining the decline in photosynthetic activity include loss of photosynthetic pigments, changes in electron transport capacity, and a decline in phosphophorylation and CO_2 fixation efficiency (Stoddart and Thomas 1982).

Studies of isolated membrane systems have shown that heat treatment of chloroplasts results in the inactivation of photochemical reactions in thermolabile thylakoid membranes, leading to the cessation of photosynthesis (Santarius 1975, Berry and Björkman 1980, Santarius 1980, Thebud and Santarius 1982). Thebud and Santarius (1982) also demonstrated that changes in the permeability of the tonoplast and plasmalemma in protoplasts from spinach (*Spinacia oleracea*) occur at temperatures above those which are sufficient to inactivate photosynthesis. Other studies suggest that high temperature limitations to photosynthesis are related to the inhibition of photosynthetic electron transport in photosystem II (Stidham et al. 1982, Quinn and

Williams 1985, Grover et al. 1986). Inhibition of photosynthesis may also result from slow regeneration of ribulose 1,5-bisphosphate (RuBP) following high temperature exposure (Farquhar 1979, Farquhar et al. 1980, Sage and Reid 1994).

Numerous studies have shown an upward shift in optimum temperature for photosynthesis in plants that have been acclimated to temperatures that are higher than their natural habitat (Berry and Björman 1980). For example, the optimum temperature for photosynthesis in *Atriplex hymennelytra, Atriplex lentiformis* (Pearcy 1977a, 1977b) and *Larrea divaricata* (Mooney et al. 1978, Björman et al. 1980) increased by 2-5°C when plants were grown at high temperature. Berry and Björman (1980) concluded that plants that normally grow in cold environments appear to have a fairly limited potential to acclimate to high temperatures, while those that grow in warm climates tend to acclimate more easily to high temperature, but have limited ability to acclimate to low temperature.

Temperature optima for net photosynthesis are often lower than the optima for growth of trees (Doehlert and Walker 1981). The optimum temperature range for photosynthesis in Douglas-fir was reported to vary from 10-22°C (Larcher 1969, Duehlert and Walker 1981) while the optimum temperatures to growth were reported to range from 18-24°C (Brix 1971). Red alder (*Alnus rubra*) grown under controlled environment in a growth chamber had an optimal photosynthetic rate at 20°C while the temperature optimum for total dry weight increase was at 25°C (Hawkins and McDonald 1994). It is not surprising that the temperature optimum for photosynthesis also shifts during the growing season as illustrated by study with *Viola* species (Mishio 1995).

2.2.3 Membranes and lipids

Leaf cell membranes are injured by extreme temperatures (Thebud and Santarius 1982). The overall rate of photosynthesis might be affected by changes in the properties of the chloroplast membranes (Berry and Björman 1980). Recently, a new concept of thermal stress in plants has been developed that links the biochemical characteristics of a plant to its optimal growth temperature range (Ferguson and Burke 1991). Mishra and Singhal (1992) studied the effects of high temperature on the structure and photosynthetic activity of wheat (*Triticum aestivum*) chloroplasts. Photosynthetic activity was reduced in stressed leaves, suggesting that lipid integrity was required for sustained photosynthetic activity under heat and irradience stress. Cell membranes consist of a lipid bilayer with various associated proteins (Horton et al. 1992). Therefore, protein denaturation, loss of enzyme stability, and loss of lipid stability can alter an overall membrane function. These processes are frequently affected by high temperature (Langridge and McWilliam 1967, Levitt 1972, Heber and Santarius 1973, Pearcy 1978, , Björman et al.1980, Santarius 1980, Raison et al.1982a, Raison et al. 1982b, Santarius and Weis 1988, Tuquet and Sallé 1996). Heat injury in cell membranes is correlated with both protein denaturation and lipid phase transition resulting in increased membrane permeability at high temperatures (Levitt 1972, Raison et al. 1980). This increased permeability could be due to either 1) excessive fluidity of membrane lipids, leading to the disruption of the lipid bilayer, or 2) denaturation and aggregation of membrane proteins, leading to "holes" in the membrane or functional loss of channel and carrier proteins (Levitt 1980). Examination of protein distribution in the thylakoid membranes of *Anacystis nidulans* confirmed that the shifts in temperature induce major changes in both lipid-lipid and

lipid-protein interactions in biological membranes (Raison et al. 1980). However, depending on the functions of each membrane system in plant cells, responses to high temperature stress and inactivation temperatures can vary. Zhang et.al. (1993) suggested that two stages may be involved in heat injury to plants. An alteration of membrane function may lead to electrolyte leakage to extracellular spaces, and structural damage may lead to fatal membrane disintegration. Thebud and Santarius (1982) reported that the plasma membrane loses its permeability at temperatures above those which cause inactivation of photosynthesis. Other potential causes of injury to membranes include the loss of photophosphorylation capability and changes in the organization of chlorophyll within the membrane (Raison et al. 1980).

The composition of membrane lipids from leaves and chloroplasts of high temperature acclimated plants were studied in several plant species. Raison et al. (1982b) reported that chloroplast membranes of *Nerium oleander* showed no significant changes in the proportion of neutral lipids, galactolipids and phospholipids during acclimation, but the proportion of linolenic acid (18:3) in total chloroplast lipids decreased in chloroplasts acclimated to high temperature (Raison et al. 1982a). On the other hand, Raison et al. (1982a) found that the proportion of unsaturated to saturated fatty acids in polar lipids decreased when *Nerium oleander* plants were grown at 45/32°C day/night conditions. Pearcy (1978) also reported that saturated fatty acids in leaf lipids of *Atriplex lentiformis* increased following an increase in growth temperature and showed that heat treatment of isolated chloroplasts from high temperature growth regimes caused no reduction in photosystem II, possibly due to an increase in saturated lipids. Berry and

Björman (1980) and Raison (1980) concluded that these decrease in the proportion of unsaturated fatty acids to saturated fatty acids of polar lipids is consistent with changes in fluidity, and provided evidence that chloroplast membrane lipids play a major role in photosynthetic acclimation to high temperature. Similar observations were also reported in algae and bacteria (Gaughran 1947, Holton et al. 1964, Kleinshmidt and McMahon 1970). In contrast, Santarius and Müller (1979) and McCourt et al. (1987) demonstrated that unsaturated fatty acids of chloroplast membranes were not associated with the increase in photosynthesis observed during heat acclimation and Gombos et al. (1994) demonstrated that saturated fatty acids of the thylakoid membranes do not enhance photosynthesis in heat-stressed *Synechocystis sp.*

2.2.4 Heat shock proteins

Plants respond to high temperature stress by synthesizing an assortment of proteins, termed heat shock proteins (HSP). These HSPs are usually not detectable at optimal growing temperatures (Krishnan 1989). However some HSPs that function as chaperones may be present in small amount in cells at all temperatures (Taiz and Zeiger 1991, Lin et al. 1984). High temperature hardening treatment induces several HSPs in developing plant tissues including germinating embryos (Kraus et al.1995, Helm and Abernathy 1990, Helm et al. 1989, Howarth 1989), mid-maturation seeds (Altschuler and Mascarenhas 1985), imbibed seed (Brodl et al. 1990, Kraus et al. 1995), and young roots (Cooper and Ho 1983, Necchi et al. 1987). Although some of the functions of HSPs in plant cells are still obscure, evidence has shown that they play a significant role in heat tolerance (Lin et al. 1984, Vierling 1991, Nguyen et al. 1994, Singla 1997). Plants exposed to a period of non-lethal high temperature, were able to tolerate higher, normally

lethal temperature (Lin et al. 1984). Helm et al. (1989) suggested that HSP expression of imbibed embryos might be related to seed vigor of wheat. Another possible role of HSPs is an association with protein synthesis during heat stress in order to sustain proteins already present and recycle other proteins (Cooper and Ho, 1983). Linquist and Craige (1988) and DeRocher et al. (1991) suggested that HSPs might play a role in preventing or repairing damage caused during stress. Singla et al.(1997) hypothesized that HSPs have 4 possible roles at the molecular level: 1) facilitating maturation of newly-synthesized proteins in their capacity as molecular chaperons (HSP 70s, HSP 60s and HSP 90s), 2) proteolysis of denatured proteins, 3) helping in disaggregation of protein aggregates formed during heat stress (HSP 100s), and 4) stabilizing mRNA molecules in the form of heat shock granules during heat shock conditions (HSP 70s and HSP 20s).

In plants, HSPs can be induced by chilling stress (Cabane et al. 1993), cold acclimation (Neven et al. 1992), cold and salt stress (McElwain and Spiker 1992), oxidative stress (Donati et al. 1990), metals (Neumann et al. 1994), arsenite (Lin et al. 1984. Kimpel and Key 1985, Ederman et al. 1988), water stress, abscisic acid, wounding (Heikkila et al.1984) and insecticides (Ree et al. 1989). Expression of HSPs in plants is not only found in controlled conditions, but can also occur in natural environments (Kimpel and Key 1985, Hernandez and Vierling 1993, Nguyen et al. 1994). These observations indicate that plants may cope with many stresses in a similar manner. However, the production of HSPs is not a universal response to stress (Vierling 1991). Moreover, HSPs have been found in non-stressed plant organs such as flowers, seeds and seed pods (Hernandez and Vierling 1993, Vierling and Sun 1987).

HSPs have been localized in many compartments of plant cells including the chloroplast stroma of barley (*Hordeum vulgare*) (Clarke and Critchley 1992), cytoplasm, plastids and endoplasmic reticulum of maize (Vierling 1991, Helm et al. 1993, Cooper and Ho 1987), golgi, mitochondria, and plasma membrane of maize and soybean (*Glycine max*) (Cooper and Ho 1987, Lin et al. 1984), nuclei and ribosomes of soybean (Lin et al. 1984). Most HSPs are encoded by nuclear genes and synthesized in the cytoplasm prior to translocation into the interior of specific organelles (Clarke and Critchley, 1992, Nover et al, 1989, Vierling, 1991). Fractionation of stromal and thylakoid membrane components showed that all chloroplast heat shock proteins were synthesized on cytoplasmic ribosomes and translocated into the stroma of the chloroplasts (Nover et al. 1989).

HSPs have been divided into 6 classes according to their molecular weights and homology of amino acid sequence (Neumann et al., 1989, Clarke and Critchley 1992): class 1, HSP 110 (95-110 kDa), class 2, HSP 90 (80-95 kDa), class 3, HSP 70 (63-79 kDa), class 4, HSP 60 (53-62 kDa), class 5, HSP 20 (10-30kDa), and class 6, HSP 8.5 (ubiquitin). Low molecular weight (LMW) HSPs (10-30 kDa) are abundant in plants during heat stress conditions and they are actively involved with thermotolerance (Lin et al.,1984, Hsieh et al. 1992, Hernadez and Vierling, 1993). Although all the functions of HSPs in plants cell have not yet been defined, the induction of HSPs appears to coincide with an increase in tolerance to several stresses (Lafuente et al. 1991).

2.3 References

Altschuler, M., and Mascarenhas, J. P. 1985. Transcription and translation of heat shock and normal proteins in seedlings and developing seeds of soybean exposed to a gradual temperature increase. Plant Molecular Biology 5:291-297.

Berry, J., and Björkman, O. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annual Review Plant Physiology **31**:491-543.

Björkman, O., Badger, M. R., and Armond, P. A. 1980. Response and adaptation of photosynthesis to high temperatures. *In* Adaptation of plants to water and high temperature stress. *Edited by* Turner N. C. and P. J. Kramer. John Wiley and Sons, Inc. New York. pp.233-249.

Boonyavetchevin, S. 1982. Analysis of tree community in mixed deciduous forest in Thailand. *In* Forestry meeting report (forest biology), Royal Forest Department, Bangkok. Thailand. 1982. 11 p.

Boyer, J. S. 1982. Plant productivity and environment. Science. **218**:443-448.

Brix, H. 1971. Growth response of western hemlock and Douglas-fir seedlings to temperature regimes during day and night. Canadian Journal of Botany **49**: 289-294.

Brodl, M. R., Belanger, F. C., and Ho, T. H. D. 1990. Heat shock proteins are not required for the degradation of α-amylase mRNA and the delamellation of endoplasmic reticulum in heat-stressed barley aleurone cells. Plant Physiology **92**:1133-1141.

Burke, J. J. 1990. High temperature stress and adaptations in crops. *In* Stress responses in plants: adaptation and acclimation mechanisms. Plant biology V.12. *Edited by* Alscher R. G. and J. R. Cumming. Wiley-Liss, Inc. pp. 295-309.

Burke, J. J., Mahan, J. R. and Hatfield J. L. 1988. Crop-specific thermal kinetic windows in relation to wheat and cotton biomass production. Agronomy Journal **80**:553-556.

Cabane, M., Calvet, O., Vincens, P., and Boudet, A. 1993. Characterization of chilling acclimation related proteins in soybean and identification of one as a member of the heat shock protein (HSP70) family. Planta **190**:346-353.

Cherry, J. H., Heuss-LaRosa, K., and Mayer, R. R. 1987. Adaptation of thermotolerance in cow pea suspension cultures, *In* Environmental stress in plants: biological and physiological mechanisms. *Edited by* Cherry J. H. springer-Verlag, Berlin. pp. 355-369.

Clarke, A. K., and Critchley, C. 1992. The identification of a heat-shock protein complex in chloroplasts of barley leaves. Plant Physiology **100**:2081-2089.

Colombo, S. J., Colclough, M. L., Timmer, V. R., and Blumwald, E. 1992. Clonal variation in heat tolerance and heat shock protein expression in black spruce. Silvae Genetica 41:234-239.

Cooper, P., and Ho, T. D. 1983. Heat shock protein in Maize. Plant Physiology **71**:215-222.

Cooper, P., and Ho, T. D. 1987. Intracellular Localization of heat shock proteins in maize. Plant Physiology **84**:1197-1203.

Daubenmire, R. E. 1974. Plant and environment: a textbook of autecology. John Wiley & Sons, New York. pp. 157-208.

DeRocher, A. E., Helm, K. W., Lauzon, L. M., and Vierling E., 1991. Expression of a conserved family of cytoplasmic low molecular weight heat shock proteins during heat stress and recovery. Plant Physiology **96**:1038-1047.

Donati, Y., Storman, D. O., and Polla, B. 1990. Oxidative injury and the heat shock response. Biochemical Pharmacology **40**:2471-2477.

Duehert, D. C., and Walker, R. B. 1981. Photosynthesis and photorespiration in Douglas-fir as influenced by irradiance, CO2 concentration, and temperature. Forest Science **27**(4):641-650.

Ederman, L., Czarnecka, E., and Key, J. L. 1988. Induction accumulation of heat shock-specific poly(A^-) RNAs and proteins in soybean seedlings during arsenite and cadmium treatments. Plant Physiology **86**:1048-1056.

Ehrler, W. L. 1975. Environmental and plant factors influencing transpiration of desert plants. *In* Environmental physiology of desert organisms. *Edited by* Hadley N. F. Dowden, Hutchingson & Ross, Inc. Pennsylvania. pp. 52-66.

Farquhar, G. D. 1979. Models describing the kinetics of ribulose biphosphate carboxylase/oxygenase. Archives of Biochemistry and Biophysics **193**:456-468.

Farquhar, G. D., von Caemmerer, S., and Berry, J. A. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta. **149**:78-90.

Ferguson, D., and Burke, J. J. 1991. Influence of water and temperature stress on the temperature dependence of the reappearance of variable fluorescence following illumination. Plant Physiology **97**:188-192.

Gaughran, E. R. T. 1947. The saturation of bacterial lipids as a function of temperature. Journal of Bacteriology **53**:506.

Gombos, Z., Wada, H., Higeg, E., and Murata, N. 1994. The unsaturation of membrane lipids stabilizes photosynthesis against heat stress. Plant Physiology **104**:563-567.

Grover, A., Sabat, S. C., and Mohanty, P. 1986. Effect of temperature on photosynthetic activities of senescing detached wheat leaves. Plant and Cell Physiology **27**(1):117-126.

Harding, S. A., Guikema, J. M., and Paulsen, G. M. 1990. Photosynthesis decline from high temperature stress during maturation of wheat. Plant Physiology **92**:654-658.

Hale, M. G., and Orcutt, D. M. 1987. The Physiology of plants under stress. John Wiley &Sons. New York. pp.58-64.

Havaux, M. 1993. Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. Plant, Cell and Environment **16**:461-467.

Hawkins, B. J., and McDonald, S. 1994.The influences of temperature and soil water on growth, photosynthesis, and nitrogen fixation of red alder (*Alnus rubra*) seedlings. Canadian Journal of Forest Research **24**: 1029-1032.

Heber, U. and Santarius., K. A. 1973. Cell death by cold and heat and resistance to extreme temperatures. Mechanisms of hardening and dehardening. *In* Temperature and life. *Edited by* Precht, H., J. Christophersen, H. Hensel, and W. Larcher. Springer, Berlin,
Heidelberg, New York. pp. 232-263.

Heikkila, J. J., Papp, J. E. T., Schultz, G. A., and Bewley, J. D. 1984. Induction of heat shock protein messenger RNA in maize mesocotyls by water stress, abscisic acid, and wounding. Plant Physiology **76**:270-274.

Hernandez, L. D., and Vierling, E. 1993. Expression of low molecular weight heat shock proteins under field conditions. Plant Physiology **101**:1209-1216.

Helm, K. W., and Abernathy, T. H. 1990. Heat shock protein and their mRNAs in dry and early imbibing embryos of wheat, Plant Physiology **93**:1626-1633.

Helm, K. W., LaFayette, P. R., Nagao, R. T., Key, J. L., and Vierling, E. 1993. Localization of small heat shock proteins to the higher plant endomembrane system. Molecular Cell Biology **13**:238-247.

Helm, K. W., Peterson, N. S., and Abernathy, T. H. 1989. Heat shock response of germinating embryos of wheat. Plant Physiology **90**:598-605.

Holton, W., Blecker, H. H., and Onore, M. 1964. Effect of growth temperature on the fatty acid composition of a blue-green alga. Phytochemistry **3**:592-602.

Horton, H. R,, Moran, L. A., Ochs, R.S., Rawn, J. D., and Scrimgeour, K. G. 1992. Principles of biochemistry. Prentice Hall, Engle Wood Chiffs:NJ. pp 9.1-9.38.

Howwarth, C. 1989. Heat shock proteins in Sorghum bicolor and *Pennisetum americanum*. I. Genotypic and developmental variation during seed germination. Plant, Cell and Environment **12**:471-477.

Hsieh, M., Chen, J., Jinn, T., Chen, Y., and Lin, C. 1992. A class of soybean low molecular weight heat shock proteins. Plant Physiology **99**:1279-1284.

Keating, W. G., and Boza, E. 1982. Characteristics, properties and uses of timbers: South-east Asia, Northern Australia and the Pacific. Vol I. CSIRO. Inkata Press. Sydney. 360 p.

Kimpel, J. A.. and Key, J. L. 1985. Heat shock in plants. Trends in Biochemistry and Science **117**:353-357.

Kleinschmidt, M. G., and McMahon, V. A. 1970. Effect of growth temperature on the lipid composition of *Cyanidium caldarium*. II. Glycolipid and phospholipid components. Plant Physiology **46**:286-289.

Koppenaal, R. S., and Colombo, S. J. 1988. Heat shock protein synthesis and thermal tolerance in wheat. Plant Physiology **90:**140-145.

Kramer, P. J. 1980. Drought stress and origin of adaptations. *In* Adaptation of plants to water and high temperature *Edited by* Turner N. C. and P. J. Kramer. 7 p.

Kraus, T. E., Pauls, K. P., and Fletcher, R. A. 1995. Paclobutrazol- and hardening-induced thermotolerance of wheat: Are heat shock proteins involved? Plant and Cell Physiology **36**(1):59-67.

Krishnan, M., Nguyen, H. T., and Burke, J. J. 1989. Heat shock protein synthesis and thermal tolerance in wheat. Plant Physiology **90:**140-145.

Lafuente, M. T., Belver, A., Guye, M. G., and Saltreit, M. E. 1991. Effect of temperature conditioning on chilling injury of cucumber cotyledons: Possible role of abscisic acid and heat shock proteins. Plant Physiology **95**:443-449.

Landis, T. D., Tinus, R. W., McDonald, S. E., and Barnet, J. P. 1992. The container tree nursery manual V. III, Atmospheric environment. USDA forest Service, Washington DC. pp. 7-19.

Lång, V. 1993. The role of ABA and ABA-induced gene expression in cold acclimation of *Arabidopsis thaliana*. Swedish University of Agricultural Sciences. pp. 1-34.

Langridge, J., and Mcwilliam, J. R. 1967. Heat responses of higher plants. *In* Thremobiology *Edited by* Rose, A. H. Academic Press. London. pp. 231-292.

Larcher, W. 1969.The effect of environment and physiological variables on the carbon dioxide gas exchange of trees. Photosynthetica **3**:167-198.

Larcher, W. 1980. Physiological plant ecology. 2nd edition. Spinger-Verlag. Berlin. 20 p.

Larcher, W., Heber, U., and Santarius., K. A. 1973. Limiting temperatures for life functions *In* temperature and life. *Edited by* Precht H., J. Christopherson, H. Hensel, and W. Larcher. Springer-Verlag, Heidelberg. pp. 195-231.

Levitt, J. 1972. Responses of plants to environmental stress. Academic Press Inc. New York. pp.229-317.

Levitt, J. 1980. Responses of plants to environmental stress. 2nd edition. Vol I: Chilling, freezing, and high temperature stress. Academic press. New York. pp. 347-445.

Lin, C., Roberts, J. K., and Key, J. L. 1984. Acquisition of thermotolerance in soybean seedlings: synthesis and accumulation of heat shock proteins and their cellular localization. Plant Physiology **74**:152-160.

Linquist, S. L., and Craig, E. 1988. The heat shock protein in plants. Annual Review of Genetics **22**:631-677.

McCourt, P., Kunst, L., Browse, J., and Somerville, C. 1987. The effects of reduced amounts of lipid unsaturation on chloroplast ultrastructure and photosynthesis in a mutant of *Arabidopsis*. Plant Physiology **84**:353-360.

McElwain, E. F., and Spiker, S. 1992. Molecular physiological analysis of a heat shock response in wheat. Plant Physiology **99**:1455-1460.

McWilliam, J. R. 1980. Adaptation to high temperature stress. *In* Adaptation of plants to water stress and high temperature *Edited by* Turner N. C. and P. J. K. Kramer. John Willey & Sons. New York. pp. 444-447.

Mishio, M. 1995. Shifts in photosynthetic responses to irradiance and temperature in *Viola* species. Photosynthetica **31** (1):127-134.

Mishra, R., and Singhal, G. 1992. Function of photosynthetic apparatus of intact wheat leaves under high temperature stress and its relationship with peroxidation of thylakoid lipids. Plant Physiology **98**:1-6.

Mooney, H. A., Björkman, O., and Collatz, G. J. 1978. Photosynthetic acclimation to temperature in the desert shrub, *Larrea divaricata*: I. Carbon dioxide exchange characteristics of intact leaves. Plant Physiology **61**:406-410.

Necchi, A., Ponga, N. E., and Mapelli, A. 1987. Early and late heat shock proteins in wheat and other cereal species. Plant Physiology **84**:1378-1384.

Neumann, D., Lichtenberger, O., Gunter, D., Tschiersch, K., and Nover, Z. 1994. Heat shock proteins induce heavy metal tolerance in higher plants. Planta **194**:360-367.

Neven, L. G., Haskell, D. W., Guy, C. L., Denslow, N., Klein, P. A., Green, G., and Silverman, A.1992. Association of 70-kilodalton heat-shock cognate proteins with acclimation to cold. Plant Physiology **99**:1362-1369.

Nguyen, H. T., Joshi, C. P., Klueva, N., Weng, J., Hendershot, K. L., and Blum, A. 1994. The heat-shock response and expression of heat-shock proteins in wheat under diurnal heat stress and field conditions. Australian Journal of Plant Physiology **21**:857-867.

Nitch, J. P. 1963. The mediation of climatic effects through endogenous regulating substances. *In* Environmental control of plant growth. *Edited by* Evans L. T. Academic Press. New York and London. 175 p.

Nover, L., Scharf, K. D., and Neumann, D. 1989. Cytoplasmic heat shock granules are formed from precursor particles and are associated with a special set of mRNAs. Molecular Cell. Biology **9**:1298-1308.

Pearcy, R. W. 1977a. Acclimation of photosynthetic and respiratory carbon dioxide exchange to growth temperature in *Atriplex lentiformis* (Torr.) Wats. Plant Physiology **59**:795-799.

Pearcy, R. W. 1977b. Effects of growth temperature on the thermal stability of the photosynthetic apparatus of *Atriplex lentiformis* (Torr.) Wats. Plant Physiology **59**:873-878.

Pearcy, R. W. 1978. Effect of growth temperature on the fatty acid composition of the leaf lipids in *Atriplex lentiformis* (Torr.) Wats. Plant Physiology **61**:484:486.

Phukittayacamee, P., Mangklarat, J., Pong-anan, K. and Nongnueng, S. 1993. Forest tree planting: The manual for Forestry Officer, Social Forest Project 1993. Royal Forest Department, Bangkok, Thailand. pp. 136-143.

Quinn P. J., and Williams, W. P. 1985. Environmentally induced changes in chloroplast membranes and their effects on photosynthesis function. *In* Photosynthetic mechanisms and the environment. *Edited by* Barber J. and N. R. Baker. Elsevier Science Publisher B.V., Amsterdam. pp.1-47.

Raison, J. K. 1980. Membrane lipids. *In* The biochemistry of plants: a comprehensive treatise: lipids structure and function. V.4. *Edited by* Stumpf P. K. Academic Press Inc. New York. pp. 57-81.

Raison, J. K., Berrry, L. A., Armond, P. A., and Pike, C. S. 1980. Membrane properties in relation to the adaptation of plants to temperature stress. *In* Adapt of plants to water and high temperature stress. *Edited by* Turner N. C. and P. J. Kramer. John Wiley & Sons, New York. pp. 261-177.

Raison, J. K., Pike, C. S., and Berry, J. A. 1982a. Growth temperature-induced alterations in the thermotropic properties of *Nerium oleander* membrane lipids. Plant Physiology **70**:215-218.

Raison, J. K., Roberts, J. K. M., and Berry, J. A. 1982b. Correlations between the thermal stability of chloroplast (thylakoid) membranes and the composition and fluidity of their polar lipids upon acclimation of the higher plant, *Nerium oleander*, to growth temperature. Biochimica et Biophysica Acta. **688**:218-228.

Ree, C. A. B., Gullons, A. M., and Walden, D. B. 1989. Heat shock protein synthesis induced by methomyl in maize (*Zea mays* L.) seedlinds. Plant Physiology **90**:1256-1261.

Sage, R. F., and Reid, C. D. 1994. Photosynthetic response mechanisms to environmental change in C3 plants. *In* Plant environment interactions *Edited by* Wilkinson R. E. Marcel Dekker, New York, pp. 413-449.

Santarius, K. A.1975. Sites of heat sensitivity in chloroplasts and differential inactivation of cyclic and noncyclic photophosphorylation by heating. Journal of Thermal Biology **1**:101-107.

Santarius, K. A. 1980. Membrane lipids in heat injury of spinach chloroplasts. Physiologia Plantarum **49**:1-6.

Santarius, K. A., and Müller, M. 1979. Investigation on heat resistance of spinach leaves. Planta. **146**:218-228.

Santarius, K. A., and Weis, E. 1988. Heat stress and membranes. *In* Plant membranes. structure, assemble and function. *Edited by* Harwood J. L, and T. J. Walton. The Biochemical Society, London, pp. 97-112.

Santisuk, T., and Niyomthama, C. 1983. Thai economic valuable trees part 3 (Thai version). Royal Forest Department, Bangkok, Thailand. pp. 154-156.

Singla, S. L., Pareek, A., and Grover, A. 1997. High temperature. *In* Plant Ecophysiology. *Edited by* Prasad M. N. V. pp. 101-127.

Smitinand, T., and Larsen, K.1985. Flora of Thailand Vol. 4 (2:131-222) pp. 149-150.

Stidham, M. A., Uribe, E. G., and Williams III, F. J. 1982. Temperature dependence of photosynthesis in *Agropyron smithii* Rydb. II. Contribution from electron transport and photophosphoration. Plant Physiology **69**: 929-934.

Stoddart, M. A.. and Thomas, H. 1982. Leaf senescence. *In* Encyclopedia of plant physiology. V.14A. *Edited by* Voulter D. and B. Perthier. Springer-Verlag, Berlin. pp.592-636.

Taiz. L.. and Zeiger, E. 1991. Plant physiology.The Benjamin/Cummings Publishing Company, Inc., California. pp. 360-362.

Thebud, R., and Sanrarius, K. A. 1982. Effects of high temperature stress on various biomembrances of leaf cells *in situ* and *in vitro*. Plant Physiology **70**:200-205.

Treshow, M. 1970. Environment and plant response. McGraw-hill Book Company. New York. pp.13, 54-56.

Tuquet, C., and Sallé, G. 1996. Characteristics of chloroplasts isolated from two mistletoes originating from temperate (*Viscum album*) and tropical (*Tapinnanthus dodoneifolius*) areas. Plant Physiology and Biochemistry **34**(2):283-292.

Vierling, E. 1991. The role of heat shock proteins in plants. Annual Review of Plant Physiology and Molecular Biology **42:** 579-620.

Vierling, E., and Sun, A. 1987. Developmental expression of heat shock proteins in higher plants. *In* Environmental stress in plants. *Edited by* Cherry J. Springer-Verlag, Berlin. pp.343-354.

Woodward, F. I. 1983. The significance of interspecific differences in specific leaf area to the growth of selected herbaceous species from different altitudes. New Phytologist **95**:313-323.

Zhang, M. I. N., Willison, J. H. M., Cox, M. A., and Hall, S. A.1993. Measurement of heat injury in plant tissue by using electrical impedance analysis. Canadian Journal of Botany **71**:1605-1660.

CHAPTER THREE

Gas Exchange of High Temperature Acclimated *Xylia xylocarpa* Seedlings in Response to Air Temperature

3.1 Introduction

Poor seedling establishment after field planting is an important reforestation problem in tropical regions. Seedlings are often raised in tree nurseries under conditions that are optimal for growth. However, these conditions do not reflect field conditions, where seedlings may experience temperature extremes and drought. Heat stress can affect many physiological and biochemical processes including photosynthesis, enzyme activities, hormone production and membrane permeability (Treshow 1970, Berry 1975, Levitt 1980, Björman et al. 1980, Sage and Reid 1994). Havaux (1993) demonstrated that an increase in temperature to 32°C can inhibit photosystem II reactions in potato (*Solanum tuberosum* L.) leaves. Plants grown at moderately high temperatures were more tolerant of subsequent stress conditions and had increased thermal stability of their photosynthetic apparatus (Chen et al. 1982). In several studied shrub species, preconditioning to high temperature over both short and long periods of time shifted the photosynthetic optimum either upward or downward (DePuit and Caldwell 1975, Pearcy 1976, Pearcy 1977, Mooney et al. 1978). The response depended on species adaptability to natural habitats and their ability to acclimate to the environmental temperature (Mishio 1995). This photosynthetic adaptation and variation in the ability to acclimate to high temperature within species and among different species has been shown for several

herbaceous plants, shrubs and forest trees (Fryer and Ledig 1972, Mooney et al. 1975, Slatyer 1977, Mooney et al. 1978, Chaisompongpan 1990, Mishio 1995). Although the relationship between temperature, optimal growth and optimal photosynthesis have been extensively studied in numerous species of trees (Hellmers and Sandahl 1959, Brix 1971, Hawkins and McDonald 1994), the literature describing variations in acclimation potential to high temperature is still limited, especially for tropical trees.

Xylia xylocarpa is an economically important native tree species in Thailand. This tree has been recognized and endorsed by the Royal Forest Department of Thailand as a silvicultural species suitable for reforestation programs. A mature tree of *Xylia xylocarpa* grows well under high temperatures in natural stands (Phukittayacamee et al. 1993) and, therefore, it appears to be suitable for the reforestation of hot and dry sites. However, the effects of high temperature and high temperature acclimation mechanisms in seedlings have never been studied.

The principal objectives of the present study were to examine gas exchange patterns and growth of *X. xylocarpa* seedlings derived from 3 seed sources and acclimated to different growth temperatures.

3.2 Materials and Methods

3.2.1 Seed sources and seed collection

Seed of *Xylia xylocarpa* were collected from 3 different locations in Thailand: Maehongson (latitude 19° 48' N and longitude 97° 55' E), Kanchanaburi (latitude 14° 01' N, longitude 99° 32' E), and Nakornratchasima (latitude 14° 30' N, longitude 101°57' E), representing northern, western, and northeastern Thailand respectively. The mean

annual temperatures averaged over 35 years are 25.5, 28.1 and 26.4 for Maehongson, Kanchanaburi and Nakornratchasima respectively. The relative humidity ranged from 50-90% throughout the year with the annual rainfall of 1115 to 1274 mm (Table 3-1). All sites have similar soil properties with sandy to sandy clay loam soil of moderate to low fertility (Table 3-2).

Mature pods were collected in March and April, 1992 from a minimum of 10 parent trees per seed source. The parent trees at each source were spatially separated by a minimum of 100 m (Table 3-3). Seed pods were opened following 5-7 days of sun drying and seeds were kept at -2°C in plastic bottles before air shipment to the University of Alberta in 1994.

3.2.2 Seed germination

Seeds were scarified by soaking for a few seconds in boiling water to enhance germination and assure its uniformity (Phukittayacamee, 1993). Pre-soaked seeds were placed on moist sand in clear polycarbonate germination boxes under the following conditions: 30/25°C day/night temperatures, 12-hour photoperiod (PAR 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 40-60% relative humidity in a growth chamber. The boxes were closed at all times during germination to maintain high humidity.

3.2.3 Growth conditions

When the first two leaves had emerged (seedlings about one-week-old), plants were transplanted into 7.5-cm diameter pots filled with Terra-Lite 2000 Metro-Mix 290 soil (Grace Horticultural Products Ltd.). The seedlings were placed in Conviron CMP 3244 growth chambers which were set at 25°C day temperature for 8 hours and 20°C

temperature for 16 hours, 12-hour photoperiod (PAR 400 $\mu mol\ m^{-2}\ s^{-1}$).Relative humidity ranged from 50 to 70 %.

After 4 weeks, seedlings were divided into two groups and placed for 8 weeks in growth chambers under different growing regimes as follows: 25 and 30°C temperature for 8 hours and 20°C temperature for 16 hours, and 35 and 40°C temperature for 6 hours and 20°C temperature for 18 hours. All treatments were given 12-hour photoperiod (PAR 400 $\mu mol\ m^{-2}\ s^{-1}$). Seedlings were fertilized weekly with 50 mL of 0.5g/L 20:20:20 (N-P-K) commercial fertilizer.

3.2.4 Gas exchange and growth

After two months, diameter at root collar and seedling height were measured. Net photosynthetic rates, transpiration rates, stomatal conductance and water use efficiency (WUE) was measured using an ADC LCA-4, infrared gas analyzer with a PLC4 (C) cuvette. Water use efficiency was calculated as the μmoles of absorbed CO_2 divided by the mmoles of transpirational H_2O. A quartz halogen lamp was positioned adjacent to the leaf surface to boost light intensity to approximately 900-1300 $\mu mol\ m^{-2}\ s^{-1}$. Plants were removed from growth chambers immediately before the measurements. Seedlings from each seed source were measured sequentially to minimize the impact of diurnal variation.

Measurements of gas exchange were performed by two different methods. In first method, gas exchange was measured at the actual day temperature of the individual growth regime (25, 30, 35 and 40°C) the second method, measurements were made at 25°C. Seedlings were placed at 25°C for at least an hour before the measurements. One

fully developed leaf from each seedling was taken for the measurements. Leaf areas were measured using a LI 3100 leaf area meter (LiCor Instruments Corp.).

3.2.5 Temperature treatments

Responses of acclimated seedlings to different temperatures were examined by placing seedlings from each growth temperature treatment in a growth chamber at 20°C for 30 minutes and increasing the temperature of the growth chamber to 50°C in 5°C increments every 30 minutes. The seedlings were removed from the growth chamber several minutes prior to the measurements and placed at room temperature (25°C). The same leaf was used for gas exchange measurements throughout the entire experiment.

For electrolyte leakage, two leaf discs, 1.5 cm in diameter, from each temperature treatment, were cut with a cork borer. Leaf discs were washed for 1 hour in 10 mL of deionized water to remove eletrolytes from cut cells. The leaf discs were then incubated in fresh deionized water at room temperature for another 5 hours and the conductivity of the solutions was measured. Total electrolyte content was determined in leaf discs plunged for 10 minutes in liquid nitrogen and then placed at -80°C over night. The next day, frozen leaf discs were incubated for 24 hours in deionized water and the total leaked electrolytes were measured using a portable electrical conductivity meter (Model C33, Fisher Scientific Ltd.).

All experiments were replicated twice with 6 seedlings per replicate and plants were placed in different growth chambers to minimize effects of growth chamber variations.

3.2.6 Data analysis

General linear model (SAS 6.1 computer package) was used to perform the analysis of variance among seed sources, growth temperatures, temperature treatments and replications for net photosynthesis, transpiration, stomatal conductance, water use efficiency, height, stem diameter, and electrolyte leakage. Seed sources, growth temperature, and temperature treatment were considered as fixed factors, while replicates were denoted as a random factor. The analysis of variance (ANOVA) model for gas exchange of acclimated seedling is :

$$Y_{ijk} = \mu + R_i + S_j + (RS)_{ij} + G_k + (RG)_{ik} + (SG)_{jk} + (RSG)_{ijk} + Error_{ijkl}$$

while the model for gas exchange of acclimated seedlings responses to temperatures is:

$$Y = \mu + R_i + S_j + (RS)_{ij} + T_k + (RT)_{ik} + (ST)_{jk} + (RST)_{ijk} + G_l + (RG)_{il} + (SG)_{jl} + (TG)_{kl} + (RTG)_{ikl} + (STG)_{jkl} + (RSG)_{ijl} + (RSGT)_{ijkl} + Error_{ijklm},$$

Where

Y = observed mean;

μ = population mean;

R_i = number of replications (i = 1,2);

S_j = seed source (j = 1,2,3),

G_k = growth temperature (k = 1, 2,3,4),

T_l = temperature treatment (l = 1, 2, 3,...7)

Student-Newman-Keuls test at $P \leq 0.05$ was used to determine statistically significant differences within the seed sources.

3. 3 Results

3.3.1 Growth and leaf morphology

Different growth temperatures induced significant differences in seedling height (P<0.00385), stem diameter squared times height (D^2H) (P<0.0181), but not stem diameter (Table 3-4). Height, diameter and D^2H among seed sources were also significantly different at P<0.0105, P<0.0303 and P<0.0215 respectively (Table 3-4). Seedlings from Maehongson and Kanchanaburi grew taller and had higher D^2H at 35/20°C and 30/20°C compared to 25/20°C and 40/20°C (Table 3-4, Fig.3-1). Seedlings grown from the Nakornratchasima seed source were smaller compared with Kanchanaburi and Maehongson seed sources (Fig. 3-1) in all growth temperatures. Leaf morphology of seedlings was affected by growth temperature. Seedlings grown at 25/20°C had different leaf shapes than those grown at 30/20°C and 35/20°C (Fig.3-2). Leaves of seedlings grown at 40/20°C had a distinctive shape and were smaller and thicker (Fig. 3-2).

3.3.2 Gas exchange

When measured at their day growth temperatures, there were no significant differences in net photosynthesis, transpiration rates, stomatal conductance, and water use efficiency in seedlings grown at different growth temperatures (Table 3-4, Fig. 3-4). In contrast, when measured at 25°C seedlings grown at higher temperature had higher net photosynthetic rates (P<0.0041), transpiration rates (P<0.0089) and stomatal conductance (P<.0064) but not water use efficiency (Table 3-4, Fig. 3-4).

In temperature treatments, net photosynthetic rates responded similarly to different temperatures in the studied seed sources. However, seedlings from different

growth temperatures differed with respect to net photosynthesis at all temperatures (Table 3-5, Fig. 3-6, 3-7, 3-8). Seedlings grown at 40/20°C day/night temperature had higher photosynthetic rates than the other growth temperatures for all temperature treatments. Net photosynthesis gradually decreased with increased temperature and was negative at 50°C in seedlings grown at 25/20°C, 30/20°C and 35/20°C. However, the seedlings grown at 40/20°C maintained positive net photosynthesis as temperatures reached 50°C (Fig. 3-6, 3-7, 3-8).

Transpiration rates and stomatal conductance were also influenced by growth temperature ($P<0.0163$, $P<0.0001$, respectively) (Table 3-5, Fig. 3-6, 3-7, 3-8), but there were no significant differences between the different seed sources. Seedlings grown at 40/20°C day/night temperature had higher transpiration rates and stomatal conductance at 20°C compared with the plants grown at lower temperatures (Fig. 3-6, 3-7, 3-8). Transpiration rates gradually decreased with increasing air temperature followed by a sharp increase again at 50°C. However, at 50°C, transpiration rates and stomatal conductance of seedlings grown at 40/20°C were lower than those of seedlings grown at the lower growth temperatures (Fig. 3-6, 3-7, 3-8).

Seedlings from the Nakornratchasima source grown at 30/20°C day/night temperature had higher water use efficiency than that in other growth temperatures but their water use efficiency declined sharply at 50°C (Table 3-5 and Fig. 3-6, 3-7, 3-8). In contrast, plants from the Kanchanaburi and Maehongson grown at 40/20°C day/night temperature had higher water use efficiency over the full range of temperature treatments

compared with the seedlings from the lower growth temperatures (Table 3-5 and Fig.3-6. 3-7, 3-8).

There were no significant differences in electrolyte leakage between the different seed sources but there was an interaction effect between different seed sources and air temperature ($P<0.0002$) (Table3-5). Seedlings grown at 40/20°C day/night temperature did not show increased electrolyte leakage throughout the range of experimental temperatures from 20 to 50°C, whereas seedlings grown under 25/20°C, 30/20°C, and 35/20°C had significantly increased electrolyte leakage at 50°C ($P<0.0001$) (Table 3-5 and Fig. 3-6, 3-7, 3-8).

3.4 Discussion

Xylia xylocarpa seedlings had the highest height growth and D^2H when grown at the day temperature of 35°C. This is higher than the optimum growth temperature recorded for seedlings of temperate trees (Hellmer 1966a, Hellmer 1966b, Larson 1967, Brix 1971, HåbjØrg 1972, Slatyer and Ferrar 1977, Hawkins and McDonald 1994). Seedlings from all three seed sources responded in the same manner to growth temperatures.

Seedlings did not show any signs of injury when grown at 40/20°C day/night conditions, but interestingly, changed their leaf morphology. This could be interpreted as a heat avoidance mechanism (Levitt 1980). Leaf dimorphism is thought to be induced by abscisic acid (ABA) (Young et al. 1990, 1995) and it is possible that high temperature induced ABA synthesis in *X. xylocarpa*, in turn, affected leaf morphology. Many plant species can acclimate to extreme temperatuare conditions by modifying leaf

characteristics and leaf orientation (Mooney et al. 1975, Wainwright 1977, Ehleringer and Mooney 1978, Rawson et al. 1978). Temperature has been shown to have a pronounced influence on specific leaf area and leaf shape in both field and controlled systems (Bensink 1971, Woodward 1983). It was shown that temperature affects growth mainly by influencing the leaf area and number of leaves rather than by changing the rate of photosynthesis per leaf unit (Brix 1967, 1969). In *Xylia xylocarpa*, the number of leaves per seedling from 25/20°C and 40/20°C growth temperatures was lower than that in 30/20°C and 35/20°C growth temperatures (data not shown). However, at their growth temperatures, net photosynthesis of seedlings grown at 25/20°C, 30/20°C, 35/20°C, 40/20°C was not significantly different. The small decrease in net photosynthesis in seedlings grown at 40/20°C compared with other growth temperatures was statistically significant only when the data were combined from seedlings of all three seed sources.

Interestingly, when measured at 25°C, seedlings grown at 40/20°C day/night temperature had higher net photosynthesis than those in other growth temperatures. This adjustment in photosynthesis of seedlings grown at 40/20°C day/night temperature may be important for growth in high temperature environments where temperatures fluctuate. This could explain why *X. xylocarpa* seedlings from Kanchanaburi and Nakornratchasima grown under high temperature regimes had the same height as seedlings grown at 25°C and 30°C day temperature even though they had fewer and smaller leaves. Seedlings grown at 40/20°C photosynthesized at higher rates in the morning before the temperature rose to 40°C.

Photosynthetic temperature response curves tend to shift as a result of acclimation to growth temperature, seasonal changes, and previous adaptations to habitat (Fryer and Ledig 1972, Pearcy 1977, Mooney et al. 1978, Mishio 1995). When plants from the same habitat were grown under different temperatures, there were differences in the optimum temperature for photosynthesis (Mooney et al. 1978). Plants and environment have complicated interactions which may involve plant genetic and metabolic adjustment (Terri 1980, Long 1985). In this study, seedlings that were grown under different temperature regimes were subjected to a range of temperatures from 20 to 50°C, which resulted in decreased net photosynthesis as temperature increased. The data obtained for *Xylia xylocarpa* did not fit a parabolic graph shown in previous studies (Slatler 1977), probably due to a high starting temperature. However this might also represent a unique photosynthetic response of *Xylia xylocarpa* seedlings or tropical tree species in general.

Seedlings grown at 30/20°C day/night temperature from the Nakornratchasima seed source had higher net photosynthetic rates and water use efficiency than plants from the remaining growth temperatures. The differences in photosynthetic temperature response are probably due to the adaptation of trees from this seed source locations to the natural habitat. Nakornratchasima has the lowest maximum temperature average of the three seed sources (Table 3-1). This is in agreement with other studies which demonstrated that plants from cooler climates have poor ability to acclimate to warm climate and vice versa (Mooney et al. 1975, Mooney et al. 1978, Berry and Björman 1980).

When exposed to moderate temperatures, transpiration rates and stomatal conductance of *X. xylocarpa* grown at different temperatures appeared to follow a trend similar to net photosynthesis. Similar observations were recorded for another tropical tree species, *Manilkara sp.*, from the evergreen forest in Venezuela (Mullerstael 1987) However, when subjected to 50°C, seedlings grown at 25/20, 30/20 and 35/20°C day/night temperatures had negative net photosynthesis while transpiration rate and stomatal conductance increased. This indicates that CO_2 limitation were not responsible for the observed decline in photosynthesis at 50°C. The increase in transpiration likely consisted of a heat dissipation mechanism, characteristic of a number of mesophytic and xerophytic plants (Ehrler 1975).

At 50°C, seedlings from 25/20°C, 30/20°C, and 35/20°C day/night growth temperatures showed signs of membrane damage, indicated by increased electrolyte leakage. Seedlings grown at 40/20°C day/night temperatures were able to maintain positive net photosynthesis and did not show an increase in ion leakage, suggesting that the increase in thermotolerance may, in part be due to increased thermostability of cell membranes (Chen et al. 1982, Chaisompongpan et al. 1990). Other changes including enzyme activities, hormone production, and the synthesis of new proteins may also contribute to an increase in thermostability (Berry and Björman 1980, Levitt 1980, Caemmerer and Farquhar 1981, Verling 1991).

In summary, the present study showed that gas exchange processes in *X. xycocarpa* were altered by different growth temperatures. Seedlings grown at 40/20°C day/night temperature showed improved thermotolerance and increased photosynthetic

stability when exposed to high temperature stress. At moderate temperatures, transpiration rate and stomatal conductance responded similarly to photosynthesis, but 50°C induced an increase in transpiration and stomatal conductance and a decline in net photosynthesis.

3.5 References

Anapanurak, W., Boonplian, S., and Puriyakorn B. 1987. Soil Survey in Thongphaphoom district, Kanchanaburi province (Thai version). Royal Forest Department, Bangkok, Thailand. pp. 1-20.

Arayarangsarit, S., and Khawsut, P. 1987. Soil use plan for Nakornratchasima province. Land development Department, Bangkok, Thailand. pp. 2-23.

Bensink, J. 1971. On morphogenesis of lettuce leaves in relation to light temperature. Meded. Landbauw. Wageningen **71**(15):1-93.

Berry, J. A. 1975. Adaptation of photosynthetic processes to stress. Science. **188**:644-650.

Berry, J. and Björman, O. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annual Review of Plant Physiology **31**:491-543.

Björkman, O., Badger, M. R., and Armond, P. A. 1980. Response and adaptation of photosynthesis to high temperatures. *In* Adaptation of plants to water and high temperature stress. *Edited by* Turner N. C. and P. J. Kramer. John Wiley and Sons, Inc. New York. pp.233-249.

Brix, H. 1967. An analysis of dry matter production of Douglas-fir seedlings in relation to temperature and light intensity. Canadian Journal of Botany **45**:2063-2072.

Brix, H. 1969. Effect of temperature on dry matter production of Douglas-fir seedlings during bud dormancy. Canadian Journal of Botany **47**:1143-1146.

Brix, H. 1971. Growth response of western hemlock and Douglas-fir seedlings to temperature regimes during day and night. Canadian Journal of Botany **49**: 289-294.

Caemmerer, S. von., and Farquhar G. D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153**:376-387

Chaisompongpan, N., Li, P. H., Davis, D. W., and Markhart, A. H. III. 1990. Photosynthetic responses to heat stress in common bean genotypes differing in heat acclimation potential. Crop Science. **30**:100-104.

Chen, H. H., Shen, Z. Y., and Li, P. H. 1982. Adaptability of crop plants to high temperature stress. Crop Science **22**:719-725.

DePuit, E. J., and Caldwell, M. M. 1975. Gas exchange of three cool semi-desert species in relation to temperature and water stress. Journal of Ecology **63**:835-858.

Ehrler, W. L. 1975. Environmental and plant factors influencing transpiration of desert plants. *In* Environmental physiology of desert organisms. *Edited by* Hadley N. F. Dowden, Hutchingson & Ross, Inc. Pennsylvania. pp. 52-66.

Ehleringer, J. R., and Mooney, H. A. 1978. Leaf hairs: effects on physiological activity and adaptive value to a desert shrub. Oecologia **40**:183-200.

Fryer, J. H., and Ledig, F. T. 1972. Microevolution of the photosynthetic temperature optimum in relation to the elevational complex gradient. Canadian Journal of Botany **50**:1231-1235.

HåbjØrg, A. 1972. Effect of photoperiod and temperature on growth and development of three latitudinal and three altitudinal populations of *Betula pudescens* Ehrh. Meld. Norg. Landbr Hogsk **51**:1-27.

Havaux, M. 1993. Characterization of thermal damage to the photosynthetic electron transport system in potato leaves. Plant Science **94**:19-33.

Hawkins, B. J., and McDonald, S. 1994. The influence of temperature and soil water on growth, photosynthesis, and nitrogen fixation of red alder (*Alnus rubra*) seedlings. Canadian Journal of Forest Research **24**:1029-1032.

Hellmers, H. 1966a. Temperature action and interaction of temperature regimes in the growth of red fir seedlings. Forest Science. **12**:90-96.

Hellmers, H. 1966b. Growth response of redwood seedlings to thermoperiodism. Forest Science **12**:276-283.

Hellmers, H. and Sundahl, W. P. 1959. Response of *Sequoia sepervirens* (D. Don) Endl. and *Pseudotsuga menziesii* (Mirb.) Franco seedlings to temperature. Nature **184**:1247-1248.

Larson, M. M. 1967. Effect of temperature on initial development of ponderosa pine seedlings from three sources. Forest Science **13**:286-294.

Levitt, J., 1980. Responses of plants to environmental stress. 2nd edition. Vol I: Chilling, freezing, and high temperature stress. Academic Press. New York. pp. 347-445.

Long, S. P. 1985. Leaf gas exchange. *In* Photosynthetic mechanisms and environment. *Edited by* Barber J. and N. R. Baker. Elsevier Science Publisher B.V. Amsterdam. pp. 481-485.

Mishio, M. 1995. Shifts in photosynthetic responses to irradiance and temperature in *Viola* species. Photosynthetica **31**(1):127-134.

Mooney, H. A., Björman, O. and Berry, J. 1975. Photosynthetic adaptations to high temperature. *In* Environmental physiology of desert organisms. *Edited by* Hadley N.F. Dowden Hutchinson & Ross Inc, Pennsylvania. pp138-151.

Mooney, H. A., Björman, O., and Colatz, G. J. 1978. Photosynthetic acclimation to temperature in the desert shrub, *Larrea divaricata*. Plant Physiology **61**:406-410.

Mullerstael, H. 1987. Water relations and gas exchange of tropical rainforest tree species under stress: *Manilkara sp.* from high caatinga forest at San Carlos de Rio Negro-Venezuela. *In* Tree crop physiology. *Edited by* Sethuraj M.R. and A.S. Raghavendra. Elsevier, Amsterdam. pp.121-138.

Pearcy. R. W. 1976. Temperature effects on growth and CO2 exchange rates in coastal and desert races of *Atriplex leniformis* (Torr.) Wats. Oecologia **26**:245-255.

Pearcy, R. W. 1977. Acclimation of photosynthesis and respiratory CO_2 exchange to growth temperature in *Atriplex leniformis* (Torr.) Wats. Plant Physiology **59**: 795-799.

Phukittayacamee, P., Mangklarat, J., Pong-anan, K. and Nongnueng, S. 1993. Forest tree planting: The manual for forestry officer, social forest project 1993. Royal Forest Department. Bangkok, Thailand. pp. 136-143 .

Rawson, H. M., Turner, N. C., and Begg, J. E. 1978. Agronomic and physiological responses of soybean and sorghum crops to water deficits IV. Photosynthesis, transpiration and water use efficiency of leaves. Australian Journal of Plant Physiology **5**:195-209.

Sage, R. F., and Reid, C. D. 1994. Photosynthetic response mechanisms to environmental change in C3 plants. *In* Plant environment interactions *Edited by* Wilkinson R.E. Marcel Dekker, New York, pp. 413-449.

Slatyer, R. O. 1977. Altitudinal variation in the photosynthetic characteristics of snow gum, *Eucalyptus pauciflora* Sieb, ex Spreng. IV: Temperature response of four populations grown at different temperatures. Australian Journal of Plant Physiology **4**:583-594.

Slatyer, R. O. and Ferrar P. J. 1977. Altitudinal variation in the photosynthetic characteristic of snow gum, *Eucalyptus pauciflora* Siev, ex Spreng. II. Effects of growth temperature under controlled conditions. Australian Journal of Plant Physiology **4**:289-299.

Tansiri, B., Muangkaew, P., Arayarangsarit, S., and Kanchachanalai, W. 1989. Soil use plan for Maehongson province. Land Development Department, Bangkok. Thailand. pp. 1-14.

Terri, J. A. 1980. Adaptation of kinetic properties of enzymes to temperature variability. *In* Adaptation of plants to water and high temperature stress. *Edited by* Turner N.C. and P.J. Kramer. John Wiley & Sons, Inc. New York 251 p.

Treshow, M. 1970. Environment and plant response. McGraw-Hill Book Company. New York. pp.13, 54-56.

Vierling, E. 1991. The role of heat shock proteins in plants. Annual Review of Plant Physiology and Molecular Biology **42:** 579-620.

Wainwright, C. M. 1977. Sun-tracking and related leaf movements in a desert lupine (*Lupinus arizonicus*). American Journal of Botany **64**(8): 1032-1041.

Woodward, F. I. 1983. The significance on interspecific differences in specific leaf area to the growth of selected herbaceous species from different altitudes. New Phytologist **95**:313-323.

Young, J. P., Dengler, N. G., Donnelly, P. M., and Dickenson, T. A. 1990. Heterophylly in *Ranunculus flabellaris*: The effect of abcisic acid on leaf ultrastucture. Annals of Botany **65**(6):603-616.

Young, J. P., Dickson, T. A., and Dengler, N. G. 1995. A morphometric analysis of heterophyllous leaf development in *Ranunculus flabellaris*. International Journal of Plant Sciences **156** (5): 590-602.

Table 3-1. Weather conditions in each seed source location averaged over 30-35 years

Seed source	Temperature°C			Relative humidity (%)			Rainfall (mm)	Year
	Max. (Apr.)	Min. (Jan)	Mean (annual)	Max.	Min.	Mean		
Maehongson	37.7	13.9	25.5	91.7	50.6	73.8	1273	(1951-1985) [1]
Kanchanaburi	37.9	17.7	28.1	87.6	51.0	68.0	1115	(1951-1980) [2]
Nakornratchasima	36.5	16.2	26.4	90.5	52.0	73.0	1137	(1951-1980) [3]

[1] Tansiri B. et.al. 1989,
[2] Anapanurak W. et.al.1987,
[3] Arayarangsarit S. and Khawsut P. 1987

Table 3-2. Altitude and soil properties of the three seed source locations

Seed source	Altitude (m)	Soil type	pH
Maehongson	300-500	Sandy soil, shallow, parent material sandstone	6-7
Kanchanaburi	100-200	Sandy, sandy loam soil mixed with granular, shallow, low to moderate fertility	6-7
Nakornratchasima	200-300	Sandy loam, sandy clay loam mixed with granular, moderate deep to shallow, low fertility	6-7

Table 3-3. Seed collection

Seed source	No. of trees	Girth (cm)	Height (m)	Age (Yrs)	Dist. among trees (m)
Maehongson	10	70-160	20-30	20-60	≥100
Kanchanaburi	10	75-150	20-30	20-50	100-500
Nakornratchasima	10	50-100	10-20	15-30	≤100

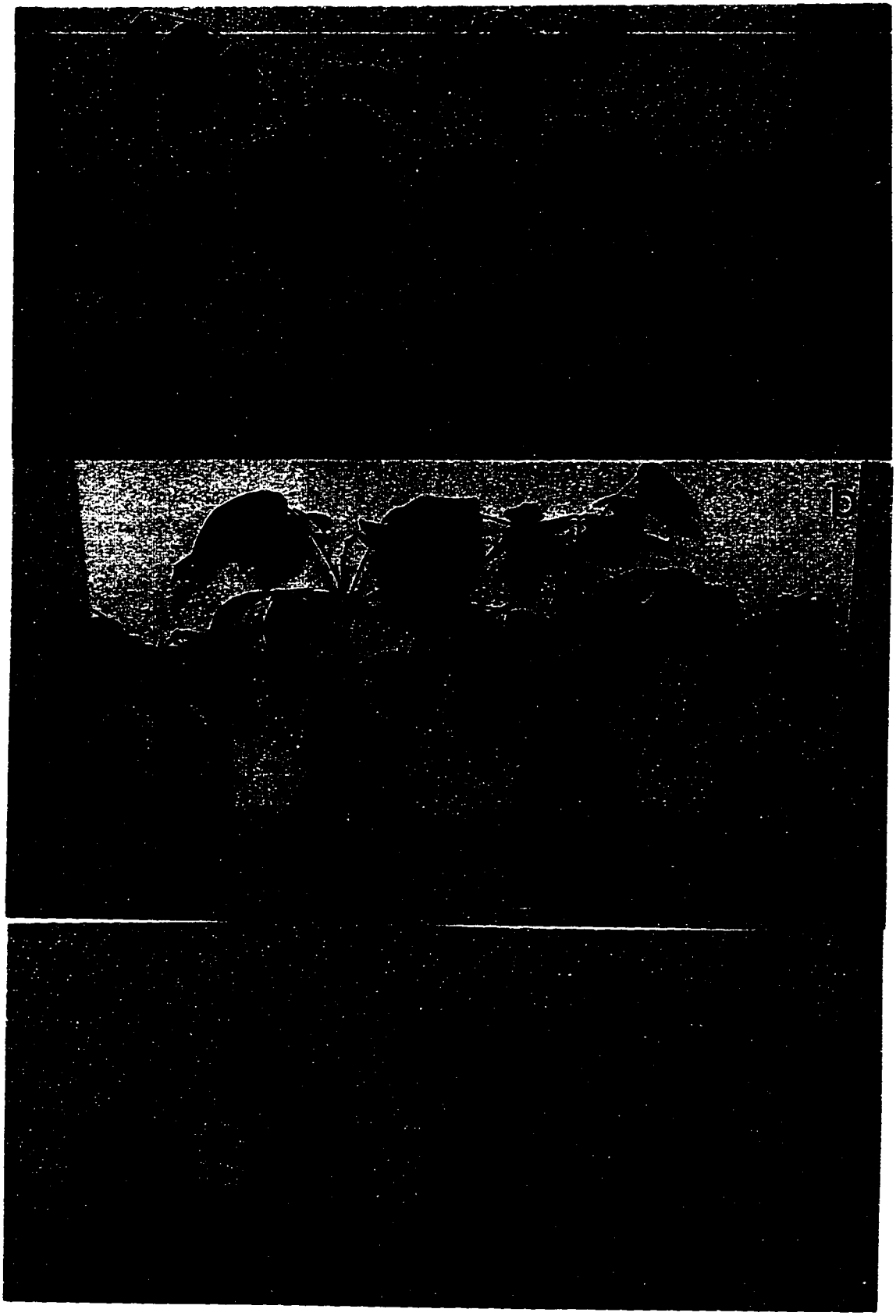
Table 3-4. Probability values from the analysis of variance for height (H), diameter (D), D^2H, net photosynthesis (A), transpiration (E), stomatal conductance (g_s), and water use efficiency (WUE) data of *Xylia xylocarpa* from three seed sources (Maehongson, Kanchanaburi, Nakornratchasima) grown at 4 different temperatures (25/20°C, 30/20°C, 35/20°C and 40/20°C day/night)

Source				Measured at day growth temperature				Measured at 25°C			
	H	D	D^2H	A	E	g_s	WUE	A	E	g_s	WUE
Replicate	0.9993	0.4426	0.3560	0.4263	0.4001	0.8694	-	0.9185	0.7809	0.8663	0.3960
Seed source	0.0105	0.0303	0.0215	0.0146	0.3866	0.5692	0.1182	0.9100	0.8578	0.8430	0.5000
Replicate X Seed source	0.4957	0.4014	0.2850	0.9648	0.3128	0.7833	0.8250	0.1833	0.0133	0.0336	0.4960
Growth temperature	0.0038	0.0724	0.0181	0.0950	0.5790	0.4114	0.0743	0.0041	0.0089	0.0064	0.0856
Replicate X Growth temperature	0.8820	0.5879	0.6260	0.0412	0.6887	0.5068	0.7136	0.6691	0.1527	0.4735	0.5211
Seed source X Growth temperature	0.2198	0.5187	0.1324	0.5679	0.8252	0.9013	0.7975	0.5461	0.5475	0.7262	0.5802
Replicate X Seed source X growth temperature	0.1699	0.5137	0.5511	0.9875	0.9118	0.7054	0.8495	0.8495	0.9718	0.8642	0.7458

Table 3-5. Probability values from the analysis of variance for net photosynthesis, transpiration, stomatal conductance, water use efficiency (WUE) and electrolyte leakage data of X*ylia xylocarpa* from three seed sources (Maehongson, Kanchanaburi and Nakornratchasima) grown at 4 different temperatures (25/20 ^{o}C, 30/20 ^{o}C, 35/20 ^{o}C, and 40/20^{o}C day/night) after seedlings were subjected to different temperatures (20, 25, 30, 35, 40, 45, 50^{o}C) for 30 minutes.

Source	Net photosynthesis	Transpiration	Stomatal conductance	WUE	Electrolyte leakage
Replicate	0.3549	0.4602	0.6843	-	0.9576
Seed source	0.3434	0.4963	0.4384	0.0086	0.3511
Replicate X Seed source	0.0769	0.1049	0.1260	0.9226	0.1151
Growth temperature	0.0058	0.1063	0.0816	0.0061	0.0191
Replicate X Growth temperature	0.7577	0.7660	0.4875	0.7399	0.4194
Seed source X Growth temperature	0.2449	0.2159	0.0144	0.0931	0.0742
Replicate X Seed source X Growth temperature	0.0004	0.0391	0.1049	0.0057	0.4025
Temperature	0.0001	0.0001	0.0001	0.0001	0.0001
Replicate X Temperature	0.6066	0.6833	0.5555	0.5430	-
Seed source X Temperature	0.0577	0.1566	0.0140	0.1232	0.0002
Replicate X Seed source X Temperature	0.0252	0.2067	0.3808	0.0138	0.9181
Growth temperature X Temperature	0.0001	0.0001	0.0001	0.0001	0.0001
Replicate X Growth temperature X Temperature	0.5873	0.7868	0.9887	0.3192	0.9826
Seed source X Growth temperature X Temperature	0.0712	0.6998	0.4109	0.0295	0.3643
Replicate X Seed source X Growth temperature X Temperature	0.9899	0.6899	0.8460	0.9974	0.3008

Figure 3-1. Effect of different growth temperatures (25/20°C, 30/20°C, 35/20°C and 40/20°C day/night) on *Xylia xylocarpa* seedlings from Maehongson (a), Kanchanaburi (b) and Nakornratchasima (c) seed sources.



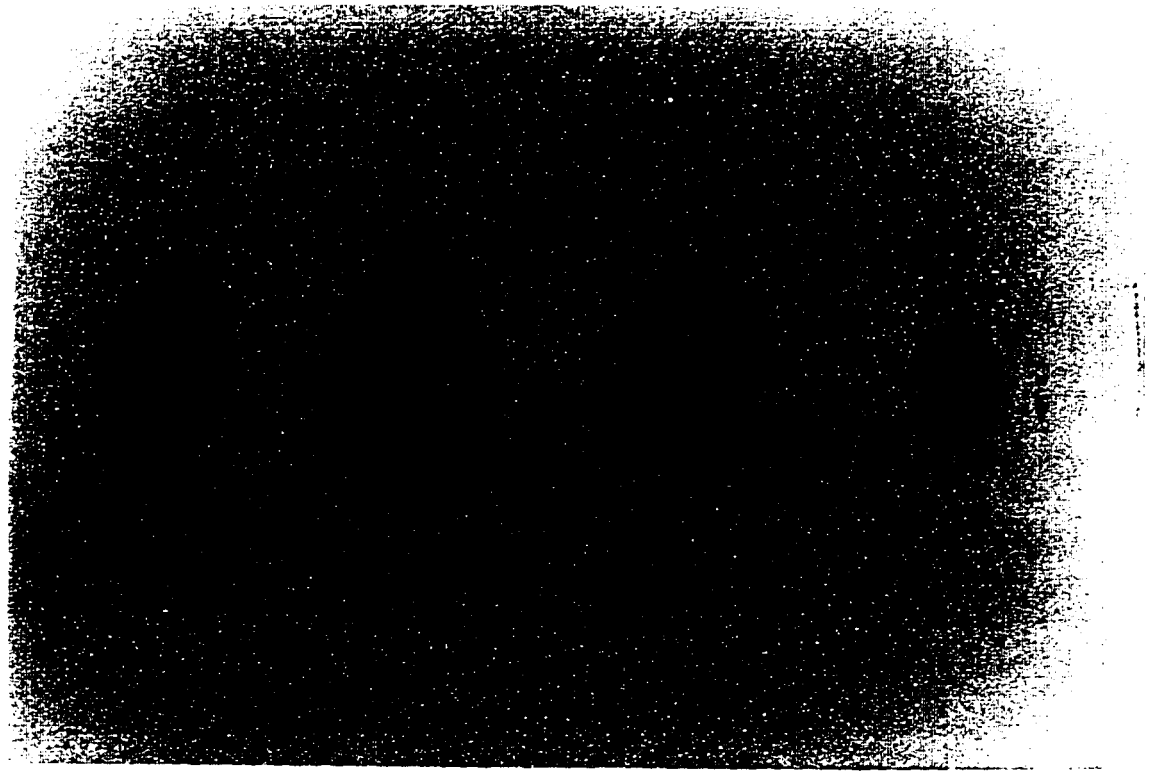


Figure 3-2. Typical leaves from *Xylia xylocarpa* seedlings grown at 25/20°C, 30/20°C, 35/20°C and 40/20°C day/night temperatures (Kanchanaburi seed source)

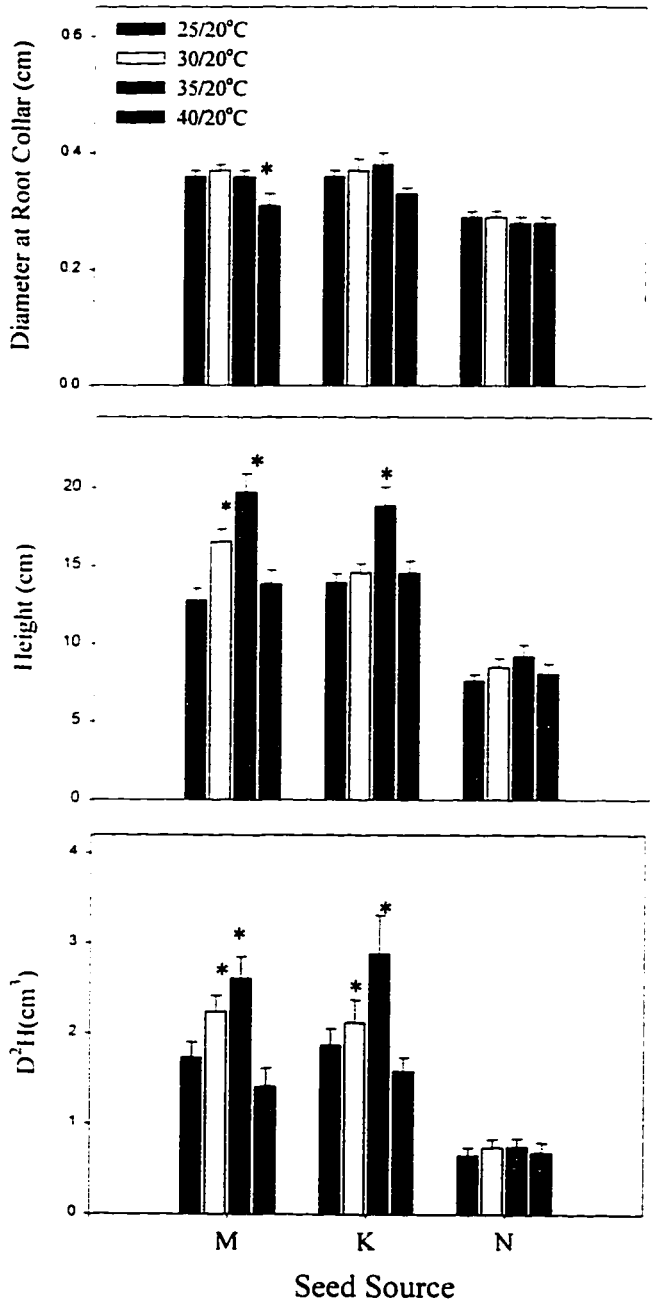


Figure 3-3. Diameter (D), height (H) and D^2H of 3-month-old *Xylia xylocarpa* seedlings from Maehongson (M), Kanchanaburi (K), and Nakornratchasima (N) grown at 25/20°C, 30/20°C, 35/20°C and 40/20°C day/night temperatures
* indicates significant difference at $P<0.05$. Means and SE are shown.

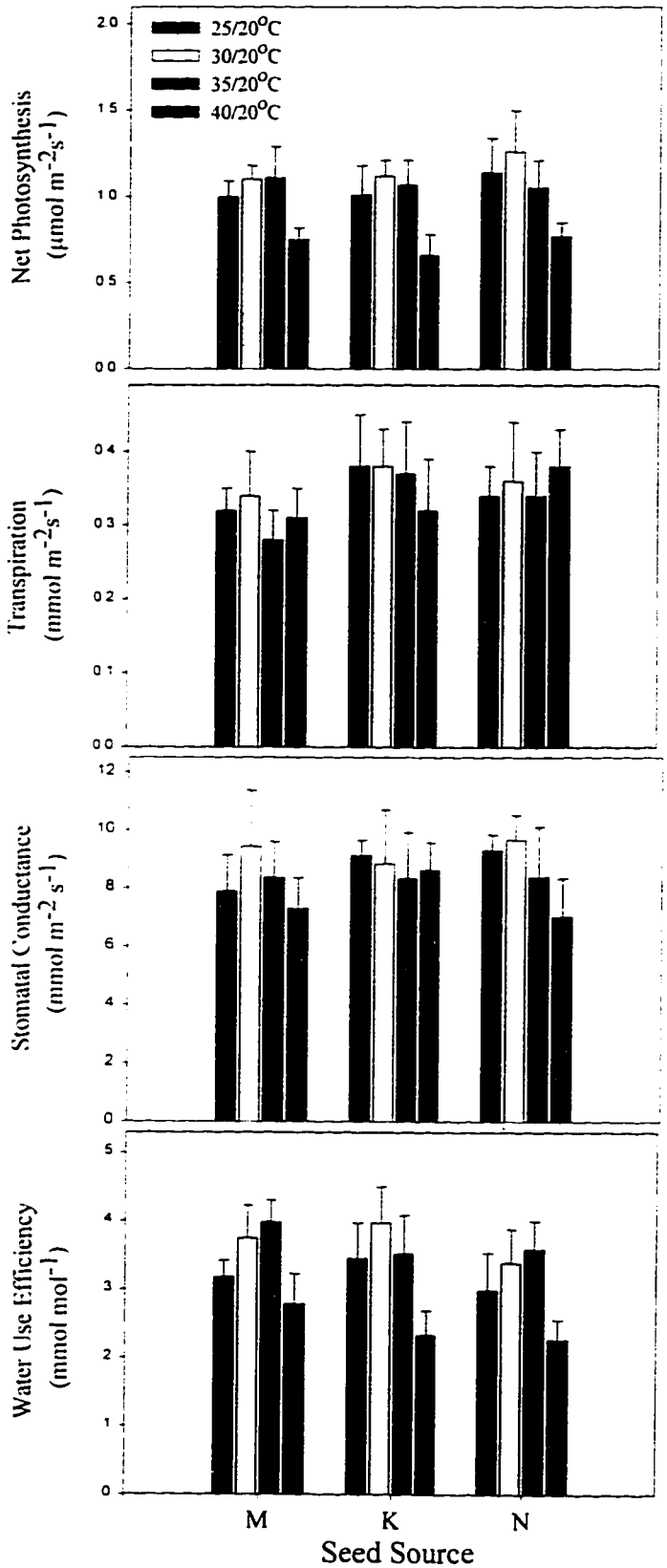


Figure 3-4. Gas exchange of 3-month-old *Xylia xylocarpa* seedlings from Maehongson (M), Kanchanaburi (K), and Nakornratchasima (N) seed sources grown at 25/20°C, 30/20°C, 35/20°C and 40/20°C day/night temperatures. Measured at the day growth temperatures. Means and SE are shown.

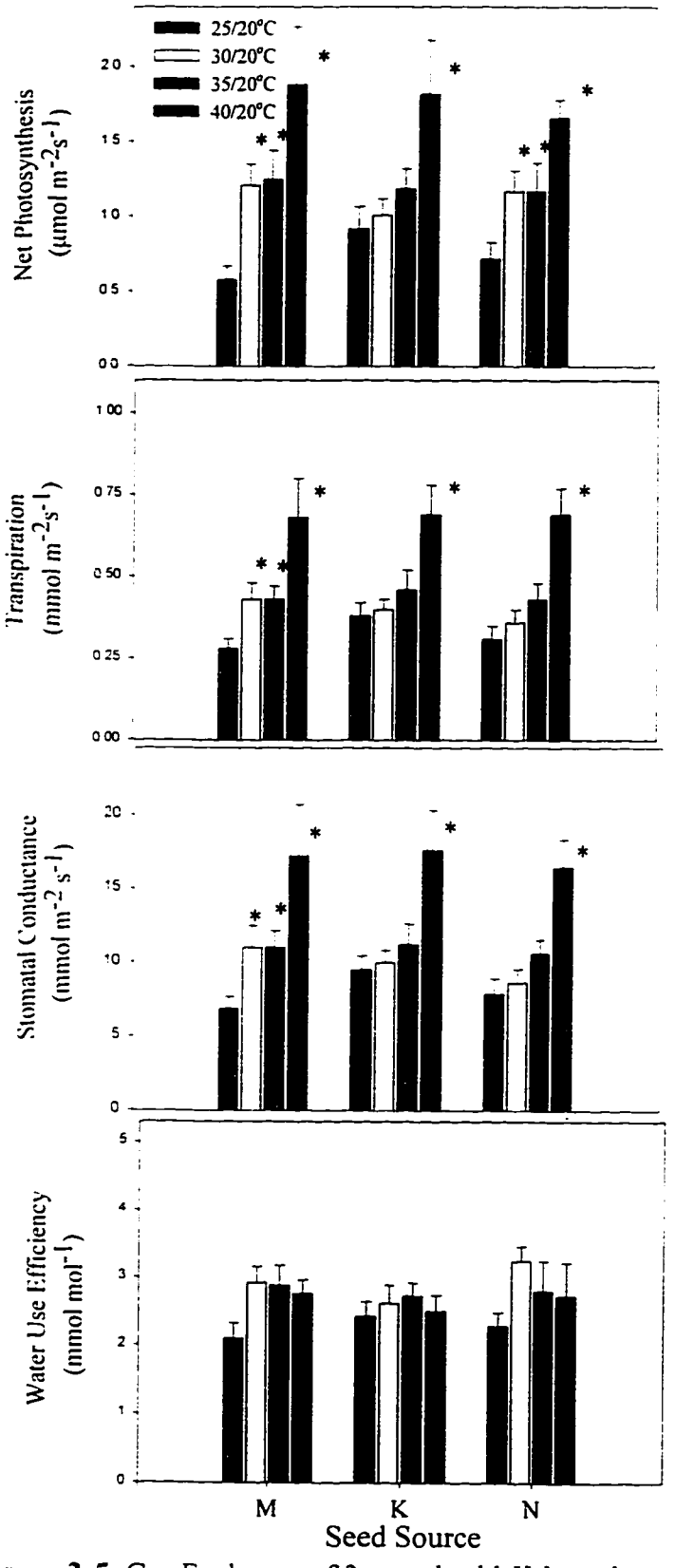


Figure 3-5. Gas Exchange of 3-month-old *Xylia xylocarpa* seedlings from Maehongson (M), Kanchanaburi (K), and Nakornratchasima (N) seed sources grown at 25/20°C, 30/20°C, 35/20°C and 40/20°C day/night temperatures. Measured at 25°C. * indicates significant difference at $P<0.05$. Means and SE are shown.

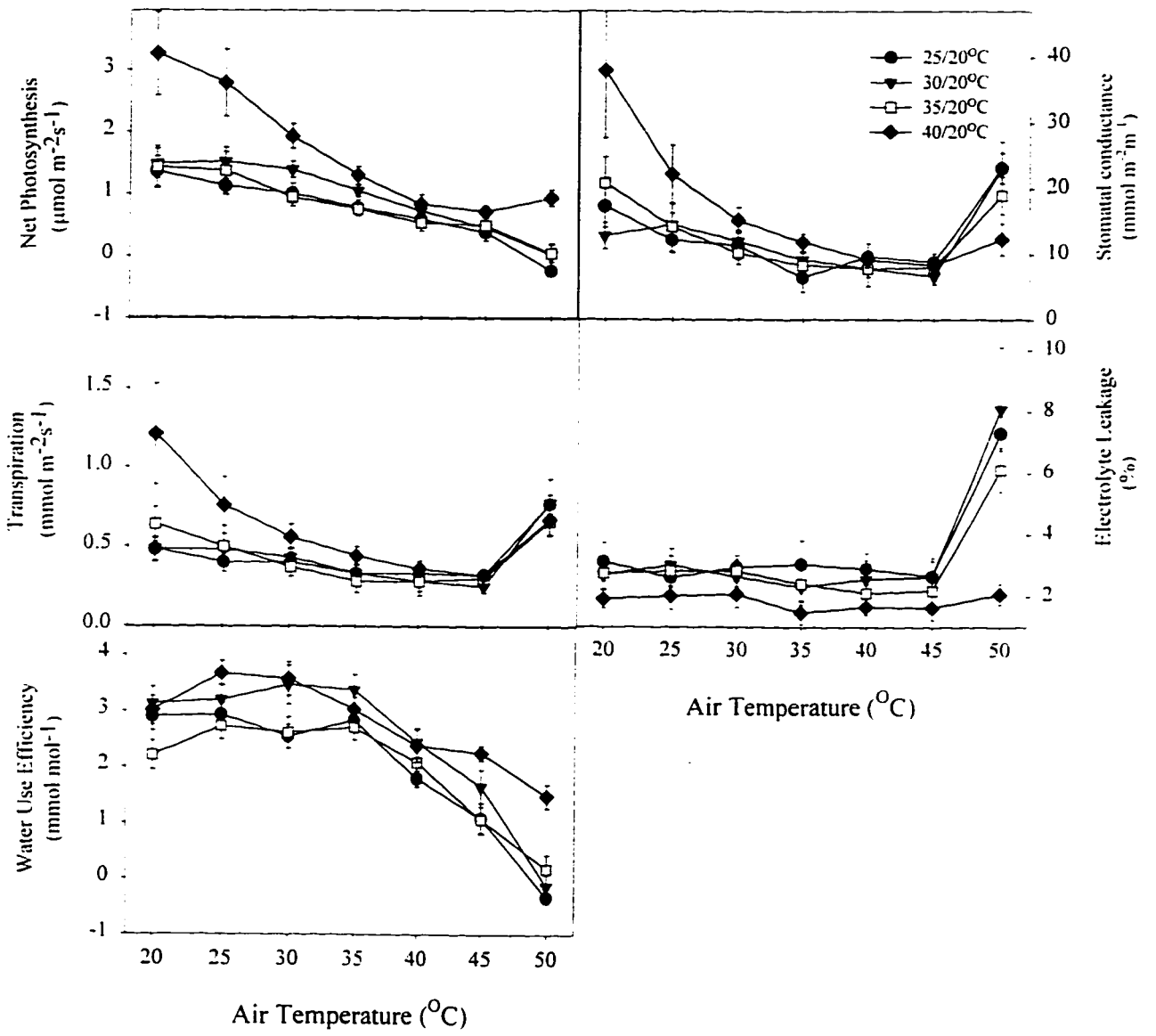


Figure 3-6. Temperature responses of 3-month-old *Xylia xylocarpa* seedlings (Maehongson seed source) grown at different temperatures (25/20°C, 30/20°C, 35/20°C, and 40/20°C day/night). Means and SE are shown.

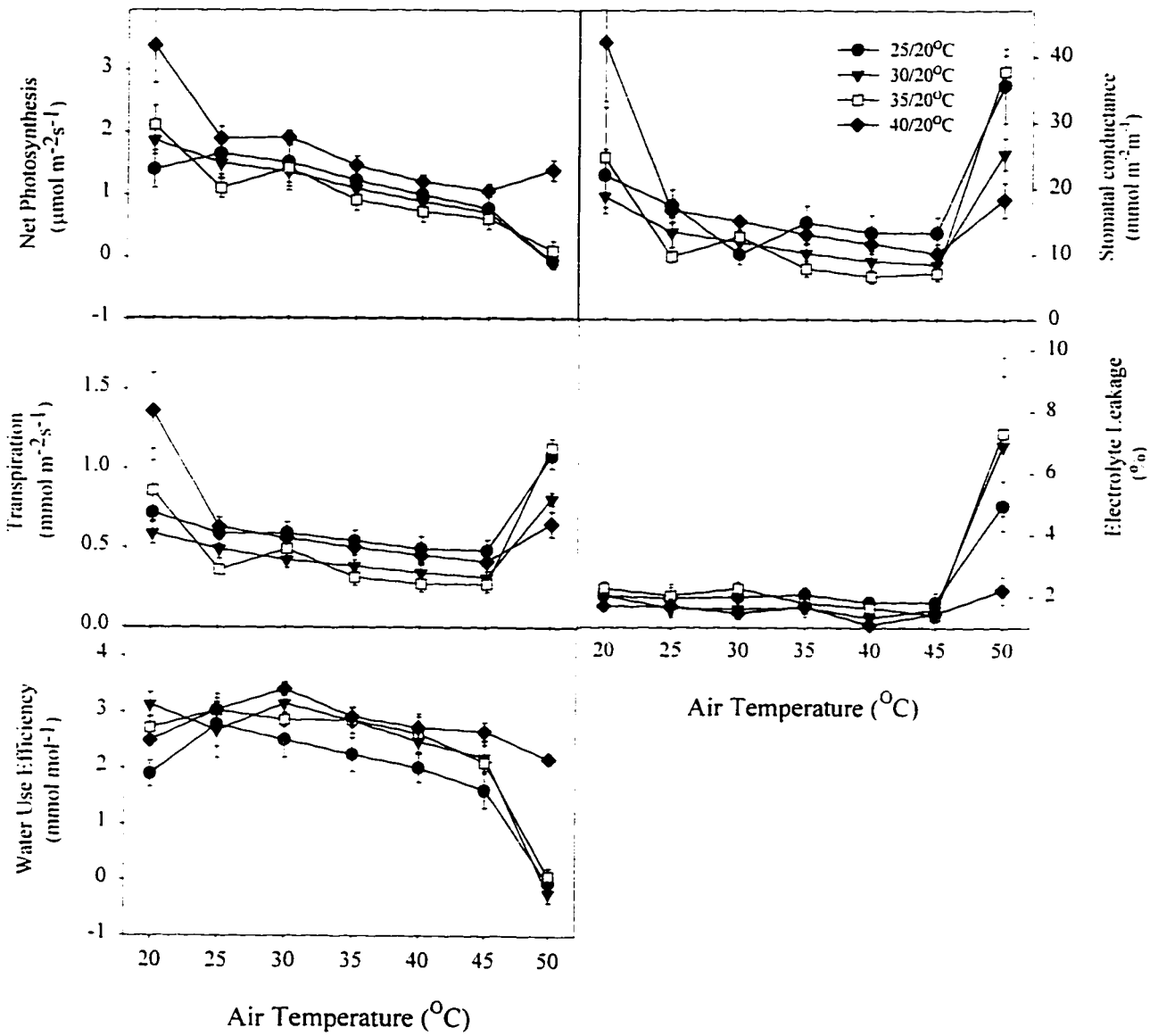


Figure 3-7. Temperature responses of 3-month-old *Xylia xylocarpa* seedlings (Kanchanaburi seed source) grown at different temperatures (25/20°C, 30/20°C 35/20°C, and 40/20°C day/night). Means and SE are shown.

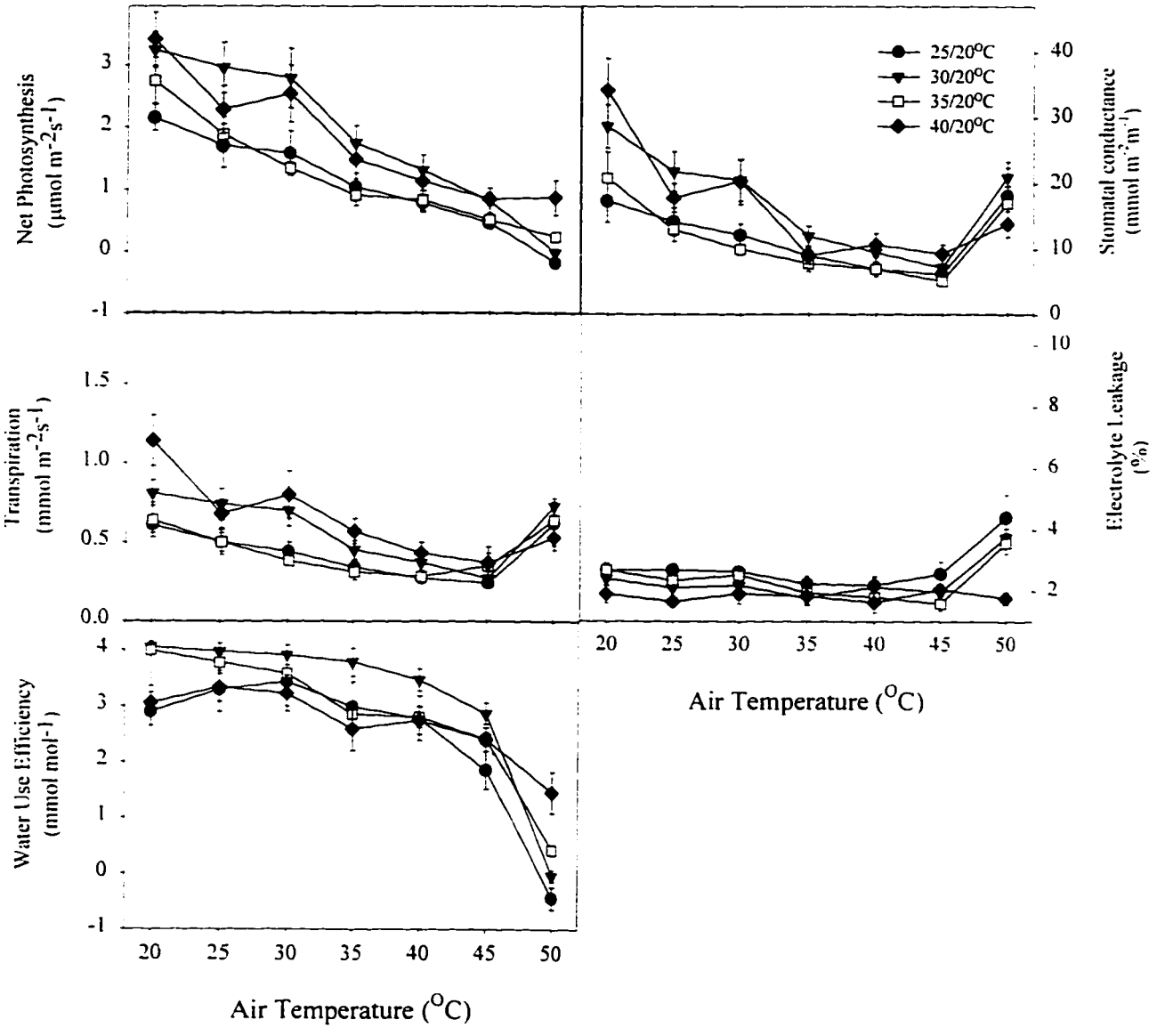


Figure 3-8. Temperature responses of 3-month-old *Xylia xylocarpa* seedlings (Nakornratchasima seed source) grown at different temperatures (25/20°C, 30/20°C, 35/20°C, and 40/20°C day/night). Means and SE are shown

CHAPTER FOUR

Protein and Lipid Changes in High Temperature Acclimated *Xylia xylocarpa* Seedlings

4.1 Introduction

The importance of high temperature stress has long been recognized for agricultural crop plants (McDaniel 1982, Krishnan et al. 1989, Burke, 1990, Ristic et al. 1991, Nguyen et al. 1994). However, the effects of high temperature stress on forest trees have received little attention. Colombo et al. (1992) speculated that heat tolerance of black spruce (*Picea mariana* Mill. B.S.P.) is heritable within families and is likely influenced by production of heat shock proteins. If this is true, these heat shock proteins may be able to increase the survival of seedlings during nursery stock production, transportation, and after planting in the forest.

Morphological, physiological, and biochemical changes occur in plants grown at high temperature (Mooney et al. 1975, Burk 1991). Photosynthesis is considered to be more thermally sensitive than other biochemical processes (Björman et al. 1980, Nash et al. 1985). Thebud and Santatius (1982) suggested that chloroplast membranes are the primary site of photosynthetic inactivation by heat stress. The tonoplast and plasmalemma remain stable at temperatures above those at which photosynthesis ceases (Thebud and Santarius 1982). The main factors determining the ability of plants to adapt to different growth temperatures are believed to be the composition of membranes (Quinn and Williams 1985), metabolic capacity (Mooney et al. 1975), and the control of enzyme activity (Larcher 1969). Changes in the properties of chloroplast membranes under high

temperature stress have been studied in many plant species and include both changes lipid composition and changes in lipid-protein associations (Langridge and McWilliam 1967, Heber and Santarius 1973, Pearcy 1978, Levitt 1980, Raison et al. 1982a, 1982b). Changes in the proportions of various lipids under high temperature stress are believed to stabilize membrane integrity, decrease membrane fluidity and decrease permeability to electrolytes (Quinn and Williams 1985). Raison et al. (1982a) reported that membrane fluidity in *Nerium oleander* grown at 20/15°C day/night temperature was higher compared to plants grown at 45/32°C day/night temperature. Changes in membrane fluidity were correlated with changes in the proportion of oleic and linolenic acids relative to other chloroplast polar lipids (Raison et al. 1982b).

In addition to changes in membrane lipid, increased thermotolerance of plants has been associated with the synthesis of heat shock proteins (HSPs) (Vierling 1991). Changes in protein expression in plants subjected to high, sub-lethal temperatures have been reported in many plant species both in the field and under controlled environments (Cooper and Ho 1983, Colombo et al. 1992, Nguyen et al. 1994). Soybean (*Glycine max* var. Wayne) seedlings subjected to 45°C for as little as 15 minutes produced a new set of proteins (Lin et al. 1984). A study of two wheat (*Triticum aestivum* L.) cultivars showed that the more heat tolerant cultivar produced more HSPs under identical conditions (Nguyen et al. 1994). It is possible that some of the acclimation effects in *X. xylocarpa* reported in Chapter 3 could be due to HSP production.

The main objective of this study was to examine the changes of heat stress tolerance of *Xylia xylocarpa* seedlings by examining the composition of chloroplast lipids

and the protein expression in chloroplasts and leaves acclimated to different growth temperatures and subjected to heat shock conditions.

4.2 Materials and Methods

4.2.1 Plant material

Three-month-old seedlings from the Maehongson, Kanchanaburi, and Nakornratchasima seed sources were grown under 4 different temperature regimes: 25/20°C, 30/20°C, 35/20°C and 40/20°C day/night as described in Chapter 3.

4.2.2 Chloroplast isolation

Four leaf samples (1g fresh weight each) from four individual seedlings representing each growth regime were used in chloroplast isolation. Seedlings were kept in the dark for 2-3 hours before harvesting to reduce chloroplast starch levels. The leaves were cut into 1-2 mm wide strips and homogenized 2 X 10 seconds at 24 000 RPM in an IKA Ultra Turrax homogenizer in 20 mL of buffer consisting of 0.33M sucrose, 0.03M Tris-HCl pH 7.8, 0.1% (w/v) fatty acid-free bovine serum albumin. The homogenate was filtered through 4 layers of cheesecloth and the filtrate centrifuged at 100 X g for 1 minute. The pellet containing cell debris was discarded and supernatant was centrifuged at 1500 X g for 5 min. to pellet the chloroplasts. The chloroplast pellet was resuspended in medium consisting of 0.33M sucrose and 0.03M Tris-HCl pH 7.8, and the suspension was layered on top of a step gradient of 10 mL 35% (w/v) sucrose over 10 mL 70% (w/v) sucrose. The gradient was centrifuged at 2 000 X g for 20 minutes and the chloroplasts were recovered from the 35%/70% interface. Chloroplasts were collected and suspended in washing medium containing of 0.33M sucrose and 0.03M Tris-HCl pH 7.8, and

pelleted at 10 000 X g for 10 min. The resulting chloroplast pellet was resuspended in washing medium and examined under the microscope to confirm chloroplast purity. The chloroplasts were stored at -80°C before lipid extraction. All steps of chloroplast isolation and purification were carried out at 3-5°C.

4.2.3 Lipid extraction

Chloroplast lipids were extracted as described by Crespi et al. (1989). Methanol (2 mL) and chloroform (1 mL) were added to chloroplast pellets followed by 1.6 mL distilled water and 200 μL HCl resulting in a ratio of $CHCl_3$/MeOH/H_2O of 1:1:0.9. The solvents were vigorously mixed for 30 seconds and centrifuged for 5 minutes at 100 X g to separate phases. The lower, chloroform phase was saved and the extraction was repeated by adding 2 mL chloroform to the remaining mixture. The chloroform layers were combined and evaporated under a stream of nitrogen to near dryness.

4.2.4 Lipid purification

Lipid samples suspended in 0.5 mL chloroform were loaded on a column of activated silicic acid (Sigma, 325 mesh). The column was sequentially eluted with 10 column volumes of chloroform to elute sterols and other neutral lipids, 10 column volumes of chloroform-acetone (1:1, v/v) to elute monogalactosyldiacylglycerols; MGDG, 30 column volumes of acetone to elute digalactosyldiacylglycerols; DGDG, and 10 column volumes of methanol to elute phospholipids; PL (Zwiazek and Blake 1990). The solvents were evaporated using a vacuum evaporator and separated lipids were suspended in 0.5 ml chloroform.

4.2.5 Lipid analysis

Fatty acid composition was determined in purified MGDG, DGDG, and PL fractions. Heptadecanoic acid (17:0) was added to all samples as an internal standard for fatty acid quantification. Chloroform was removed from samples under a stream of nitrogen to minimize oxidation and the samples were trans-esterified with 0.5 M sodium methoxide for 20 minutes at 70°C. Fatty acid methyl esters were extracted 3 times with 3 mL hexane, pooled and dried under a stream of nitrogen to approximately 10 μL. The samples were injected into a Hewlett Packard gas chromatograph equipped with a 30-m long and 0.25-mm inside diameter DB225 capillary column. The initial oven temperature of 180°C was increased 2°C/min to the final temperature of 210°C. The injector and detector temperatures were set at 250°C and the carrier (helium) flow was 15 mL/min with head pressure set at 150 kPa. Integration of chromatographic peaks was performed using a 3390A Hewlett Packard electronic integrator. Chromatographic peaks were identified by gas chromatography-mass spectrometry (GC-MS) and retention times were used to calculate the equivalent chain length of fatty acid methyl esters as described by Jamieson and Reid (1969). Fatty acid composition of lipids was calculated as percent weight of total fatty acids. Two-way ANOVA was used to test for significant differences among seed sources and growth regimes. The means represent 4 independent chloroplast isolations. Student-Newman-Keuls test at $P \leq 0.05$ was used to determine statistically significant differences.

4.2.6 Plant materials for chloroplast isolation following heat shock

Leaves of *Xylia xylocarpa* from each seed source and growth temperature were collected before and after seedlings were subjected to heat shock conditions (47°C) for 1 hour. Mature, fully expanded leaves were selected from each seedling to minimize variation due to leaf age. Chloroplast isolations followed the same procedure as for lipid analyses. Two independent chloroplast isolations were done.

4.2.7 Total leaf and chloroplast protein extraction

Leaves or isolated chloroplasts were crushed in a mortar with liquid nitrogen. Samples containing 40 mg dry weight of leaves or isolated chloroplasts. were washed three times with diethyl ether to remove lipids and other organic compounds interfering with electrophoresis. The dried powder was suspended in extraction buffer containing 2.5 mM Tris pH 6.8, 2-4% (w/v) sucrose, 8% (w/v) SDS. 10% (w/v) polyvinylpolypyrrolidone. Five percent (v/v) β-mercaptoethanol was added just before use. Samples were heated for 2 minutes at 70°C, and cooled to room temperature. Protamine sulfate (0.01%) was added prior to 2-D electrophoresis and samples were centrifuged at 12 000g for 10 minutes. Proteins in the supernatant were precipitated at -80°C for 2 hours with 90% (v/v) acetone containing 10 mM dithiothreitol. Protein pellets obtained by centrifugation were vacuum-dried prior to processing for SDS PAGE.

4.2.8 Electrophoresis

Dried protein pellets were solubilized in 200 μL sample buffer containing 0.0625 M Tris HCL (pH 6.8), 1.25% SDS, 12.5% glycerol, 1.25% β-mercaptoethanol, and 0.001% bromophenol blue. Protein concentrations were determined using the

bicinchoninic acid (BCA) assay (Pierce Chemical Co.) using bovine serum albumin as a standard. Samples containing 10 μg of total protein were separated by 10% and 12.5% polyacrylamide gels using the buffer system of Laemmli (1970) and a mini-gel electrophoresis system (Bio-Rad).

Two-dimensional polyacrylamide gel analyses of total leaf and chloroplast proteins from the Kanchanaburi seed source were performed following the method of O'Farrell (1975). 20 μg of total protein were used for two-dimensional polyacrylamid gel. Separated proteins were visualized by silver staining (Merrill and Goldman 1984).

4.2.9 Analysis of protein profiles

Silver stained gels were scanned at 1200 dpi using a Microtek Scanmaker III scanner equipped with a transparent media adapter. Image files were analyzed using Molecular Analyst software from Bio Rad.

4.2.10 Expression of HSP 18.1

Protein samples from each of the three seed sources grown at different growth temperatures and subjected to heat shock were separated on 12.5% acrylamide gels and electroblotted to nitrocellulose (Towbin et al. 1979). The blotted proteins were probed with rabbit anti-HSP 18.1 antiserum in 1:500 dilution (Hernandez and Vierling 1993). Alkaline phosphatase conjugated antibodies (Sigma) raised against rabbit were used to detect immunoreactive bands.

4.3 Results

4.3.1 Chloroplast lipids

The predominant fatty acids in total chloroplast lipids were linolenic acid (18:3), linoleic acid (18:2), and palmitic acid (16:0), which together comprised about 80-90 % of the total lipids (Table 4-1). Growth temperature affected the total amount of chloroplast lipids measured on a fresh weight basis. The effect was statistically significantly for the MGDG ($P<0.0001$) and DGDG ($P<0.0053$) but not for PL (Table 4-1, 4-2). The amount of MGDG per gram fresh weight of leaves was higher for the Nakornratchasima seed source than for the other two seed sources grown at 35/20°C day/night temperature ($P<0.0048$). In addition, total MGDG and DGDG chloroplast lipid content in seedlings grown at 40/20°C day/night temperature was higher compared to that of in the remaining growth temperatures ($P<0.05$).

Oleic acid (18:1) content of the MGDG in the Kanchanaburi seed source was significantly higher than the other seed sources ($P<0.0001$). In contrast, linolenic (18:3) content was lower for the Kanchanaburi than for Nakornratchasima and Maehongson seed sources in all of the studied growth temperatures ($P<0.0098$) (Table 4-1, 4-2) . In all three seed sources, 18:2 showed an increasing trend with increasing day temperature (Table. 4-2).

In all three seed sources, seedlings grown at 25/20°C day/night temperature had the lowest content of 18:0 in the DGDG lipids, followed by 40/20°C, 30/20°C, and 35/20°C day/night temperature (Table 4-2). However, the Kanchanaburi seed source had a higher content of 18:1 in DGDG lipids than the other two seed sources ($P<0.0001$)

(Table 4-1, 4-2). Linolenic acid (18:3) was significantly influenced by growth temperature ($P<0.0024$), and seedlings grown at 25/20°C had the greatest content of 18:3 followed by 30/20°C, 40/20°C, and 35/20°C (Table. 4-1, 4-2). The content of 16:0 from the three seed sources was similar in the different growth temperatures and in all lipid classes. The ratio of unsaturated to saturated fatty acids did not show significant differences between growth temperature and among seed sources in any of the lipid classes (Table 4-1, 4-2). There were also no significant changes in the chloroplast phospholipid content between growth temperatures and among seed sources (Table 4-1, 4-2).

4.3.2 Gel electrophoresis

Leaves from the three seed sources grown at 40/20°C day/night temperature contained a distinct 27-kD protein band as shown in the 1-D gel (Lane 4 on Fig. 4-1a, 4-1b and 4-1c) and 2-D gel (indicated with a small square on Fig. 4-3d). Interestingly, the 27-kD protein band disappeared under heat shock conditions (Fig. 4-1, 4-3d, 4-4d). A 50 kD and a 39 kD protein band also increased in staining intensity in seedlings grown at 40/20°C day/night from the Maehongson seed source but not in the Kanchanaburi and Nakornratchasima seed sources (Fig. 4-1). Chloroplast protein profiles from all growth temperatures and HS treatments appeared similar in all three seed sources (Fig. 4-2).

Two dimensional PAGE gels of leaf proteins from the different growth temperatures and HS treatments from Kanchanaburi seed source are showed about 10 protein spots, mostly below 45 kD (indicated with small circles on Fig. 4-3), which could

not be detected after heat shock. The quantities of several proteins at approximately 90 kD and 28 kD increased after heat shock (indicated with small triangles on Fig. 4-4).

4.3.3 HSP 18.1

The HSP 18.1 protein was present in leaves grown at the 35/20°C and 40/20°C day/night temperatures and in heat-shocked leaves(Lanes 3, 4, 5, 6, 7, 8 in Fig. 4-5a, 4-5b and 4-5c respectively). HSP18.1 was not detected in leaves grown at the 25/20°C and 30/20°C temperatures, except after heat shock (Lanes 1, 2 in Fig. 4-5a, 4-5b, 4-5c). Leaves from the 40/20°C growth temperature contained more HSP18.1 protein compared with 35/20°C temperature (Fig. 4-5). Purified chloroplast proteins from the different growth regimes and heat shock treatment did not react with the HSP18.1 antiserum (data not show).

4.4 Discussion

Changing the physical properties of membranes may play an important role in plant acclimation mechanisms to either high or low temperatures (Pearcy 1978). Raison et al. (1982a) reported that membrane lipids of *Nerium oleander* grown at 20/15°C day/night temperature, had higher fluidity than those in plants grown at 45/32°C day/night temperature likely due to the higher ratio of unsaturated to saturated fatty acids (Raison et al. 1982b). Similar changes have also been observed in bacteria and algae (Gaughran 1947, Holton et al. 1964, Kleinschmidt and McMahon 1970). In contrast, Gombos et al. (1994) suggested that under heat stress, the proportion of unsaturated fatty acids increases and it likely enhances the stability of the photosynthetic system. Santarius and Müller (1979) also demonstrated that increased high temperature tolerance of

photosynthesis during high temperature acclimation in spinach was not related to the levels of saturated fatty acids in membrane lipids. In the present study, *X. xylocarpa* seedlings grown at 40/20°C showed an increase in the photosynthetic tolerance of high temperature (Chapter 3). In all three seed sources similar changes in fatty acid profiles occurred, including an increased content of 18:2 in MGDG and DGDG of chloroplasts from 40/20°C growth temperature. However, the ratio of unsaturated to saturated fatty acids in MGDG, DGDG and PL of chloroplasts did not show significant changes. Therefore, in *X. xylocarpa*, the overall proportion of unsaturated and saturated fatty acids does not appear to be directly associated with the photosynthetic response to temperature. The increase in the stability of photosynthesis under elevated temperature and in thermotolerance of *X. xylocarpa* seedlings grown at 40/20°C day/night temperature is probably due to other mechanisms than simply changes in the chloroplast lipid composition.

Heat shock proteins, predominantly of low molecular weight, are produced by plants in response to elevated temperature. *Xylia xylocarpa* seedlings accumulated low molecular weight (LMW) heat shock protein during high temperature acclimation. One of the accumulated proteins belongs to the class I LMW HSPs (DeRocher et al. 1991, Hernandez and Vierling 1993). Antibodies against HSP18.1 were shown to recognize multiple polypeptides in some plants (Hernandez and Vierling 1993). In *X. xylocarpa*, a single immunoreactive protein band was present in seedlings grown at 35/20°C and 40/20°C day/night temperatures and in seedlings of all growth temperatures subjected to 47°C heat shock for 1 hour. However, seedlings grown at 40/20°C did not appear

synthesize increased amounts of HSP18.1 after heat-shocked, compared to unshocked growth temperatures. The production of HSP18.1 coincided with the increase in high temperature tolerance of *X. xylocarpa* grown at 40/20°C day/night temperature as evidenced by low electrolyte leakage and thermostable photosynthesis. Hernandez and Vierling (1993) reported that the LMW HSP18.1 was also found in the flowers and seeds of several pea species and *Acacia constratricta* at optimum growth temperatures. Hernandez and Vierling (1993) concluded that the possible role of this protein might be in developmental regulation. Another possible role of HSP18.1 may involve the prevention of cellular damage under high temperature stress (DeRocher et al. 1991). The absence of HSP18.1 from the chloroplast samples indicates that, in *X. xylocarpa*, this protein is likely not directly involved in the photosynthetic mechanisms. However, it is possible that seedlings grown at higher temperatures also produced other HSPs which may have a direct role in thermoprotection of photosynthesis. Seedlings grown at 40/20°C produced a unique 27 kD protein which was not found in seedlings grown at other temperatures. Unlike HSP18.1, this protein decreased after heat shock. It is possible that this protein is a heat shock protein, but has a very narrow induction temperature range. The presence of this protein also coincides with increased thermotolerance of *X. xylocarpa*. Further study will be needed to determine the function and structure of this protein.

In the present study, several proteins, from *X. xylocarpa* seedlings grown at different growth temperatures, decreased or disappeared and several proteins increased in quantity following heat shock. Although the reduction of existing proteins during heat

shock has been reported (Cooper and Ho 1983 and Lin et al. 1984), the mechanism of how this phenomenon might benefit plants under stress conditions is still unclear. Pea seedlings synthesized HSP18.1 at temperatures as low as 30°C. After the removal of heat shock conditions this protein disappeared within a few days (DeRocher et al. 1991). Changes in protein expression have been demonstrated for a number of other heat-shocked higher plants (Cooper and Ho 1983 and Lin et al. 1984). In soybean (*Glycine max* var Wayne), the amount of normal proteins decreased while a new set of proteins was produced when the temperature was shifted from 28°C to 40°C (Lin et al. 1984). Cooper and Ho (1983) also reported that maize (*Zea mays* L.) seedlings produced 10 identifiable HSPs within 20 minutes of exposure to 40°C. When seedlings were left at 40°C for longer than 20 minutes, the 10 HSPs increased in quantity to a maximum while another set of new HSPs was produced.

In summary, growth temperatures had a pronounced effect on the composition of leaf proteins but little affect on chloroplast lipids and chloroplast proteins in *X. xylocarpa*. These changes coincided with the increased thermotolerance observed. The predominant heat-induced proteins observed in *X. xylocarpa* were those of 18.1 kD, and 27 kD and there were several proteins identified by silver staining, that decreased or disappeared following heat shock exposure of 47°C for 1 hour.

4.5 References

Burke, J. J. 1990. High temperature stress and adaptations in crops. *In* Stress responses in plants:Adaptation and acclimation mechanisms. *Edited by* Alscher R.G. and J. R. Cumming. Wiley-Liss, Inc. New York. pp. 295-309.

Crespi, P., Crevecoeur, M., Penel, C., and Greppin, H. 1989. Changes in spinach plasmalemma after gibberellic acid treatment. Plant Science **62**:63-71.

Colombo, S. J., Colclough, M. L., Timmer, V. R., and Blumwald, E. 1992.Clonal variation in heat tolerance and heat shock protein expression in black spruce. Silvae Genetica **41**, (1-5):234-239.

Cooper, P., and Ho, T. D. 1983. Heat shock protein in Maize. Plant Physiology **71**:215-222.

DeRocher, A. E., Helm, K. W., Lauzon, L. M., and Vierling, E. 1991. Expression of a conserved family of cytoplasmic low molecular weight heat shock proteins during heat stress and recovery. Plant Physiology **96**:1038-1047.

Gaughran, E. R. L. 1947. The saturation of bacterial lipids as a function of temperature. Journal of Bacteriology. **53**:506.

Gombos, Z., Wada, H., Hideg, E., and Murata, N. 1994. The unsaturation of membrane lipids stabilizes photosynthesis against heat stress. Plant Physiology **104**:563-567.

Heber, U., and Santarius, K. A. 1973. Cell death by cold and heat and resistance to extreme temperatures. Mechanisms of hardening and dehardening. *In* Temperature and life. *Edited by* Precht, H., J. Christophersen, H. Hensel, and W. Larcher. Springer, Berlin, Heidelberg, New York. pp. 232-263.

Hernandez, L. D., and Vierling, E. 1993. Expression of low molecular weight heat shock proteins under field conditions. Plant Physiology **101**:1209-1216.

Holton, W., Blecker, H. H., and Onore, M. 1964. Effect of growth temperature on the fatty acid composition of a blue-green alga. Phytochemistry. **3**:595-602.

Jamieson, G.R., and Reid, E. H. 1969. The analysis of oils and fats by gas chromatography. VIII. Correlation of retention data and polarity of stationary phase. Journal of Chromatography **42**:304-310.

Kleinschmidt, M. G., and McMahon, V. A. 1970. Effect of growth temperature on the lipid composition of *Cyanidium caldarium*. Plant Physiology **46**:286-289.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**:680-685.

Langridge, J., and Mcwilliam, J. R. 1967. Heat responses of higher plants. *In* Thermobiology *Edited by* Rose A. H. Academic Press. London. pp. 231-292.

Larcher, W. 1969. The effect of environmental and physiological variables on the carbon dioxide gas exchange of trees. Photosynthetica **3**(2):167-198.

Levitt, J., 1980. Responses of plants to environmental stress. 2nd edition. Vol I: Chilling, freezing, and high temperature stress. Academic press. New York. pp. 347-445.

Lin, C., Roberts, J. K., and Key, J. L. 1984. Acquisition of thermotolerance in soybean seedlings: synthesis and accumulation of heat shock proteins and their cellular localization. Plant Physiology. **74**:152-160.

McDaniel, R. G. 1982. The physiology of temperature effects on plants. *In* Breeding plants for less favorable environments. *Edited by* Christiansen M.N. and C.F. Lewis. Wiley. New York. pp. 13-45.

Merrill, C. R., and Goldman, D. 1984. Detection of polypeptides in two-dimensional gels using silver staining. *In* Two-dimensional Gel Electrophoresis of proteins: Methods and Applications. *Edited by* J. E. Celis, R. Bravo. Academic Press, New York. pp. 93-109.

Mooney, H. A., Björman, O., and Berry, J. 1975. Photosynthetic adaptations to high temperature. *In* Environmental physiology of desert organisms. *Edited by* Hadley N. F. Dowden Hutchinson & Ross, Inc. Pennsylvania. pp138-151.

Nash. D., Miyao, M., and Murata, N. 1985. Heat inactivation of oxygen evolution in photosystem II particles and its acceleration by chloride depletion and exogenous manganese. Biochimica et Biophysica. Acta **805**:127-133.

Nguyen, H. T., Joshi, C. P., Klueva, N., Weng, J., Hendershot, K. L., and Blum, A. 1994. The heat-shock response and expression of heat-shock proteins in wheat under diurnal heat stress and field conditions. Australian Journal of Plant Physiology **21**:857-867.

O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. Journal of Biological Chemistry **250**:4007-4021.

Pearcy, R. W. 1978. Effect of growth temperature on the fatty acid composition of the leaf lipids in *Atriplex lentiformis* (Torr.) Wats. Plant Physiology **61**:484:486.

Quinn, P. J., and Williams, W. P. 1985. Environmentally induced changes in chloroplast membranes and their effects on photosynthetic function. *In* Photosynthetic mechanisms and the environment. *Edited by* Barber J. and N. R. Baker. Elsevier. Amsterdam. pp. 1-47.

Raison, J. K., Pike, C. S., and Berry, J. A. 1982a. Growth temperature-induced alterations in the thermotropic properties of *Nerium oleander* membrane lipids. Plant Physiology **70**:215-218.

Raison, J. K., Roberts, J. K. M., and Berry, J. A. 1982b. Correlations between the thermal stability of chloroplast (thylakoid) membranes and the composition and fluidity of their polar lipids upon acclimation of the higher plant, *Nerium oleander*, to growth temperature. Biochimica et Biophysica Acta **688**:218-228.

Ristic, Z., Gifford, D. J., and Cass, D. D. 1991. Heat shock proteins in two lines of Zea may L. that differ in drought and heat resistance. Plant Physiology **97**:1430-1434.

Santarius, K. A., and Müller, M. 1979. Investigation on heat resistance of spinach leaves. Planta. **146**:218-228.

Thebud, R., and Santarius, K. A., 1982. Effects of high-temperature stress on various biomembranes of leaf cells in situ and in vitro. Plant Physiology **70**:200-205.

Towbin, H., Staehelin, T., and Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some application. Proceedings of the National Academy of Sciences. **76**:4350-4354.

Vierling, E. 1991. The role of heat shock proteins in plants. Annual Review of Plant Physiology and Molecular Biology **42:** 579-620.

Zwiazek, J. J., and Blake, T. J. 1990. Effects of preconditioning on electrolyte leakage and lipid composition in black spruce (*Picea mariana*) stressed with polyethylene glycol. Physiologia Plantarum. 79:71-77.

Table 4-1. Changes in the fatty acid composition of chloroplast lipids of *Xylia xylocarpa* seedlings from three seed sources grown at different temperatures. Means (n=4) are from 4 experiments ± standard error.

Lipid species	Seed Source	Growth temperature (d/n ^{0}C)	Fatty Acids (% of total)						
			16:00	18:0	18:1	18:2	18:3	Unsat/sat	Total Amount (ug/g leaves (fw))
Monogalactoacyldiglycerol (MGlDG)	Machongson	25/20	18.37±0.58	7.83±0.37	4.56±0.62	9.96±0.85	59.28±0.41	2.82±0.10	46.49±2.70
		30/20	13.85±1.65	8.24±1.31	5.79±0.32	11.96±0.87	60.17±3.69	3.82±0.75	53.06±4.88
		35/20	21.40±2.39	13.09±1.51	6.05±0.92	10.30±0.86	49.15±4.28	1.98±0.27	39.53±3.50
		40/20	13.14±1.57	8.80±2.28	5.51±1.42	14.57±2.17	57.99±3.23	4.04±0.97	59.36±4.27
	Kanchanaburi	25/20	16.38±1.86	9.19±3.36	7.24±1.30	9.27±1.04	57.93±5.68	3.39±0.83	65.66±8.72
		30/20	20.69±1.79	12.84±1.96	9.14±0.92	11.45±0.67	45.61±3.14	2.08±0.32	95.48±13.24
		35/20	22.40±3.93	11.72±1.54	9.62±0.35	11.22±1.28	45.04±4.60	2.12±0.42	49.91±0.30
		40/20	21.61±2.00	18.45±2.49	9.23±0.79	13.73±0.48	36.99±5.08	1.58±0.27	99.97±6.74
	Nakornratchasima	25/20	21.34±2.45	10.33±3.55	4.30±0.47	8.21±0.56	55.81±6.60	2.44±0.77	71.32±5.95
		30/20	19.37±1.36	12.70±1.13	4.88±0.11	10.81±2.09	52.25±0.77	2.15±0.21	69.45±19.91
		35/20	19.69±5.63	12.36±3.73	7.00±1.33	13.76±1.64	47.18±7.61	2.76±1.15	76.19±14.30
		40/20	12.58±3.85	7.11±2.38	4.63±0.59	19.63±2.19	56.05±4.83	5.23±1.21	124.64±4.78
Digalactoacyldiglycerol (DGlDG)	Machongson	25/20	30.45±0.92	9.55±0.96	5.54±0.99	8.76±0.22	45.66±1.22	1.50±0.06	63.23±5.02
		30/20	25.78±2.23	19.51±1.74	9.35±1.53	12.37±1.71	33.00±4.64	1.22±0.11	92.71±13.91
		35/20	27.38±1.86	19.33±1.80	7.33±1.13	11.11±1.46	34.84±3.19	1.16±0.13	57.57±3.27
		40/20	24.35±0.96	15.26±1.35	7.64±1.03	13.77±0.94	38.99±1.83	1.53±0.08	86.49±3.82
	Kanchanaburi	25/20	28.17±0.80	13.56±2.50	9.03±1.55	9.83±1.26	39.41±3.43	1.43±0.16	92.09±4.95
		30/20	26.36±1.09	17.59±0.62	9.28±0.70	10.90±0.93	35.87±1.66	1.28±0.06	70.46±6.29
		35/20	27.31±3.07	17.22±1.59	17.38±3.16	14.63±2.05	23.45±5.70	1.26±0.11	78.76±9.52
		40/20	29.15±1.12	17.86±1.97	10.45±0.94	14.53±0.80	28.01±2.15	1.15±0.14	101.10±6.74
	Nakornratchasima	25/20	30.61±2.65	11.42±1.92	4.81±0.32	8.24±0.56	44.93±4.15	1.43±0.25	85.26±4.21
		30/20	30.83±3.56	13.91±0.45	6.68±0.93	11.63±0.55	36.96±4.67	1.28±0.18	80.96±7.24
		35/20	27.38±1.86	19.33±1.80	7.33±1.13	11.11±1.46	34.85±3.18	1.16±0.13	86.09±1.99
		40/20	27.80±3.71	12.59±0.33	4.43±0.82	18.23±2.88	36.96±2.03	1.54±0.22	105.09±6.40

Table 4-1. Cont.

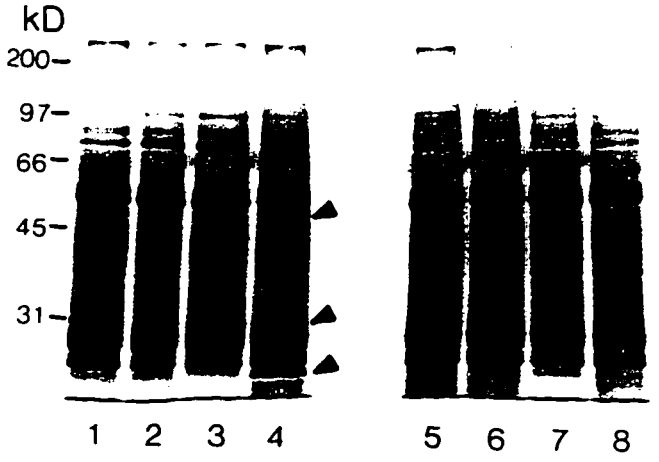
Lipid species	Seed Source	Growth temperature (d/n ^{0}C)	Fatty Acids (% of total)						
			16:00	18:0	18:1	18:2	18:3	Unsat/sat	Total Amount (ug/g leaves (fw))
Phospholipids (PL)	Maehongson	25/20	28.66±1.78	9.66±3.51	11.60±2.02	26.26±2.96	22.40±2.52	1.67±0.27	122.03±13.42
		30/20	25.65±1.10	14.97±2.39	17.96±0.95	16.85±1.10	24.58±2.41	1.49±0.15	118.78±4.41
		35/20	29.07±3.39	15.64±3.19	14.10±2.26	18.47±3.91	24.71±1.85	1.39±0.28	136.82±11.86
		40/20	29.03±1.03	9.96±0.77	18.92±3.27	18.88±1.49	23.21±1.80	1.57±0.06	129.77±6.62
	Kanchanaburi	25/20	27.31±0.36	10.39±2.82	14.41±2.38	22.49±2.42	27.89±3.56	1.76±0.21	135.84±22.04
		30/20	26.23±1.46	11.91±1.11	19.93±1.61	16.13±0.24	25.80±2.29	1.66±0.18	124.58±15.52
		35/20	26.92±1.83	7.98±0.88	18.83±2.00	17.08±1.93	28.36±2.14	1.87±0.20	125.57±5.31
		40/20	30.26±3.74	13.20±1.72	19.58±2.15	17.31±1.32	19.65±2.22	1.34±0.17	154.40±16.21
	Nakornratchasima	25/20	27.70±0.71	15.67±0.18	13.34±2.86	19.77±1.01	23.52±2.49	1.31±0.04	142.09±18.24
		30/20	28.12±1.05	16.16±2.28	18.68±0.69	16.46±1.03	20.60±2.25	1.29±0.16	147.85±9.60
		35/20	26.38±1.74	10.57±1.04	18.34±2.42	23.26±3.17	21.45±2.66	1.76±0.23	147.32±25.28
		40/20	27.49±0.76	9.01±1.92	18.34±0.96	22.99±1.11	22.18±2.47	1.77±0.20	185.02±34.52

Table 4-2. Probability values from the analysis of variance for fatty acid data of *Xylia xylocarpa*. Chloroplast lipids were divided into 3 classes (MGDG, DGDG, PL) from three seed sources (Maehongson, Kanchanaburi, and Nakornratchasima) from seedlings grown at 4 different growth temperatures (25/20°C, 30/20°C, 35/20°C, and 40/20°C day/night).

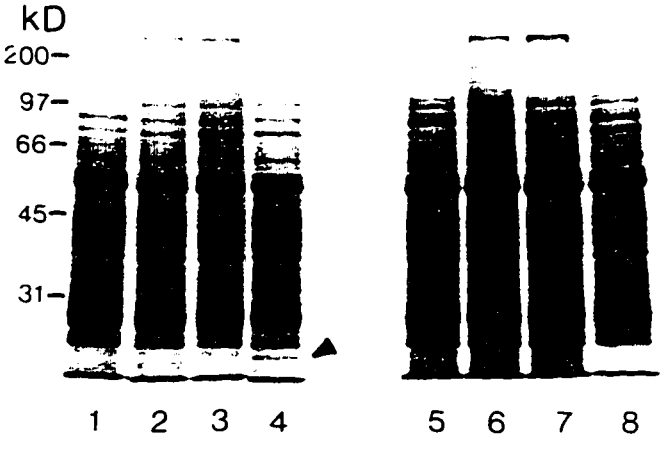
Lipid Class	Source	Fatty Acid						
		16:0	18:0	18:1	18:2	18:3	Unsat/sat	Total (lipids)
Monogalactosyldiacylglycerol (MGDG)	Seed source	0.1722	0.0997	0.0001	0.2141	0.0098	0.1538	0.0048
	Growth temperature	0.1226	0.4109	0.0524	0.0001	0.0671	0.1514	0.0001
	Seed source X Growth temperature	0.1850	0.0715	0.8680	0.1057	0.2227	0.0394	0.4165
Digalactosyldiacylglycerol (DGDG)	Seed source	0.3936	0.1374	0.0001	0.6711	0.0542	0.4362	0.2252
	Growth temperature	0.4981	0.0001	0.0037	0.0001	0.0024	0.4853	0.0053
	Seed source X Growth temperature	0.6170	0.1063	0.0205	0.1802	0.2516	0.5243	0.0124
Phospholipids (PL)	Seed source	0.7902	0.6605	0.0439	0.4828	0.2382	0.7137	0.0717
	Growth temperature	0.3342	0.5522	0.0020	0.1293	0.4751	0.7941	0.0867
	Seed source X Growth temperature	0.8016	0.0806	0.9452	0.1996	0.4171	0.2224	0.8790

Figure 4-1. 10% SDS PAGE gel analysis of total leaf proteins (10 µg per lane) from 3-month-old *Xylia xylocarpa* seedlings from Maehongson (A), Kanchanaburi (B), and Nakornratchasima (C) grown at 25/20°C, 30/20°C, 35/20°C, 40/20°C day/night temperatures (lanes 1, 2, 3, and 4 respectively). Lanes 5, 6, 7, and 8 show leaf proteins of seedlings grown at 25/20°C, 30/20°C, 35/20°C, and 40/20°C day/night temperatures, respectively, after they were subjected to 47°C heat shock for 1 hour. Molecular weight markers from Bio-Rad are indicated on the left. Proteins that changed under experimental conditions are indicated by arrows. Protein bands were visualized with silver stain.

A


kD
200
97
66
45
31
1 2 3 4
5 6 7 8

B


kD
200
97
66
45
31
1 2 3 4
5 6 7 8

C

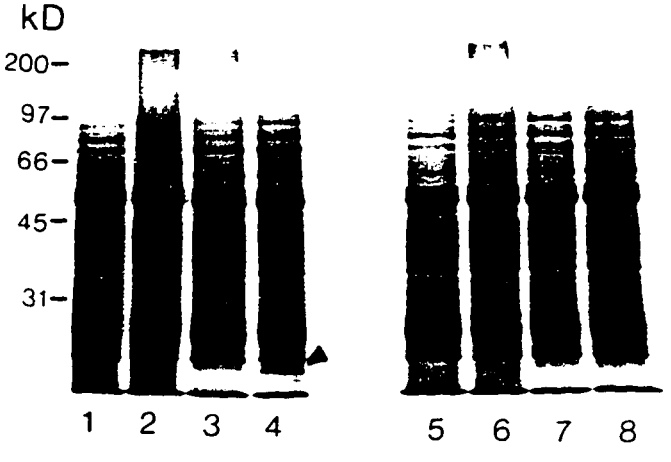
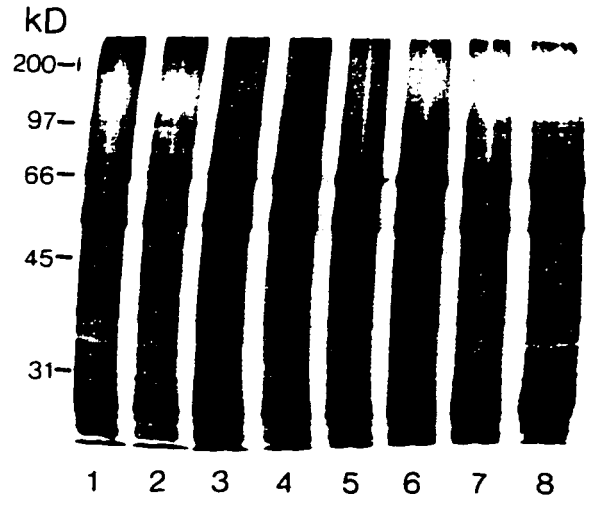
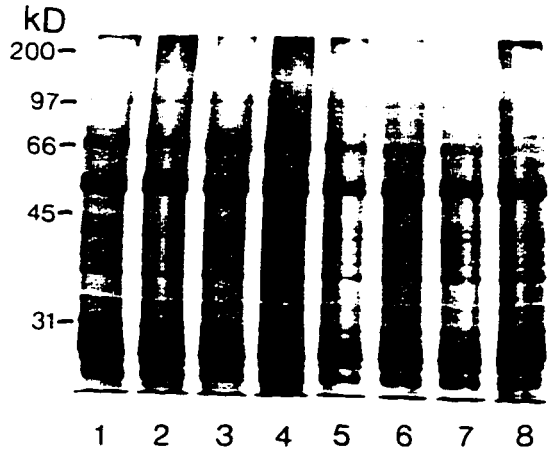

kD
200
97
66
45
31
1 2 3 4
5 6 7 8

Figure 4-2. 10% SDS PAGE gel analysis of chloroplast proteins (10 μg per lane) from 3-month-old *Xylia xylocarpa* seedlings from Maehongson (A), Kanchanaburi (B), and Nakornratchasima (C) grown at 25/20°C, 30/20°C, 35/20°C, 40/20°C day/night temperatures (Lane 1, 2, 3, and 4 respectively). Lanes 5, 6, 7, and 8 show chloroplast proteins from seedlings grown at 25/20°C, 30/20°C, 35/20°C, and 40/20°C day/night temperatures, respectively, after they were subjected to 47°C heat shock for 1 hour. Molecular weight markers from Bio-Rad are indicated on the left. Protein bands were visualized with silver stain.

A


kD
200
97
66
45
31
1 2 3 4 5 6 7 8

B


kD
200
97
66
45
31
1 2 3 4 5 6 7 8

C

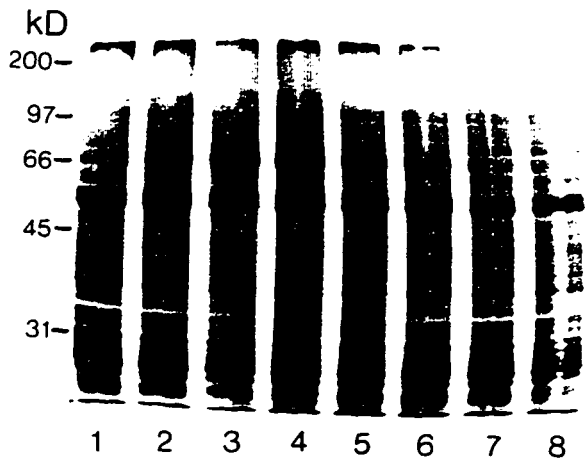

kD
200
97
66
45
31
1 2 3 4 5 6 7 8

Figure 4-3. 2-D PAGE gel analysis of 20 μg total leaf protein from 3-month-old *Xylia xylocarpa* seedlings from the Kanchanaburi seed source grown at 25/20°C (A), 30/20°C (B), 35/20°C (C), and 40/20°C (D) day/night temperatures. Molecular weight makers from Bio- Rad are shown on the left. Protein spots which changed in staining intensity when seedlings were subjected to 47°C heat shock for 1 hour are circled (Fig.4-4). The square shows a 27 kD protein produced only in seedlings grown at 40/20°C day/night temperature. Protein bands were visualized with silver stain.

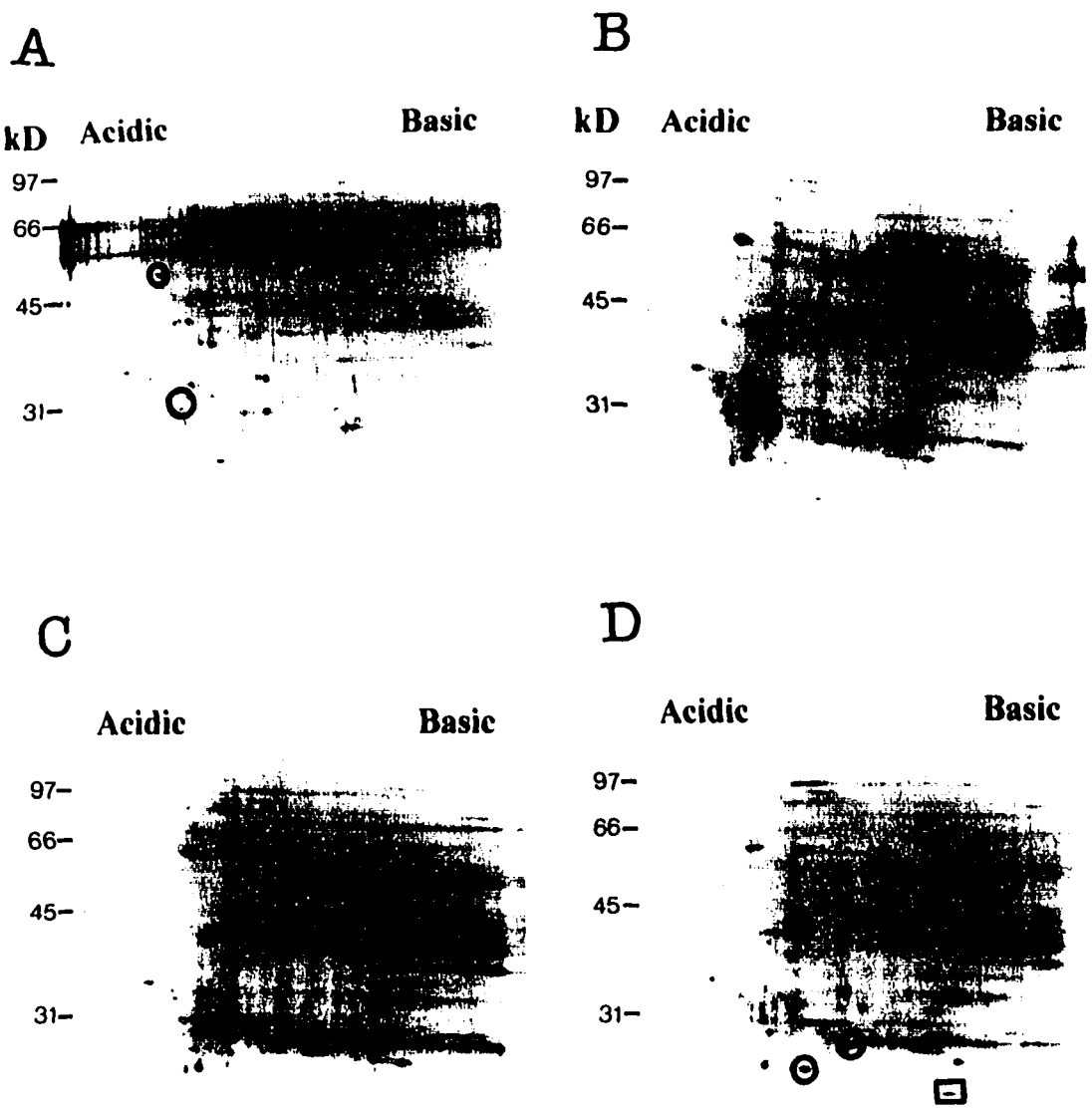

A
kD
Acidic
Basic
97–
66–
45–
31–
B
kD
Acidic
Basic
97–
66–
45–
31–
C
Acidic
Basic
97–
66–
45–
31–
D
Acidic
Basic
97–
66–
45–
31–

Figure 4-4. 2-D PAGE gel analysis of 20 µg total leaf proteins from 3-month-old *Xylia xylocarpa* seedlings from the Kanchanaburi seed source grown at 25/20°C (A), 30/20°C (B), 35/20°C (C), and 40/20°C (D) day/night temperatures after they were subjected to 47°C heat shock for 1 hour. Molecular weight markers from Bio-Rad are shown on left. Triangles indicate spots that changed in staining intensity following heat shock. Protein bands were visualized with silver stain.

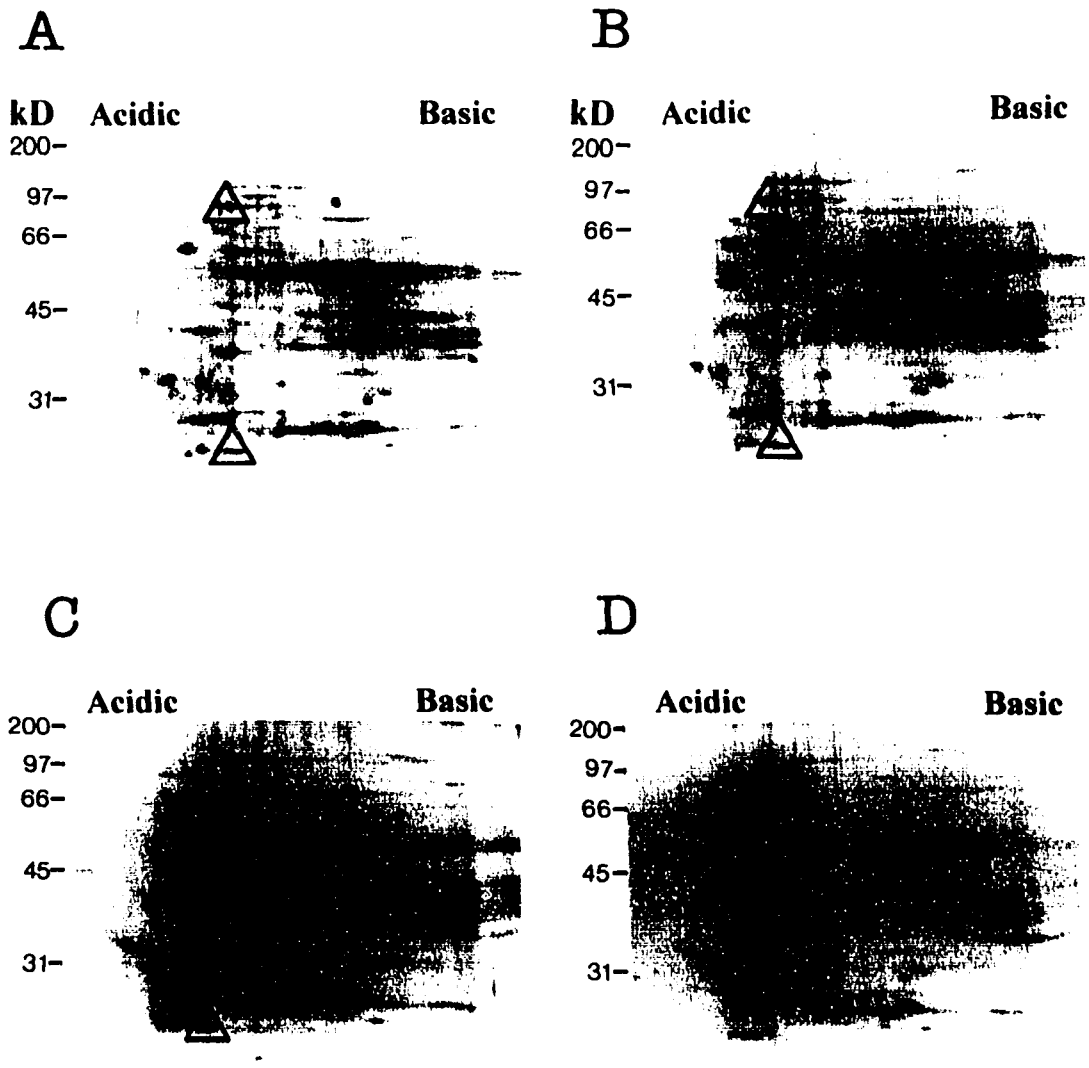
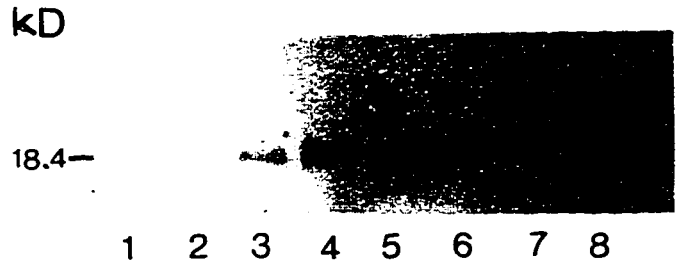

A
kD
Acidic
Basic
200–
97–
66–
45–
31–
B
kD
Acidic
Basic
200–
97–
66–
45–
31–
C
Acidic
Basic
200–
97–
66–
45–
31–
D
Acidic
Basic
200–
97–
66–
45–
31–

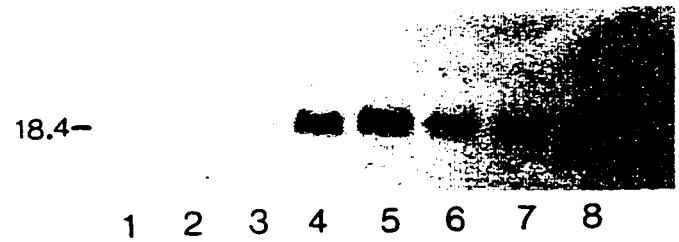
Figure 4-5. Western blots of 10 µg total leaf proteins from the Mahongson (A), Kanchanaburi (B), and Nakornratchasima (C) seed sources following immunodetection with an HSP18.1 antiserum. Preimmune antiserum shown for Kanchanaburi proteins (D). Lanes 1, 2, 3, and 4 are total leaf protein samples from seedlings grown at 25/20°C, 30/20°C, 35/20°C, 40/20°C day/night temperatures respectively. Lanes 5, 6, 7, and 8 are leaf protein samples from seedlings grown at 25/20°C, 30/20°C, 35/20°C, 40/20°C day/night temperatures following treatment at 47°C heat shock for 1 hour. Molecular weight markers from Bio-Rad are indicated on the left. Alkaline phosphatase conjugated secondary antibody was used for detection.

A

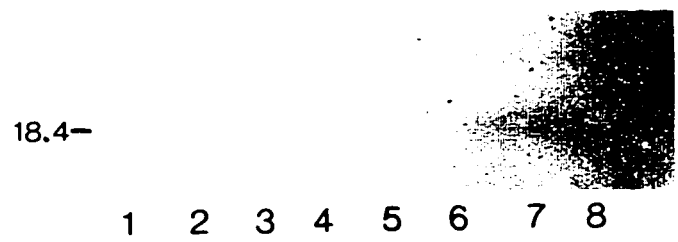


B

C



D



CHAPTER FIVE

General Discussion

Responses of plants to high temperature have been studied at different levels of plant organization (Paull 1992). Cell processes and structures known to be altered by high temperature include membrane integrity and fluidity (Raison et al 1982), electrolyte leakage (Chen et al. 1982, Inaba and Crandall 1988), photosynthetic electron transport, carbon fixation (Berry 1975), and metabolic rates (Burke 1990). Changes at the whole plant level range from growth, gas exchange, and nutrient distribution to those in plant morphology. However, there have been few studies that combined both whole plant and biochemistry approach to examine plant responses to high temperature stress.

The present study demonstrated that *X. xylocarpa* seedlings grown in high temperature can tolerate potentially injurious heat stress. The acclimation of plants to 40°C increased the stability of photosynthesis, increased plasma membrane integrity, altered chloroplast lipid composition, and produced HSP 18.1 and other heat stress proteins. Lipid composition of chloroplasts was affected by growth temperature while chloroplast proteins remained unchanged. Changes in chloroplast galactolipids, especially those in linolenic acid could directly influence photosynthetic reactions. In the present study, heat-acclimated plants produced HSP18.1 protein which could also indirectly affect photosynthetic processes and protect the integrity of the plasma membranes.

The present study suggests that long-term acclimation can increase thermotolerance of *X. xylocarpa* seedlings. Therefore, long-term acclimation of *X. xylocarpa* seedlings to high temperatures is recommended in nursery practices. However,

further studies should be conducted in both a controlled environment and the field to examine the recovery of seedlings from long-term heat stress.

In this study, seedlings from different seed sources showed differences in growth and photosynthesis during the acclimation to various growth temperatures. Based on these results I suggest that Maehongson and Kanchaburi seed sources should be used for planting at moderate and high temperature sites.

In summary, the conclusions and recommendations of the present study are:

1. Plants from Kanchanaburi and Maehongson seed sources were able to adapt to high temperature conditions.

2. Growth temperature significantly affected seedling growth, leaf morphology and protein and lipid composition of *Xylia xylocarpa* seedlings.

3. *Xylia xylocarpa* seedlings had the optimum day growth temperatures ranging from 30-35°C. Seedlings from the Maehongson and Kanchanaburi seed sources had higher growth rates than the seedlings from the Nakornratchasima seed source. When measured at the actual ambient treatment temperature, seedlings from all three seed sources did not show significant differences in gas exchange. Seedlings grown at 40/20°C day/night temperature significantly changed their gas exchange patterns. Net photosynthetic rate, transpiration rate, and stomatal conductance increased when the seedlings grown at 40/20°C day/night temperature were subjected to 25°C.

4. At moderate temperatures, transpiration and stomatal conductance of *X. xylocarpa* responded similarly to photosynthesis. When measured at 50°C, seedlings grown at 25/20°C, 30/20°C and 35/20°C had increased transpiration rates and stomatal

conductance, while net photosynthesis declined below zero. Seedlings grown at 40/20°C day/night temperature had higher net photosynthesis after exposure to various short-duration temperatures compared with seedlings from other growth temperatures. At 50°C, net photosynthesis was still positive.

5. Seedlings grown at 40/20°C day/night temperature and subjected to heat stress showed significantly increased thermotolerance and reduced leaf electrolyte leakage.

6. Changes in chloroplast lipids occurred mainly in the galactolipid fraction with little changes in phospholipids. The major changes included those in the contents of oleic, linoleic and linolenic acids. Different seed sources grown under different growth temperatures appeared to have different chloroplast lipid compositions. However, the overall ratio of unsaturated to saturated fatty acid remained constant in all temperature treatments.

7. Leaf protein expression varied in plants grown at different temperatures. Leaves from plants grown at 35/20°C and 40/20°C day/night temperatures contained the HSP18.1 protein.

8. To prepare *X. xylocarpa* seedlings for planting at high temperature sites, high temperature growth conditions could be used to increase thermotolerance. A follow-up study should be conducted to determine how seedlings grown at high temperatures in tree nurseries perform under field conditions where they are likely to encounter both high temperatures and drought.

9. For the future study, samples and replications should be increased to reduce the impact of exposure time under heat stress during the measurement and the variation

among individual seedlings. More seed sources should be included since numerous studies of crop species have demonstrated that high temperature tolerance is genetically controlled (Chaisomponpan 1990). Other economically important native tree species should be examined for high temperature tolerance using the conductivity test of electrolyte leakage (Martineau et al 1979, Chen 1982).

5.1 References

Berry, J. A. 1975. Adaptation of photosynthetic processes to stress. Science. **188**:644-650.

Burke, J. J. 1990. High temperature stress and adaptations in crops. *In* Stress responses in plants:Adaptation and acclimation mechanisms. *Edited by* Alscher R.G. and J. R. Cumming. Wiley-Liss, Inc. New York. pp. 295-309.

Chen, H. H., Shen, Z. Y., and Li, P. H. 1982. Adaptability of crop plants to high temperature stress. Crop Science **22**:719-725.

Inaba, M., and Crandall, P. G. 1988. Electrolyte leakage as an indicator of high temperature injury to harvested mature green tomatoes. Journal of American Society of Horticulture Science **113**:96-99.

Martineau, J. R., Specht, J. E., Williams, J. H., and Sulliva, C. Y. 1979. Temperature tolerance in soybean. I. Evaluation of technique for assessing cellular membrane thermostability. Crop Science. **19**:75-78.

Paull, R. E. 1992. Heat stress in whole plants. *In* Plant stress in the tropical environment. *Edited by* T. L. Davenport and H. M. Harrington, Proceedings of a Workshop held in Kailua-Kona Hawaii. pp102-106.

Raison, J. K., Roberts, J. K. M., and Berry, J. A. 1982. Correlations between the thermal stability of chloroplast (thylakoid) membranes and the composition and fluidity of their polar lipids upon acclimation of the higher plant, *Nerium oleander*, to growth temperature. Biochimica et Biophysica Acta **688**:218-228.