American Journal of Botany 93(1): 102–109. 2006.

The interactive effects of light and temperature on heat-shock protein accumulation in *Solidago altissima* (Asteraceae) in the field and laboratory[1]

Deepak Barua[2,3,4] and Scott A. Heckathorn[3]

[2]Department of Biology, Syracuse University, 130 College Place, Syracuse, New York 13244 USA; and [3]Department of Earth, Ecological, and Environmental Science, University of Toledo, Toledo, Ohio 43606 USA

Heat-shock proteins (HSPs) protect cells from abiotic stresses. However, most work on HSPs in plants has been carried out in laboratory-grown crop or model species. Few studies have examined field expression of HSPs or HSP expression in response to multiple stresses that often occur simultaneously in nature. Heat stress in nature is frequently accompanied by high light, and photoinhibition is a major limitation for photosynthesis. Light induction of HSPs may help ameliorate damage from excess light. In this study, we asked if accumulation of representative HSPs differed in naturally occurring *Solidago altissima* (goldenrod) in contrasting light microclimates (open sun vs. shade) and on cool vs. warm days. Our results show that HSP content in field-grown plants, undergoing natural temperature stress, was greater in open sun than shaded environments. Supporting these results, both light and temperature significantly affected accumulation of HSPs in the laboratory. This is the first study to show that the interaction of light microclimate and temperature can significantly influence HSP accumulation in field-grown plants.

Key words: goldenrod; HSP70; photoinhibition; small heat-shock protein; stress proteins.

Heat-shock proteins (HSPs) protect proteins, membranes, and other cellular components during heat-stress and facilitate repair or degradation of damaged proteins following a stressful event (Parsell and Lindquist, 1994; Wang et al., 2004). Extensive research has led to a detailed understanding of HSP regulation and their mechanisms of action (Feder and Hoffman, 1999; Wang et al., 2004), but in spite of this, there is limited information about HSP expression in native plants in their natural habitat. The vast majority of the work on HSPs in plants has been carried out in laboratory-grown plants of either crops or the model plant, *Arabidopsis thaliana*. Of the few studies that have examined field expression of HSPs in plants (Burke et al., 1985; Kimpel and Key, 1985; Hernandez and Vierling, 1993; Ortiz et al., 1995; Bhadula et al., 1997; Stout et al., 1997; Al-Niemi and Stout, 2002; Merquiol et al., 2002), only three have addressed field expression in native non-commercial plants (Stout et al., 1997; Al-Niemi and Stout, 2002; Merquiol et al., 2002). Further, all these previous studies of HSPs have examined only the importance of temperature on HSP production in field-grown or naturally occurring plants.

Importantly, while HSPs were so named because they are up-regulated by an acute increase in temperature (Lindquist, 1980), a variety of other stresses, including photoinhibitory high light, also induce HSPs (Feder and Hoffman, 1999; Wang et al., 2004). Light stress often occurs under field conditions, and photoinhibition is a major limitation for photosynthesis in the field (Long et al., 1994). Photoinhibition occurs when excessive radiation leads to the over-reduction of the components of photosynthetic electron transport. This results in the impairment of electron transport, and ultimately, in irreversible damage to the photosynthetic reaction centers (Ohad et al., 1990), and high temperatures exacerbate these effects of high light. HSPs can protect against photoinhibition (e.g., by protecting photosystem II [PSII] or stabilizing thylakoid membranes), and light induction of HSPs may ameliorate the damaging effects of excess light (Schuster et al., 1988; Stapel et al., 1993; Downs et al., 1999; Schroda et al., 1999).

The effect of light on the induction of HSPs has been demonstrated in cyanobacteria (Glatz et al., 1997; Hihara et al., 2001; Asadulghani et al., 2003), algae (von Gromoff et al., 1989; Dryzmalla et al., 1996), and higher plants (Stapel et al., 1993; Debel et al., 1994; Rossel et al., 2002). Light regulation of constitutive HSPs (i.e., heat-shock cognates) has also been demonstrated in spinach (Li and Guy, 2001). A regulatory pathway independent of heat shock probably mediates light induction of HSPs (Kropat et al., 1997). These studies show that light can induce HSPs independent of temperature, and in most cases, there is an additive effect of high light (high light vs. dark) and high temperature (high vs. low) on accumulation of HSPs. For example, increased light levels decreased the minimum temperature required to induce mitochondrial small HSP in *Chenopodium rubrum* (Knack and Kloppstech, 1992). However, these previous studies have all been conducted under laboratory conditions, and thus, with plants that have not been acclimated to natural high light or dynamic light regimes, which is likely to alter the response of HSPs to light (e.g., decrease the light-responsiveness of HSPs to light, due to acclimation to high or variable light). Further, most of the previous studies investigated light effects by comparing light vs. no-light treatments, rather than low vs. high light. Consequently, the relative importance of temperature vs. light stress in determining expression of HSPs in naturally occurring plants is not known.

To investigate heat and light controls on HSP production in the field, in this study, we examined accumulation of HSPs in naturally occurring *Solidago altissima* (goldenrod). We were especially interested in examining HSP accumulation in wild plants over multiple consecutive hot days, as often occurs in

[1] Manuscript received 9 March 2005; revision accepted 25 September 2005.

The authors thank C. Klotz for help with the field work. This work was supported in part by grants from the U.S. National Science Foundation to S.A.H.

[4] Author for correspondence (e-mail: dbarua@oeb.harvard.edu), phone: 617-496-8646, fax: 617-495-9484

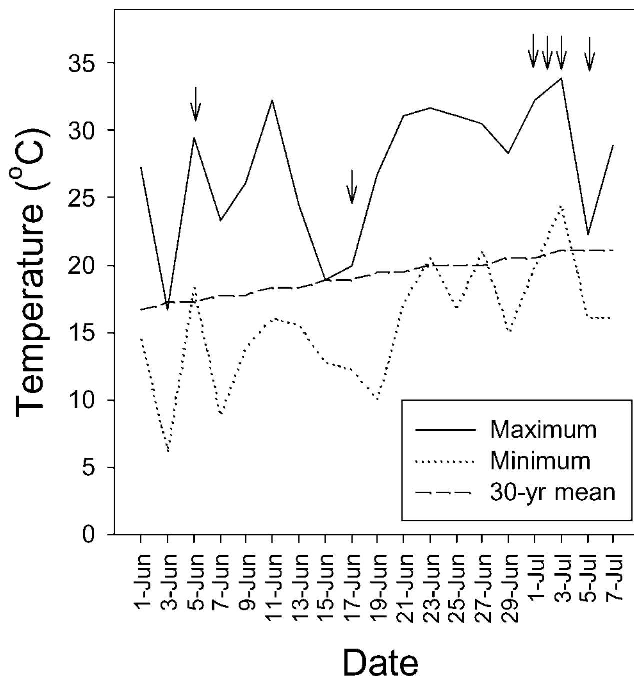

Fig. 1. Maximum and minimum daily temperatures during the sampling period (June–July 2002), and 30-year mean daily temperatures for Syracuse, New York, USA. Arrows indicate sampling days. Temperature data were obtained from the National Climate Data Center (NCDC).

natural habitats during a heat wave (in contrast to abrupt heat stress, as imposed in most laboratory studies). Specifically, we asked if representatives of two of the five major families of HSPs (Wang et al., 2004), HSP70 and small HSPs (sHSPs), differed in their accumulation in goldenrods from contrasting light microclimates (open sun vs. shade) and on cool vs. warm days. A second component of the study was conducted in the laboratory, under more controlled conditions, to confirm field results regarding light and heat interaction on HSP content. Here we examined the temperature response of HSPs to determine temperatures required to induce HSPs, temperatures of maximal HSP accumulation, and shut-off temperatures for HSP accumulation. We asked whether low or moderately elevated light levels influence these set points for HSPs or the magnitude of the response. Also, to determine the effects of higher light intensities on HSPs (we were limited to moderate light levels of 600 μmol · m^{-2} · s^{-1} PPFD in the controlled-environment growth chambers) and to determine if high light can induce HSPs independent of temperature in goldenrod, we examined the light response of HSP accumulation to low, moderate, and high light, at control and heat-shock temperatures. We monitored PSII function (dark-adapted F_v/F_m) to assess the effects of temperature and light on plant performance, as PSII is extremely sensitive to both light and temperature stress (Weiss and Berry, 1988; Ohad et al., 1990).

MATERIALS AND METHODS

Field measurements and sample collection—To determine the effect of light on HSP content in naturally occurring plants, measurements were made on and samples collected from, sun and shade plants on six sampling days during the summer of 2002, in Syracuse, New York, USA (Fig. 1). The first sampling day was representative of a hot summer day. The second day was cool, overcast, and preceded by three cool days, thus serving as a reference for basal HSP content in the field in the absence of light and heat stress. We then sampled on 3 days during a 4-day heat wave and on a cooler day immediately following this heat wave. Field sampling and measurements were carried out between 1500 and 1600 hours. An open area and an adjacent forested (shaded under-canopy) area with abundant goldenrod (*Solidago altissima* L.) were chosen for sampling. Air and leaf temperature and incident light at leaf height (approximately 1.5 m from the ground) were measured for five replicate open (sun) and shade plants each day. Air and leaf temperatures were averaged over 30 s immediately prior to sampling the plant. Mature plants, approximately 1.5 m in height, were chosen, and different plants were sampled each day. Care was taken to ensure that plants were >5 m from each other to avoid sampling the same genet (vegetative clone). The first fully expanded leaf from five plants was collected and frozen in liquid nitrogen for protein analysis. The second fully expanded leaf from the same five plants was collected and placed in a moist darkened container for chlorophyll fluorescence measurements. Leaf temperatures were determined with a LI-COR 1000 datalogger (LI-COR, Lincoln, Nebraska, USA) and a fine-gauge thermocouple attached to the underside of the leaf. Stomatal conductance was measured for a mature leaf on each plant (as in Heckathorn et al., 1996), using a LI-COR 6200 portable photosynthesis system (LI-COR). Leaf water potential was measured for a mature leaf on each plant, using a pressure bomb (PMS Instrument, Albany, Oregon, USA). Photochemical efficiency of PS II was measured by the chlorophyll fluorescence parameter, F_v/F_m (PAM chlorophyll fluorometer, Walz, Germany), after leaves were allowed to dark-adapt for 30 min (as in Heckathorn et al., 2002).

Laboratory growth conditions, temperature, and light treatments—Goldenrod seedlings were collected from the field and transplanted into 1-L pots containing topsoil. Sixty plants were grown in controlled-environment growth chambers (Conviron, Winnipeg, Canada), with 350 ± 50 μmol · m^{-2} · s^{-1} photosynthetic photon flux density (PPFD), and a 25°C/18°C day/night temperature cycle. The plants were allowed to acclimate to the growth chamber for a month before use.

Temperature and light response of HSP accumulation—For examining the temperature response of HSPs at low and moderate light levels, mature whole leaves from separate chamber-grown plants were detached and placed on moistened paper towels in an open petri plate. These were placed in a growth chamber for 2 h at one of the following temperatures: 25 (control), 30, 33, 36, 39, 42, or 45°C and given either low (60 μmol m^{-2} · s^{-1} PPFD) or moderate (600 μmol · m^{-2} · s^{-1} PPFD) light. Preliminary experiments were performed to determine the required set temperature for the growth chamber to obtain the desired treatment temperature for the leaves. Leaf temperatures were monitored by fine-gauge thermocouples, and a LICOR-1000 data logger (LI-COR) during the experiment. Leaf temperatures were ±0.5°C of treatment temperatures. Three replicate leaves from each light and temperature combination were frozen in liquid nitrogen for protein analysis. Another three replicate leaves were dark adapted for 30 min and photochemical efficiency of PSII measured as before.

We were limited in our temperature response experiment to maximum light levels of 600 μmol · m^{-2} · s^{-1} PPFD. To further examine the light response of HSPs at higher light levels, we used a high intensity, cold light source and fiber optic cables. Leaf discs (2.2 cm diameter) were punched from separate mature leaves of chamber-grown plants and placed in a glass cuvette on a moistened filter paper disc. The cuvette was immersed in a temperature controlled, circulating water bath for 2 h at either control (25°C) or heat-shock (39°C) temperatures, and given either low (60 μmol · m^{-2} · s^{-1} PPFD), moderate (600 μmol · m^{-2} · s^{-1} PPFD), or high (1800 μmol · m^{-2} · s^{-1} PPFD) light. A high intensity, cold light source (KL1500 LCD, Schott, Germany) and fiber optic cable were used to provide even illumination without overheating the leaf surface. Preliminary experiments for each light and temperature combination were performed to determine the set temperature required to obtain the desired treatment leaf temperature. Leaf temperatures, monitored with a LICOR-1000 data logger (LI-COR) and fine-gauge thermocouples attached to the underside of the leaf discs, were ±0.2°C of the treatment temperature. Three replicate leaf discs were frozen in liquid nitrogen for protein

analysis and another three replicate leaf discs were dark adapted for 30 min, and photochemical efficiency of photosystem II measured as before.

Protein extraction and electrophoresis—Proteins were extracted by grinding leaves to a fine powder in liquid nitrogen with a mortar and pestle, then in extraction buffer containing 1% sodium dodecyl sulfate, 100 mM Tris (pH 8.0), 10 mM EDTA, 1 mM phenylmethylsulfonylfluoride, 10 mM ascorbate, 10 mM dithiothreitol, 1 μM leupeptin, 10% glycerol (v : v), 2% polyvinylpolypyrrolidone (w : v), and 0.05% bromophenol blue. Protein extractions were boiled for 3 min and then centrifuged at 21 000 × *g* for 3 min to remove insoluble debris. Protein concentration of each sample was determined in triplicate by the Coomassie dye-binding method of Ghosh et al. (1988) using bovine serum albumin (BSA) as a standard.

Leaf proteins were fractionated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using 16 × 20 × 0.1 cm 12% gels; equal total protein was loaded per lane (40 μg). Following SDS-PAGE, the proteins were transferred to polyvinylidinedifluoride (PVDF) membranes by electrophoresis, and then the membranes were probed with protein-specific antibodies, and secondary antibodies conjugated to alkaline phosphatase. The membranes were cut in half at the 45-kDa marker, and the top portion of the membrane probed with HSP70 antiserum, and the bottom portion probed with antiserum to sHSPs. Antibodies to sHSPs were polyclonal rabbit antibodies raised against oligopeptides of conserved sequences (described in Downs et al., 1998). Preliminary experiments with whole-leaf and isolated chloroplast protein samples indicated that this antibody detected both chloroplastic and non-chloroplastic sHSPs, which were independently resolvable and similarly responsive to experimental treatments (not shown). For the purposes of this paper, all sHSP bands (1 chloroplast, 2 non-chloroplast) detected on immunoblots were quantified in sum, and referred to as sHSPs, to ascertain the general response of the sHSP family to experimental treatments. The HSP70 antibody used (SPA-820, Stressgen, Victoria, British Columbia, Canada) detects multiple members of the HSP70 family of proteins (e.g., constitutive and induced). These multiple proteins have similar molecular masses and were not resolvable on the one-dimensional gels used in this study; thus, as with sHSPs, HSP70 results in this paper represent a general response of the HSP family to treatments. Secondary antibodies were detected with a colorimetric system consisting of nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate. The developed immunoblots were scanned using a desktop scanner, and the intensity of the protein bands quantified using NIH imaging software (Scion Image, Scion Corp., Frederick, Maryland, USA). A standard heat-shock sample was prepared from leaves of *Chenopodium album* heat-shocked at 40°C for 4 h. One lane of this standard was loaded on every gel run, and protein abundance is expressed as a percentage of this heat-shock standard to allow comparisons across gels. Preliminary gels and immunoblots with serial dilutions of tissue extracts were analyzed to determine appropriate tissue protein concentrations to insure that samples were within the linear range of detection. All samples were run in triplicate in three separate gels.

Statistical analysis—In the field experiment, differences between light climate (sun and shade) and days were analyzed by two-way ANOVA, with light, day, and the interaction of the two as factors. The laboratory temperature response experiment was analyzed by two-way ANOVA, with temperature, light, and the interaction of the two as factors. The laboratory light response experiment was analyzed by two-way ANOVA, with light, temperature, and the interaction of the two as factors. Tukey's post-hoc tests were used to analyze differences between specific treatment means.

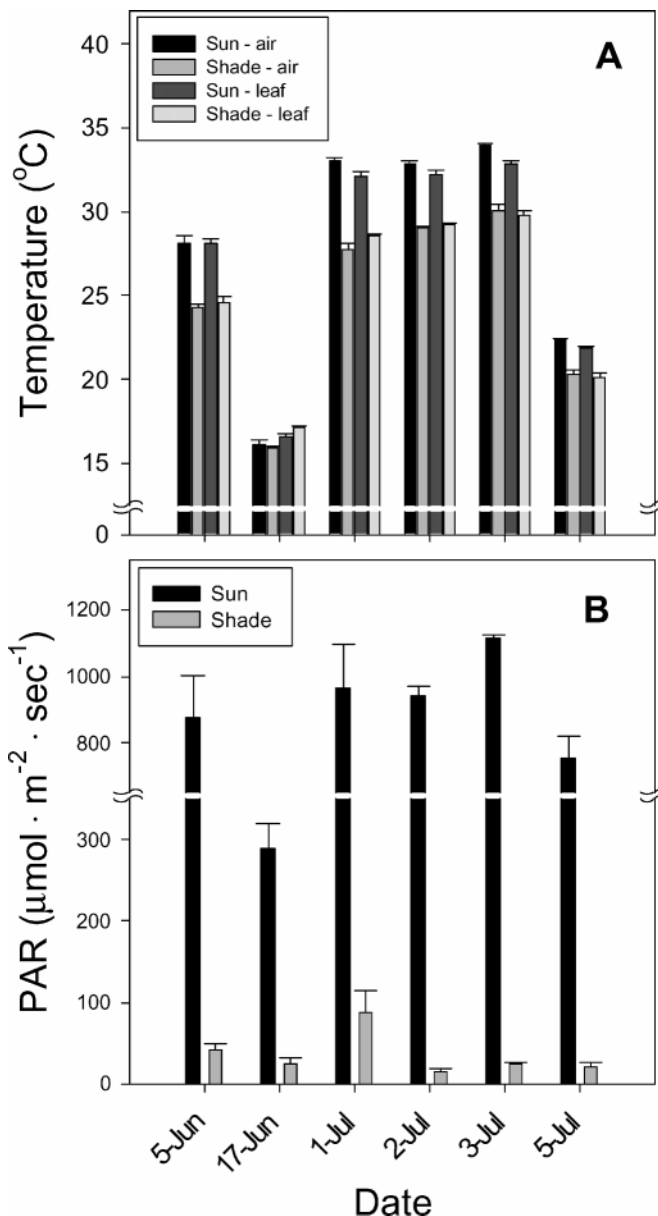

Fig. 2. Temperatures and incident light levels measured at leaf height in field plants of *Solidago altissima*. (A) Air and leaf temperatures in the sun and shade. (B) Incident light levels, measured as photosynthetically active radiation (PAR), for sun and shade. Error bars represent 1 SE (N = 5).

RESULTS

Maximum and minimum air temperatures during the sampling period, and the 30-year daily mean temperatures for Syracuse, New York, are shown in Fig. 1. The first sampling day, representative of a hot summer day, was followed by sampling on a cool and overcast day, and finally, the 4 days of the heat wave. Average air temperatures in the sun were 3°C higher than the shade (Fig. 2A). Likewise average leaf temperatures in the sun were 2.4°C higher than the shade. Leaf temperatures were marginally higher than air temperatures in the shade and marginally lower than air temperatures in the sun, though these differences were not statistically significant. The average incident light level in the open during sampling was 863.5 ± 33.7 μmol · m^{-2} · s^{-1} PPFD. Light levels were significantly lower under the forested canopy, and averaged 36.1 ± 32.9 μmol · m^{-2} · s^{-1} PPFD. Except for the second sampling day, all other days were sunny or mostly sunny and had fairly high light levels (Fig. 2B).

Plants in the field accumulated considerable levels of HSP70 (Fig. 3A), which was approximately half of the heat-shocked standard at the lowest. sHSPs content was comparatively lower, approximately a third of the heat-shocked control

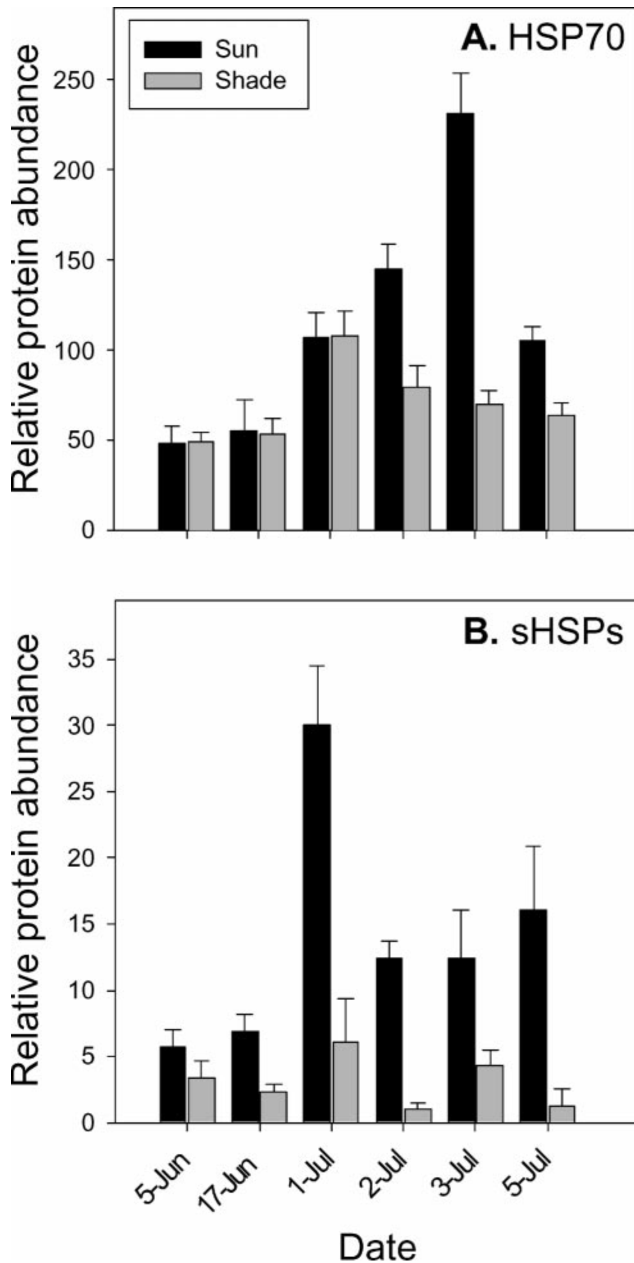

Fig. 3. Heat-shock protein (HSP) content in sun and shade leaves in field plants of *Solidago altissima*, for (A) HSP70 and (B) small HSPs. Protein content is expressed as a percentage of a heat-shock standard. Error bars represent 1 SE ($N = 5$).

at the highest levels (Fig. 3B). HSP70 and sHSP content differed significantly between days ($P < 0.0001$ for HSP70 and $P < 0.0001$ for sHSPs) and was higher in sun plants than in shade plants ($P = 0.0004$ for HSP70, and $P < 0.0001$ for sHSPs). HSP70 content in sun plants increased over the heat wave and dropped to lower levels following the heat wave, while HSP70 content in shade plants was elevated only on the first day of the heat wave. sHSPs in sun plants were elevated on the first day of the heat wave, but dropped to lower levels after that. Plants in the shade had barely detectable levels of sHSPs on all the days examined.

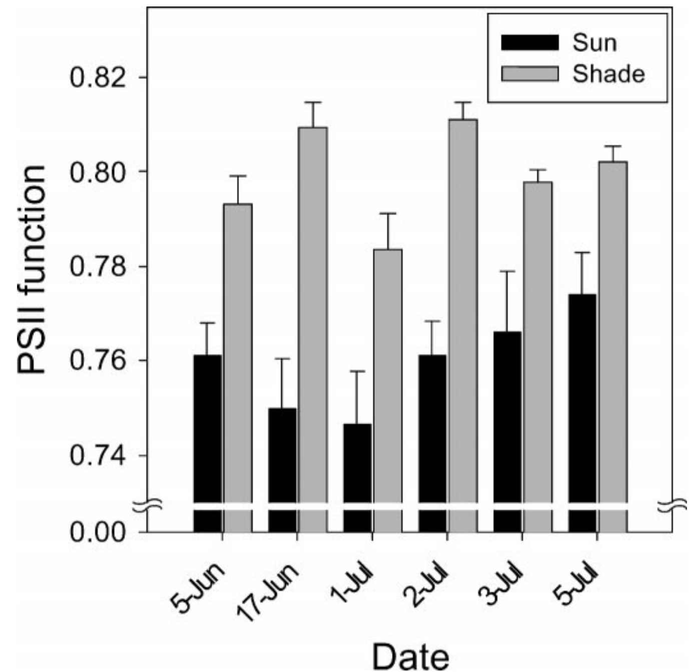

Fig. 4. Photosystem II function, measured as dark-adapted F_v/F_m, for sun and shade leaves in field plants of *Solidago altissima*. Error bars represent 1 SE ($N = 5$).

PS II function, measured as dark-adapted F_v/F_m, was significantly lower for plants in the sun than the shade ($P < 0.0001$) (Fig. 4). There were no significant differences in PSII efficiency ($P = 0.0829$) between days or an interaction between day and light climate ($P = 0.2663$). Leaf water potential was significantly lower ($P < 0.0001$) for plants in the sun as compared to those in the shade (Fig. 5A), which mirrored the results of stomatal conductance in plants from the sun and shade ($P < 0.0001$, Fig. 5B).

In the laboratory temperature-response experiment, there was a significant effect of temperature on HSP70 content in detached leaves ($P < 0.0001$). HSP70 was induced at 33°C, peaked at 39°C, and was barely detectable at 45°C (Fig. 6A). While there was no main effect of light on HSP70 content ($P = 0.2548$), there was a significant light × temperature interaction ($P = 0.0006$), and at 39°C, HSP70 content was higher at moderately elevated light ($P < 0.05$; Tukey's honest significant difference). Effects of light and temperature, and the interaction between the two, were significant for sHSPs ($P = 0.0001$, $P < 0.0001$, and $P = 0.0002$, respectively). At both low and moderately elevated light, sHSPs were clearly induced at 33°C, peaked at 39°C and were not detectable at 45°C (Fig. 6B). sHSPs content at 36 and 39°C was greater at moderately elevated light than low light ($P < 0.05$; Tukey's honest significant difference). As expected, there was a significant decline in PSII function with increasing temperature ($P < 0.001$, Fig. 7). While PSII function was lower at moderately elevated light ($P < 0.001$), there was no temperature × light interaction ($P = 0.3864$).

Results from the laboratory light-response experiment show significant effects of light and temperature, and the interaction of the two, on HSP70 content in leaf discs ($P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively). There was no significant effect of increased light on HSP70 content at control tem-

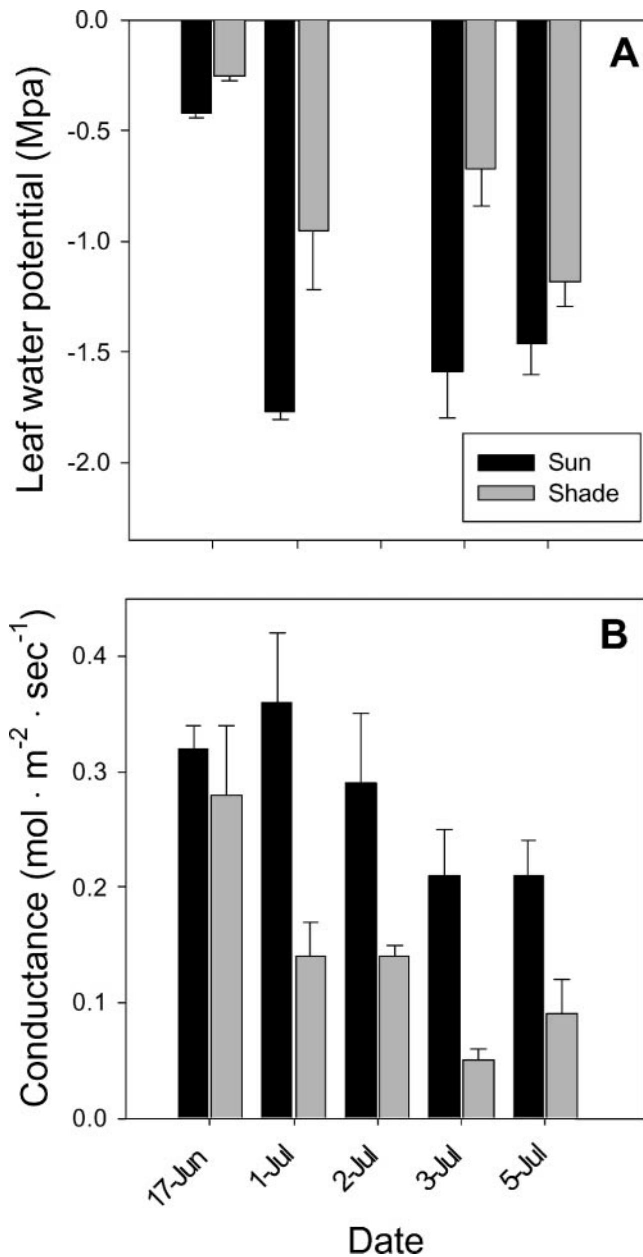

Fig. 5. (A) Leaf water potential and (B) stomatal conductance for sun and shade leaves in field plants of *Solidago altissima*. Error bars represent 1 SE (N = 5).

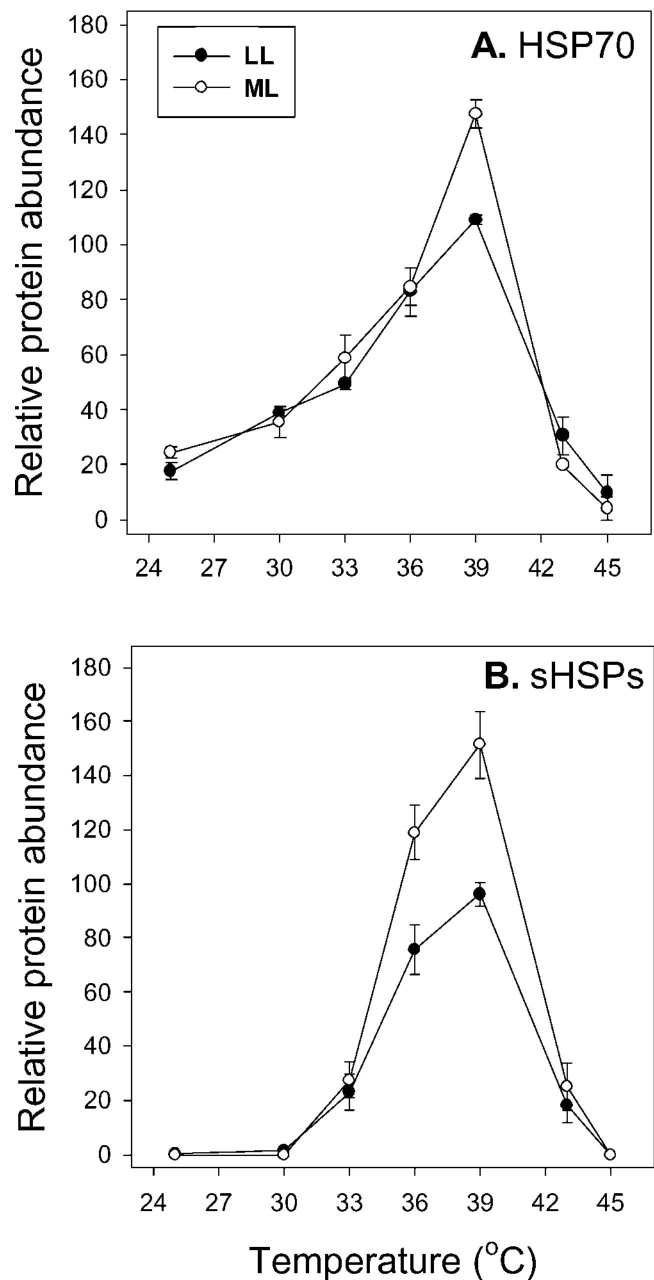

Fig. 6. Temperature response of heat shock protein (HSP) accumulation, in excised leaves of *Solidago altissima* in the laboratory, for (A) HSP70 and (B) small HSPs, at low (LL = 60 μmol · m^{-2} · s^{-1} PPFD) and moderately high (ML = 600 μmol · m^{-2} · s^{-1} PPFD) light. Protein content is expressed as a percentage of a heat-shock standard. Error bars represent 1 SE (N = 3).

peratures (Fig. 8A). At the heat-shock temperature there was an increase in HSP70 with increasing light. As with HSP70, there were significant effects of light, temperature, and the interaction of the two, on sHSPs ($P < 0.0001$, $P < 0.0001$, and $P = 0.0002$, respectively). There was no detectable accumulation of sHSPs at control temperatures at any of the light levels. Similar to HSP70, increasing light resulted in greater accumulation of sHSPs at the heat-shock temperature (Fig. 8B). PSII function was significantly affected by temperature, light, and the interaction between the two ($P < 0.0001$, $P <$ 0.0001, and $P = 0.0009$, respectively), with decreased PSII function at higher light and temperatures (Fig. 9).

DISCUSSION

Plants in the field are often exposed to multiple stresses simultaneously, and heat stress in nature is frequently accompanied by high light. Our results show that plant responses to heat stress are strongly influenced by light climate. HSP content in plants in the field, undergoing naturally occurring temperature stress, was significantly greater in open sun than shaded environments despite small differences in leaf temperature between sun and shade. In accordance with these results, we saw a significant effect of light and temperature on accumu-

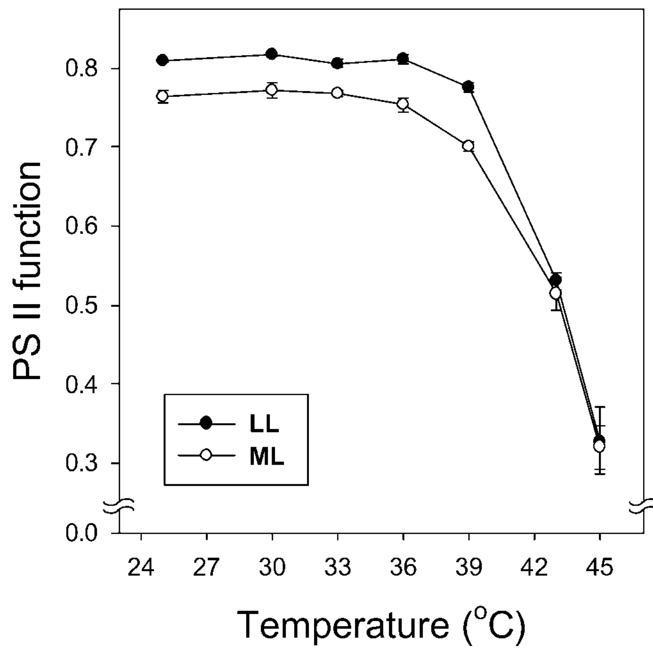

Fig. 7. Temperature response of photosystem II function in excised leaves of *Solidago altissima*, measured as dark-adapted F_v/F_m, at low (LL = 60 μmol · m^{-2} · s^{-1} PPFD) and moderately high (ML = 600 μmol · m^{-2} · s^{-1} PPFD) light, in the laboratory. Error bars represent 1 SE (N = 3).

lation of HSPs in the laboratory. This is the first study to show that the interaction of light and temperature significantly influences HSP accumulation in naturally growing plants in the field.

In the field, HSP70 content remained relatively high on all days examined, in both sun and shade plants. In contrast, sHSPs were barely detectable in the shade plants on all of the days examined and were only elevated in sun plants on the first day of the heat wave. Previous results in plants undergoing natural temperature stress in the field (Burke et al., 1985; Kimpel and Key, 1985; Keeler et al., 2000; Merquiol et al., 2002) have shown significant accumulation of HSPs, though others have found lack of field accumulation of HSPs (Hernandez and Vierling, 1993). It is well established that exposure of organisms to a mild heat stress results in increased accumulation of HSPs in a subsequent heat shock (Vierling, 1991); however, few studies have examined HSP accumulation over multiple consecutive days as often occurs during a heat wave in natural conditions. Our results show that HSP70 content increased through the 3 days of the heat wave, suggesting an additive effect of exposure to hot days, on accumulation of HSP70. A previous laboratory study of a 4-day heat-shock reports diminished HSP accumulation with each additional heat-shock (Howarth, 1991), though the heat-shock temperatures used (50°C) were extremely severe.

The observed differences in HSP content between sun and shade plants were more pronounced on hot days, indicative of a light × temperature interaction. However, because leaf temperature was greater in sun plants, we cannot conclusively attribute differences in HSP content solely to differences in light climate. Leaf temperatures in the sun, during the heat wave, were within experimentally determined temperatures required to induce HSPs (i.e., between 30 and 33°C), while leaf temperatures in the shade were considerably lower. However, it is

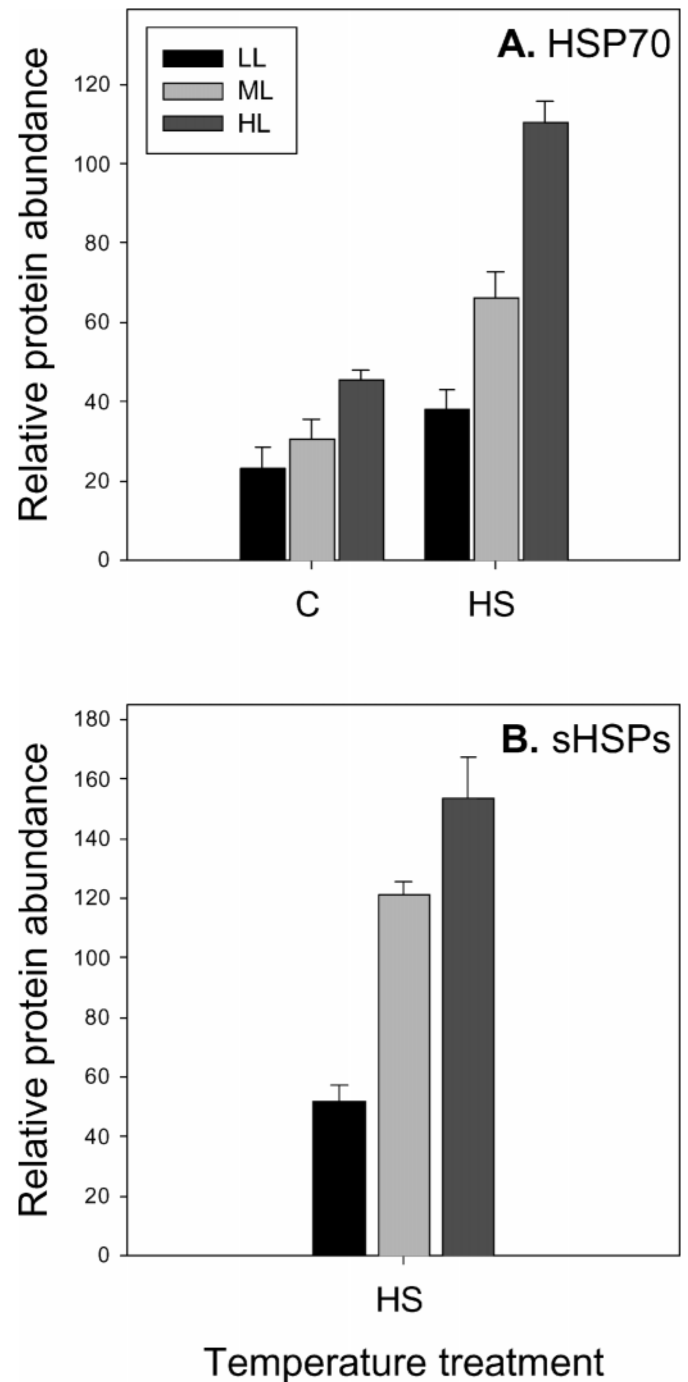

Fig. 8. Light response of heat shock protein (HSP) accumulation, in leaf-discs of *Solidago altissima* in the laboratory, for (A) HSP70 and (B) small HSPs. LL = low light (60 μmol · m^{-2} · s^{-1} PPFD), ML = moderately elevated light (600 μmol · m^{-2} · s^{-1} PPFD), and HL = high light (1800 μmol · m^{-2} · s^{-1} PPFD) at control (C = 25°C) and heat-shock (HS = 39°C) temperatures. There were no detectable sHSPs at control temperatures. Protein content is expressed as a percentage of a heat-shock standard. Error bars represent 1 SE (N = 3).

difficult to directly compare induction temperatures or accumulated protein content in the field to the experimental treatments because plants in the field experience a dynamic temperature regime through the day, as opposed to the fixed treatment temperatures for 2 h in the experimental laboratory treat-

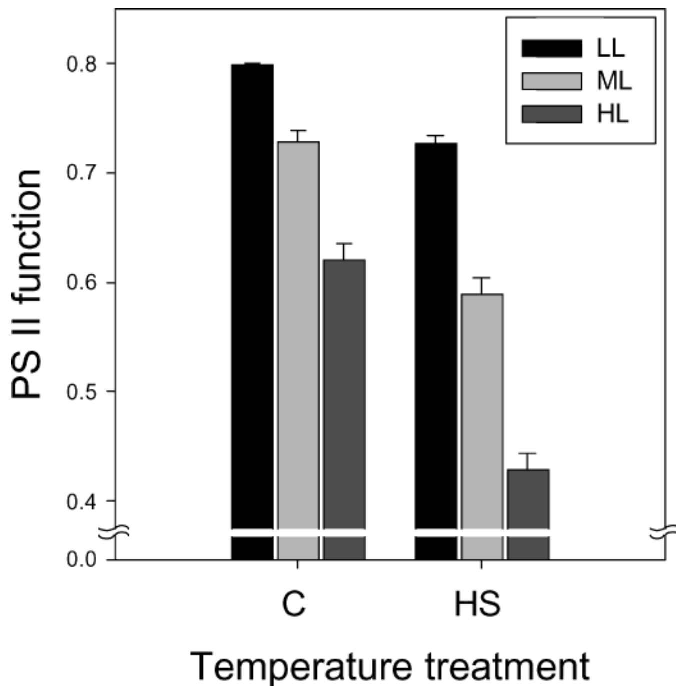

Fig. 9. Light response of photosystem II function in leaf-discs of *Solidago altissima* in the laboratory, measured as dark-adapted F_v/F_m in the laboratory. LL = low light (60 μmol · m^{-2} · s^{-1} PPFD), ML = moderately elevated light (600 μmol m^{-2} s^{-1} PPFD), and HL = high light (1800 μmol · m^{-2} · s^{-1} PPFD) at control (C = 25°C) and heat-shock (HS = 39°C) temperatures. Error bars represent 1 SE (*N* = 3).

ments. Plants in the sun had lower photosystem II function on all days examined, indicative of damage at the higher light levels. While leaf water potential was lower for sun plants, we believe that this was due to increased leaf transpiration in the sun because conductance did not differ among days. It is unlikely that the plants were water stressed or that the differences in water potential contributed to significant differences in HSP content, as days of peak HSP accumulation did not coincide with days of lowest water potential or highest stomatal conductance.

The laboratory experiments at controlled light levels and temperature confirmed that light influences HSP accumulation. The interaction of high light and heat-shock temperatures resulted in increased HSP accumulation. However we found that light in itself was not sufficient to induce either of the two HSPs examined. It has been suggested that temperature-independent light induction of HSPs is specific to certain HSPs (Debel et al., 1994). We did not detect any differences in the set points of induction, maximal production, or shut-off of the HSP response at low vs. moderately elevated light levels, as has been shown before (Knack and Kloppstech, 1992). It is possible that the resolution of the temperature intervals was too coarse to detect smaller changes in temperature set points of the heat shock response. PSII function decreased with increasing light levels and heat-shock temperatures, indicative of light and temperature caused damage, and an additive effect of the interaction of both high light and temperature was evident.

These results add to the surprisingly small number of studies that have examined HSP accumulation in plants in the field, most of which examine agricultural or commercially important crop plants and none of which addresses the effect of light (Burke et al., 1985; Kimpel and Key, 1985; Hernandez and Vierling, 1993; Ortiz et al., 1995; Wisniewski et al., 1996; Stout et al., 1997; Bhadula et al., 1997; Keeler et al., 2000; Al-Niemi and Stout, 2002; Merquiol et al., 2002). Overall, few studies have examined the effect of light on HSPs, and in a limited number of organisms—a species of cyanobacteria (Glatz et al., 1997; Hihara et al., 2002), an alga (von Gromoff et al., 1989; Kropat et al., 1997; Dryzmalla et al., 1996), and three higher plants (Knack et al., 1992; Stapel et al., 1993; Debel et al., 1994; Rossel et al., 2002). Fewer still have studied the interaction of light and temperature on HSP accumulation (Stapel et al., 1993; Debel et al., 1994; Glatz et al., 1997; Asadulghani et al., 2003). Our results confirm previous results on the effect of light on HSP accumulation, and importantly, show that light climate can be an important factor controlling HSP accumulation of plants in their natural habitat.

LITERATURE CITED

AL-NIEMI, T. S., AND R. G. STOUT. 2002. Heat-shock protein expression in a perennial grass commonly associated with active geothermal areas in western North America. *Journal of Thermal Biology* 27: 547–553.

ASADULGHANI, Y. SUZUKI, AND H. NAKAMOTO. 2003. Light plays a key role in the modulation of heat shock response in the cyanobacterium *Synechocystis* sp. PCC 6803. *Biochemical and Biophysical Research Communications* 306: 872–879.

BHADULA, S. K., G. YANG, A. STERZINGER, AND Z. RISTIC. 1997. Synthesis of a family of 45Ku heat shock protein in a drought and heat resistant line of maize under controlled and field conditions. *Journal of Plant Physiology* 152: 104–111.

BURKE, J. J., J. L. HATFIELD, R. R. KLEIN, AND J. E. MULLET. 1985. Accumulation of heat shock proteins in field grown cotton. *Plant Physiology* 78: 394–398.

DEBEL, K., G. KNACK, AND K. KLOPPSTECH. 1994. Accumulation of plastid HSP 23 of *Chenopodium rubrum* is controlled post-translationally by light. *Plant Journal* 6: 79–85.

DOWNS, C. A., S. A. HECKATHORN, J. K. BRYAN, AND J. S. COLEMAN. 1998. The methionine-rich low-molecular-weight chloroplast heat-shock protein: evolutionary conservation and accumulation in relation to thermotolerance. *American Journal of Botany* 85: 175–183.

DOWNS, C. A., S. L. RYAN, AND S. A. HECKATHORN. 1999. The chloroplast small heat-shock protein: evidence for a general role in protecting photosystem II against oxidative stress and photoinhibition. *Journal of Plant Physiology* 155: 488–496.

DRZYMALLA, C., M. SCHRODA, AND C. F. BECK. 1996. Light-inducible gene HSP70B encodes a chloroplast-localized heat shock protein in *Chlamydomonas reinhardtii*. *Plant Molecular Biology* 31: 1185–1194.

FEDER, M. E., AND G. E. HOFFMAN. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Review of Physiology* 61: 243–282.

GHOSH, S., S. GEPSTEIN, J. J. HEIKKILA, AND E. B. DUMBROFF. 1988. Use of a scanning densitometer or an ELISA plate reader for measurement of nanogram amounts of protein in crude extracts from biological tissues. *Analytical Biochemistry* 169: 227–233.

GLATZ, A., I. HORVATH, V. VARVASOVSZKI, E. KOVACS, Z. TOROK, AND L. VIGH. 1997. Chaperonin genes of the *Synechocystis* PCC 6803 are differentially regulated under light-dark transition during heat stress. *Biochemical and Biophysical Research Communications* 239: 291–297.

HECKATHORN, S. A., G. J. POELLER, J. S. COLEMAN, AND R. L. HALLBERG. 1996. Nitrogen availability alters patterns of accumulation of heat stress-induced proteins in plants. *Oecologia* 105: 413–418.

HECKATHORN, S. A., S. A. RYAN, J. A. BAYLIS, D. WANG, E. W. HAMILTON III, L. CUNDIFF, AND D. S. LUTHE. 2002. In vivo evidence from an *Agrostis stolonifera* selection genotype that chloroplast small heat-shock proteins can protect photosystem II during heat stress. *Functional Plant Biology* 29: 935–946.

HERNANDEZ, L. D., AND E. VIERLING. 1993. Expression of low molecular heat shock proteins under field conditions. *Plant Physiology* 101: 1209–1216.

HIHARA, Y., A. KAMEI, M. KANEHISA, A. KAPLAN, AND M. IKEUCHI. 2001. DNA microarray analysis of cyanobacterial gene expression during acclimation to high light. *Plant Cell* 13: 793–806.

HOWARTH, C. J. 1991. Molecular responses of plants to an increased incidence of heat shock. *Plant, Cell & Environment* 14: 831–841.

KEELER, S. J., C. M. BOETTGER, J. G. HAYNES, K. A. KUCHES, M. M. JOHNSON, D. L. THUREEN, C. L. KEELER JR., AND S. L. KITTO. 2000. Acquired thermotolerance and expression of the HSP100/ClpB genes of lima bean. *Plant Physiology* 123: 1121–1132.

KIMPEL, J. A., AND J. L. KEY. 1985. Presence of heat shock mRNAs in field grown soybeans. *Plant Physiology* 79: 672–678.

KNACK, G., AND K. KLOPPSTECH. 1992. The heat-shock response in a photoautotrophic cell-culture of *Chenopodium rubrum*: the effects of temperature and light. *Journal of Plant Physiology* 140: 489–493.

KROPAT, J., U. OSTER, W. RUDIGER, AND C. F. BECK. 1997. Chlorophyll precursors are signals of chloroplast origin involved in light induction of nuclear heat-shock genes. *Proceedings of the National Academy of Sciences, USA* 94: 14168–14172.

LI, Q.-B., AND C. L. GUY. 2001. Evidence for non-circadian light/dark-regulated expression of Hsp70s in spinach leaves. *Plant Physiology* 125: 1633–1642.

LINDQUIST, S. 1980. Varying patterns of protein synthesis in *Drosophila* during heat shock: implications for regulation. *Developmental Biology* 77: 463–479.

LONG, S. P., S. HUMPHRIES, AND P. G. FALKOWSKI. 1994. Photoinhibition of photosynthesis in nature. *Annual Review of Plant Physiology and Plant Molecular Biology* 45: 633–662.

MERQUIOL, E., L. PNUELI, M. COHEN, M. SIMOVITCH, S. RACHMILEVITCH, P. GOLOUBINOFF, A. KAPLAN, AND R. MITTLER. 2002. Seasonal and diurnal variations in gene expression in the desert legume *Retama raetam*. *Plant, Cell & Environment* 25: 1627–1638.

OHAD, I., N. ADIR, H. KOIKE, D. KYLE, AND Y. INOUE. 1990. Mechanism of photoinhibition in vivo. A reversible light-induced conformational change of reaction center II is related to an irreversible modification of the D1 protein. *Journal of Biological Chemistry* 265: 1972–1979.

ORTIZ, C. A., L. A. BRAVO, M. PINTO, AND L. CARDEMIL. 1995. Physiological and molecular responses of *Prosopis chilensis* under field and simulation conditions. *Phytochemostry* 40: 1375–1382.

PARSELL, D. A., AND S. LINDQUIST. 1994. Heat shock proteins and stress tolerance. *In* R. Morimoto, A. Tissieres, and C. Georgopoulos [eds.], The biology of heat shock proteins and molecular chaperones, 457–494. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

ROSSEL, J. B., I. W. WILSON, AND B. J. POGSON. 2002. Global changes in gene expression in response to high light in Arabidopsis. *Plant Physiology* 130: 1109–1120.

SCHRODA, M., O. VALLON, F.-A. WOLLMAN, AND C. F. BECK. 1999. A chloroplast-targeted Heat shock protein 70 (HSP70) contributes to the photoprotection and repair of photosystem II during and after photoinhibition. *Plant Cell* 11: 1165–1178.

SCHUSTER, G., D. EVEN, K. KLOPPSTECH, AND I. OHAD. 1988. Evidence for protection by heat-shock proteins against photoinhibition during heatshock. *EMBO Journal* 7: 1–6.

STAPEL, D., E. KRUSE, AND K. KLOPPSTECH. 1993. The protective effect of heat shock proteins against photoinhibition under heat shock in barley (*Hordeum vulgare*). *Journal of Photochemistry and Photobiology B* 21: 211–218.

STOUT, R. G., M. L. SUMMERS, T. KERSTETTER, AND T. R. MCDERMOTT. 1997. Heat- and acid-tolerance of a grass commonly found in geothermal areas within Yellowstone National Park. *Plant Science* 130: 1–9.

VIERLING, E. 1991. The roles of heat shock proteins in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 42: 579–620.

VON GROMOFF, E. D., U. TREIER, AND C. F. BECK. 1989. Three light-inducible heat shock genes of *Chlamydomonas reinhardtii*. *Molecular and Cellular Biology* 9: 3911–3918.

WANG, W., B. VINOCUR, O. SHOSEYOV, AND A. ALTMAN. 2004. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends in Plant Science* 9: 244–252.

WEIS, E., AND J. A. BERRY. 1988. Plants and high temperature stress. *In* S. P. Long and F. I. Woodward [eds.], Plants and temperature, 329–346. Company of Biologists, Cambridge, UK.

WISNIEWSKI, M., T. J. CLOSE, T. ARTLIP, AND R. ARORA. 1996. Seasonal patterns of dehydrins and 70-kDa heat shock proteins in bark tissues of eight species of woody plants. *Physiologia Plantarum* 96: 496–505.