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Morphogenesis of Douglas-fir buds is altered at elevated temperature but not at elevated CO_2[1]

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Abstract

Global climatic change as expressed by increased CO_2 and temperature has the potential for dramatic effects on trees. To determine what its effects may be on Pacific Northwest forests, Douglas-fir (*Pseudotsuga menziesii*) seedlings were grown in sun-lit controlled environment chambers at ambient or elevated (+ 4°C above ambient) temperature, and at ambient or elevated (+ 200 ppm above ambient) CO_2. In 1995–1996 and 1996–1997, elevated CO_2 had no effect on vegetative bud morphology, while the following unusual morphological characteristics were found with greater frequency at elevated temperature than at ambient: rosetted buds with reflexed and loosened outer scales, convoluted inner scales, clusters of small buds, needles elongating between scales, needle primordia with white, hyaline apical extensions, and buds with hardened scales inside of unbroken buds. Buds became rosetted in elevated temperature chambers after temperatures exceeded 40°C in July, 1996. Rosettes were induced within 48-h in buds placed in a 40°C oven; fewer rosettes formed at 20°C. Induction was reversible in buds transferred from 40 to 20°C, implying that rosetting is a physical rather than a growth phenomenon. It appears that rosettes form after long-term exposure to elevated temperature and after shorter periods of exposure to intense heat. Elevated temperature influences bud morphology and may therefore influence the overall branching structure of Douglas-fir seedlings.

Keywords: Climatic change; *Pseudotsuga menziesii*; Douglas fir; Bud; Morphology; Temperature

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1. Introduction

Elevations in atmospheric CO_2 and temperature are associated with climatic change (Cannell and Smith, 1986; Billington and Pelham, 1991; Gates et al., 1995). Climatic change may influence the

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physiology, morphology and phenology of buds if it occurs more rapidly than the adaptations of trees to changes in climate (Cannell and Smith, 1986; Billington and Pelham, 1991; Chomba et al., 1993; Beuker, 1994; Murray et al., 1994; Myking and Heide, 1995; Olszyk D., pers. commun.). Buds are the points of growth and branching for the aerial portions of trees, therefore, alterations in bud development may lead to changes in the morphology, architecture and geometry of trees. It has been demonstrated that elevated temperature influences the shape of meristematic cells in Douglas fir needle primordia (MacDonald and Owens, 1993) which are produced by the shoot apical meristem inside of the bud (Owens and Molder, 1973). Therefore, elevated temperatures may contribute to developmental and morphological changes by influencing other meristematic cells within the bud.

Bud development is an active period of metabolic, ultrastructural and morphological change. In Douglas-fir (*Pseudotsuga menziesii*), temperature dependent changes in ultrastructure, such as the catabolism-associated enclosure of cytoplasmic structures into vacuoles (Matile, 1975), have been found in bud apices during the transition from bud scale to needle initiation (Kupila-Ahvenniemi et al., 1978; Krasowski and Owens, 1990). During needle initiation, apical cell numbers, apical height and width, and mitotic activity are all maximal (Owens and Molder, 1973). High respiration rates associated with bud development suggest that an active metabolism is necessary for the synthesis of new cell materials (Fielder and Owens, 1989, 1992).

Few studies exist on the morphology of buds in response to climatic change (MacDonald and Owens, 1993). In Douglas-fir, vegetative buds had needle primordia with acute rather than obtuse apices when subjected to temperature and moisture stress in growth chambers (MacDonald and Owens, 1993). Because buds appear to be sensitive to temperature, more knowledge is needed on bud morphogenesis in response to climatic change.

To assess the effects of predicted climatic change in the Pacific Northwestern US on forest trees (Tingey et al., 1996), Douglas-fir seedlings were grown at the Environmental Protection Agency (EPA) facility in Corvallis, OR in sun-lit controlled-environment chambers at ambient and elevated CO_2 (ambient $+200$ ppm) and temperature (ambient $+4$°C) regimes. The $+4$°C increase in temperature was chosen because it represents a value within the range of predicted temperatures that would occur with an effective doubling of CO_2, which accounts for the corresponding increase in other greenhouse gases such as methane (Tingey et al., 1996).

To determine whether specific morphological characteristics of vegetative buds of Douglas fir are related to elevated CO_2 and/or temperature, we examined the morphology of buds from each climatic treatment and from on-site unenclosed chambers. In situ monitoring of buds and exposure of harvested buds to elevated temperatures were used to explore the mechanisms of bud development. We hypothesize that due to temperature stress, buds with abnormal characteristics will be present with greater frequency at elevated temperature than at ambient temperature, and that at elevated CO_2, buds will not experience temperature stress and will therefore be similar to buds at ambient CO_2 and temperature.

2. Materials and methods

2.1. Experimental design

Fourteen *P. menziesii* (Mirb.) Franco (Douglas-fir) seedlings were grown in each of 14 sun-lit controlled-environment chambers known as terracosms, at ambient and elevated CO_2 (ambient $+200$ ppm) and temperature ($+4$°C above ambient; Tingey et al., 1996). These levels were maintained year-round, and followed dynamic tracking of ambient CO_2 and temperature levels to maintain diurnal and seasonal patterns. The temperature levels were within 2°C of the target temperature for 85–100% of the hours between 1 November 1993 and 30 November 1994, and CO_2 concentration was within ± 50 μmol mol^{-1} for 92–100% of those hours (Tingey et al., 1996). The seedlings were exposed to these climatic treatments for the 4 years from their planting in the summer of 1993 as bare-root, 2-year-old 'woods

run' stock (Weyerhauser Company) until their harvest in the summer of 1997. The dewpoint was adjusted in spring, 1994 to maintain the same vapor pressure deficit in ambient and elevated CO_2 and temperature treatments. Trees were watered with reverse-osmosis water on a schedule that maintained soil moisture typical of the seasonal variations in the forests of the southern Willamette Valley (Tingey et al., 1996).

Three chambers each were devoted to the following climatic treatments: ambient CO_2 and temperature (ACAT); elevated CO_2 and ambient temperature (ECAT); ambient CO_2 and elevated temperature (ACET); and elevated CO_2 and temperature (ECET). The two remaining terracosms were unenclosed (chamberless (CL)), but otherwise resembled the climatic treatment chambers. Terracosm buds were compared with buds from sites in the Cascade Mountains of Oregon which were chosen to reflect present climatic conditions for the Cascades.

2.2. Morphology and development of in situ buds

The externally visible morphological characteristics of 20 vegetative buds per chamber were monitored in order to trace their external morphological development over time (Table 1 and Figs. 1 and 2). On each tree per chamber, one to two branch terminal buds on the second to the fourth whorls down from the terminal leader were labeled with brightly colored paper tags. Buds were monitored at approximately 6-week intervals from November, 1995 until bud break in 1996, and a new set of buds were tagged in June, 1996 and monitored until bud break in 1997.

Table 1
Externally and internally visible unusual morphological characteristics of buds

Characteristic	Description and visibility
Cluster	External—group of small (<3 mm) buds
Expanded	External—needles grow outward between bud scales
Rosette	External—outermost bud scales are reflexed
Scale-needles	External—expanded needles have white, scale-like margins
Convoluted	Internal—bud scales are rolled and curled
New bud	Internal—new bud with scales inside unbroken bud
Primordia	Internal—no needle primordia
Stalk	Internal—buds arise from an elongated stalk
White	Internal—apices of needle primordia have white, hyaline extensions

2.3. Morphological characteristics of harvested buds

Harvested and dissected vegetative buds were classified according to their externally and internally visible morphological characteristics (Table 1 and Fig. 1). Twenty terminal branch buds or adjacent lateral buds were harvested per chamber at approximately 6-week intervals from October, 1995 until March, 1996, and from June, 1996 until February, 1997. One bud was taken from trees 1–8, and two buds were taken from trees 9–14. After measuring bud length and width, we dissected the buds longitudinally with a single-edged razor blade and photographed them through an Olympus SZ-PT dissecting microscope with a Dolan–Jenner fiber-optic light source and a Nikon AF N6006 camera with Fugichrome T64 or Ektachrome T100 film.

2.4. Induction of rosettes

Twenty harvested buds from each chamber were classified as N (normal) or R (rosetted buds with reflexed and loosened outer scales). Ten of the buds from each chamber were placed in vials in a 20°C oven; the remaining ten were placed in vials in a 40°C oven, which approximates the temperature of ACET and ECET chambers during summer heat waves. Half the vials at each temperature were uncapped, and half were capped to control water loss. The percentage of change from N to R was calculated at 48 and 96 h for each terracosm and for capped versus uncapped vials. To determine whether rosetting was reversible, 20 buds that became rosetted at 40°C were capped, placed at 20°C, and the percentage of reversion to normal was recorded at 48 h. As a

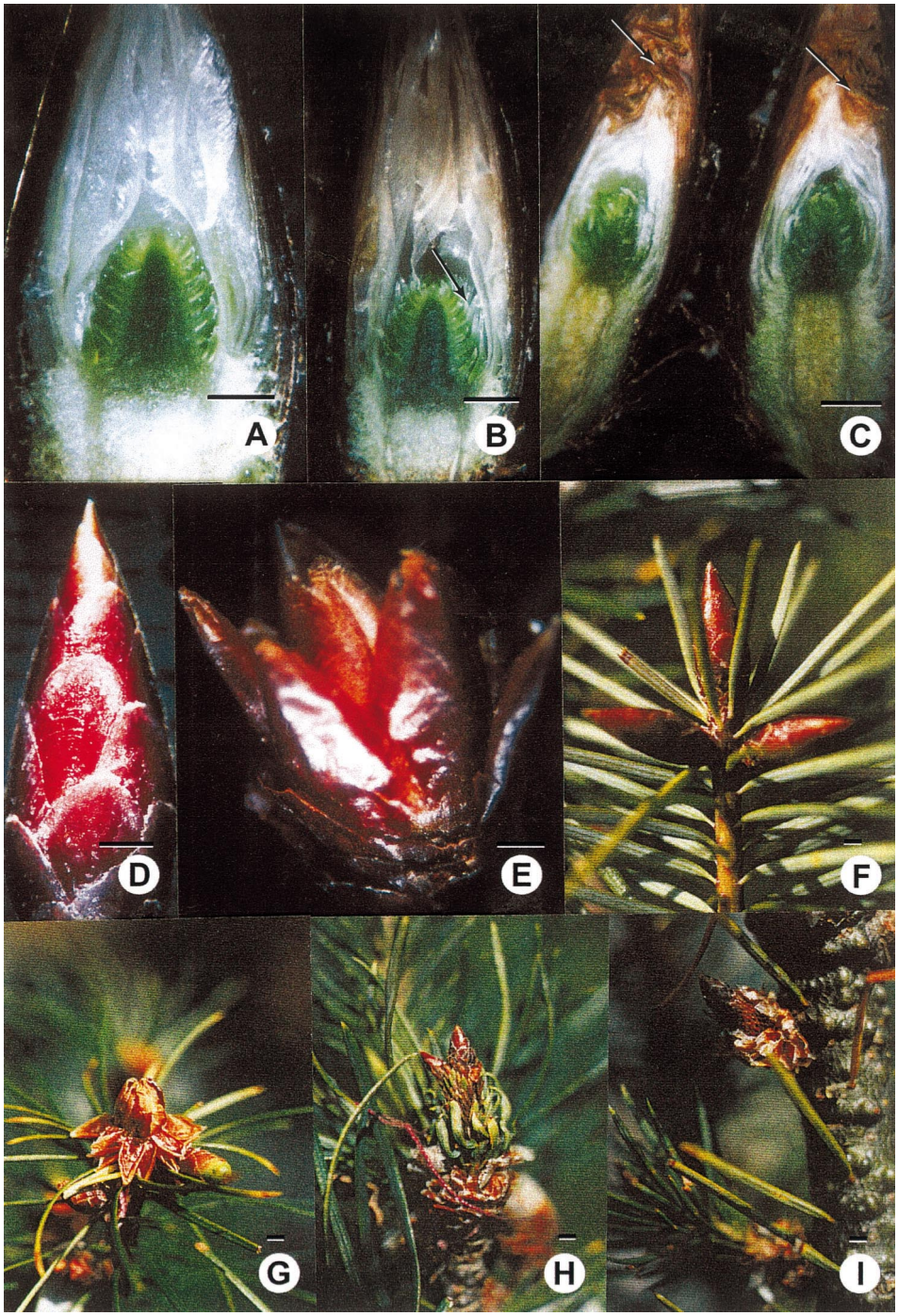

Fig. 1. Douglas-fir vegetative buds. Dissected normal bud from an ambient CO_2 and temperature terracosm (A). Dissected abnormal buds from elevated temperature terracosms with: bud scale-like needle primordia (arrow), (B), convoluted bud scales (arrows), (C). Exterior of normal bud from an ambient CO_2 and temperature terracosm (D), and exterior of rosetted bud from an elevated temperature terracosm (E). Normal buds from an ambient CO_2 and temperature terracosm (F). Abnormal buds from ambient CO_2 and elevated temperature terracosms: rosetted (G), shoot with two small buds and reduced needles (H), bud with reduced needles and elongated stalk originating from tree trunk (I). Scale = 1 mm.

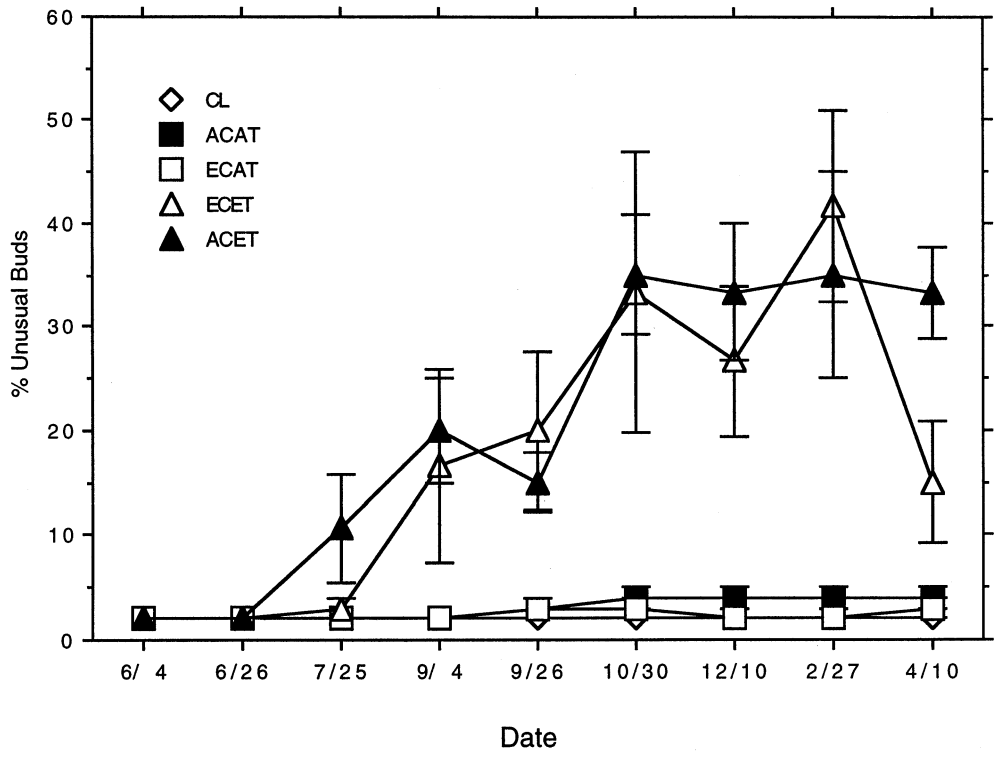

Fig. 2. Unusual tagged buds from June, 1996 to April, 1997. Symbols are averages ± standard errors for six replicate chambers per temperature treatment across CO_2 levels. Treatments are: ACAT, ECAT, ECET and ACET.

control, two groups of ten buds that had become rosetted were uncapped and returned to 40°C.

2.5. Statistical analyses

The experimental unit was the average of all buds observed per terracosm, with three replicate terracosms per treatment. Qualitative data was converted to quantitative data by assigning a value of 1 to a bud when it exhibited a characteristic, and a 0 when it did not. Buds were assigned a value of 1 for each characteristic they exhibited. The incidence of each characteristic for each chamber was expressed as an arcsine transformed percentage. Two-way analyses of variance (ANOVA) were used to determine: (1) the effects of temperature and CO_2 (elevated vs. ambient) on the incidence of characteristics in each climatic treatment; and (2) the induction of rosetting at 20 and 40°C in capped versus uncapped vials. Terracosm # 2 (ACAT) was not included in the 1996–1997 analyses because of aphid infestation.

3. Results

3.1. Morphological characteristics of buds

Bud morphology varied among climatic treatments, with a greater frequency of unusual buds occurring at elevated temperature (ACET and ECET) than at ambient temperature (Tables 1–3 and Figs. 1–4). No significant morphological differences were found with elevated compared to ambient CO_2 (Tables 2 and 3), and there were no interactive effects of temperature and CO_2. Although a few unusual buds were found in the ambient temperature treatments and in the chamberless terracosms, they were not present in statistically significant quantities. Buds from the CL

Table 2
Two-way ANOVA (P-values) of anatomical characteristics of Douglas-fir buds, 1995–1997

Characteristic	Date						
	12/4/95	1/12/96	2/28/96	6/11/96	8/19/96	11/7/96	2/7/97
Cluster							
Temperature	0.067	0.195	0.202	—	0.407	0.159	—
CO_2	0.500	0.974	0.808	—	0.407	1.000	—
Convoluted							
Temperature	—	0.362	0.017	0.121	0.000	0.101	0.017
CO_2	—	0.579	0.610	0.407	0.104	0.717	0.104
Long stalk							
Temperature	0.032	0.067	0.024	—	0.252	0.033	0.092
CO_2	0.863	0.500	0.845	—	1.000	0.155	0.788
Needle-scales							
Temperature	—	0.022	0.003	—	—	—	—
CO_2	—	0.973	0.506	—	—	—	—
New bud in old							
Temperature	—	0.006	—	0.407	0.407	0.155	0.017
CO_2	—	0.356	—	0.407	0.407	0.492	0.104
Rosettes							
Temperature	0.000	0.002	—	—	0.021	0.013	0.011
CO_2	0.107	0.503	—	—	0.849	0.741	0.289
White primordia							
Temperature	0.001	0.075	0.002	—	0.034	0.008	0.000
CO_2	0.563	0.360	0.481	—	0.345	0.454	0.705

There was no significant interaction between temperature and CO_2. Dashes indicate that the characteristic was not observed on that date.

and ambient (ACAT) treatments resembled each other and were similar to buds from sites in the Cascade Mountains. In a two-way ANOVA of CL and ambient (ACAT) buds, there was no significant difference ($P = 0.94$). More pronounced morphological differences were often noted on buds closer to the tops of trees.

In 1995–1996 and again in 1996–1997, these morphological traits were found with significantly greater frequency at elevated temperature than at ambient temperature: rosettes, convoluted scales, elongated stalks, needle primordia with white, hyaline apical extensions that resembled bud scales, and new buds with hardened scales inside of old buds (Table 2).

These unusual traits were observed infrequently at elevated temperature and were not found at ambient temperature: clusters of buds, multiple buds enclosed in one set of bud scales, and buds without needle primordia. Buds without needle primordia had normal bud scales and were found late in the 1996–1997 season when other buds had needle primordia. Possibly due to their infrequency, these traits did not yield statistically significant differences between treatments.

As the season progressed after the peak of summer in 1996, the percentage of rosetted buds (Figs. 2–4), buds with convoluted scales, and buds with scale-like needle primordia became increasingly significant at elevated temperature (Table 2). The incidence of these factors was also influenced by branch position, with terminal branch buds on 19 August, 1996 having a significantly greater frequency of these characteristics ($P < 0.05$) than that of lateral branch buds on 13 August, 1996 ($P > 0.05$).

On 11 June, 1996, needle primordia were visible in buds from elevated temperature chambers (where bud break took place earlier), but were not yet apparent in buds from ambient temperature chambers. On 11 June and on 13 August, 1996, the significantly greater length and width of elevated temperature buds probably reflected the earlier break at elevated temperature. The greater width of elevated temperature buds on 7 November, 1996, may have been due to distortions in morphology of rosettes and other unusual buds.

3.2. Bud change over time

In 1995–1996, sampling did not begin until November, when the percentage of unusual buds had already reached nearly maximal levels. It remained high at elevated temperature until March, when it began to decline with the onset of bud break. In 1996–1997, buds were normal when monitoring began in June (Fig. 2). At elevated temperature, the percentage of unusual buds began to rise in July and became statistically significant ($P < 0.05$) by 4 September, 1996 (Table 3), following several months of exposure to +4°C above ambient summer temperatures. This upward trend continued until December, when the percentage dipped by approximately 5%. This can be attributed to the unseasonal, late lammas break of some rosetted buds during December. By February, new buds had formed on shoots derived from the break of rosetted buds in December, and the percentage of unusual buds rose to reflect the incidence of unusual buds on these new shoots. The percentage of unusual buds declined with bud break.

Table 3
Two-way ANOVA *P*-values of % unusual tagged Douglas-fir buds, 1996–1997[a]

Date	Temperature	CO_2	Temperature/CO_2
11/95	0.001	0.761	0.761
1/96	0.001	0.320	0.320
2/96	0.001	0.210	0.210
3/25/96	0.001	0.043[b]	0.043[b]
4/15/96	0.001	0.321	0.321
7/7/96	0.131	0.261	0.261
9/4/96	0.018	0.788	0.788
10/30/96	0.007	0.887	0.962
12/10/96	0.001	0.444	0.723
2/2/97	0.002	0.797	0.574
4/10/97	0.001	0.061[c]	0.082[c]

[a] Measurements were also made on 4 and 26 June, 1996, but on these dates the small, developing buds did not have unusual characteristics.
[b] These values are probably statistically anomalous, as the percentage of unusual tagged buds was zero at elevated CO_2 on this date.
[c] These values may reflect the lowered percentage of unusual buds at elevated temperature as spring bud break proceeded.

Once buds became malformed, they remained so until bud break. Shoots originating from rosetted buds either elongated normally or produced short shoots which often had needles with brown edges that may have originated as extensions of needle primordia. While by definition an in situ bud cannot be harvested and remain on the tree, examination of growth from malformed buds revealed insights as to their internal structure prior to bud break. The exterior of elongated bud stalks had small brown scales which may have originated as reduced needles (Fig. 1). Upon break these buds presented only a few new needles. Clusters of small buds which terminated shoots remained in place at least until the following spring. Needle growth between the outer bud scales was most common in late fall, after which there was no growth until spring. Dissection revealed that these needles developed and grew from the usual position of a bud scale.

The fate of buds that were monitored in 1995–1996 was assessed on 18 July, 1996. As not all tags remained in place by this date, this is a partial account. In the chamberless, ambient temperature and elevated CO_2 chambers, all buds had broken and formed new shoots with new buds. However, in all elevated temperature chambers, at least some tagged buds had not broken or were clusters that had broken but underwent little elongation.

Most buds formed in 1996 had broken and produced shoots with new buds by 27 June, 1997. In the ACAT and ECAT chambers, all buds of healthy trees had broken. In the ACET

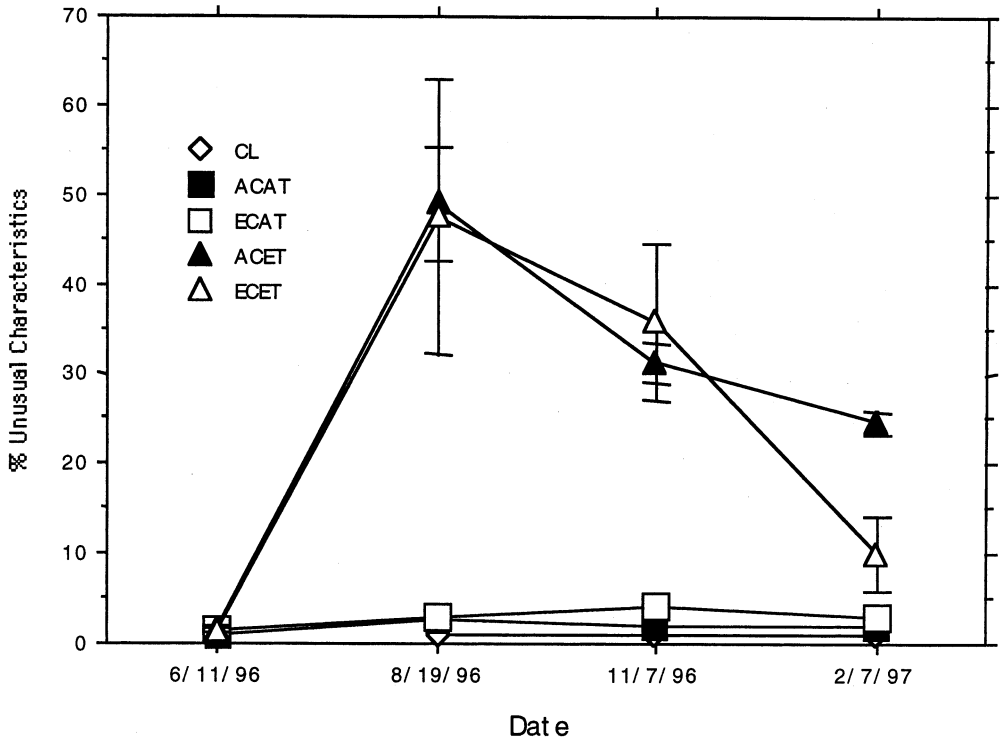

Fig. 3. Percent of unusual dissected buds in 1996–1997 from the climatic treatments (ACAT, ECAT, ECET and ACET) and the CL. For each climatic treatment, $n = 60$ dissected buds per date, with $n = 40$ for the unenclosed chambers. Symbols are averages $\pm$ standard errors for six replicate chambers per temperature treatment across CO_2 levels.

chambers, 100% of the tagged buds had broken, and in the ECET chambers, 91% of the tagged buds had broken. In two ECET chambers, all tagged buds had broken, but in the third ECET chamber, 25% of the tagged buds remained unbroken. Unbroken buds often belonged to clusters that were unbroken rosettes.

There is evidence that other stresses besides high temperature are associated with bud abnormalities. As of June, 1997, many trees in an ambient temperature chamber died or were unhealthy due to aphids and root weevils. Of the 20 tagged buds in this chamber, 10% became rosetted and did not break, and an additional 25% remained normal but did not break. In an ECAT chamber where root weevils were found in spring, 1997, one bud became rosetted. Rosetted buds were found on broken branches in an ECAT chamber and in an ACET chamber.

3.3. Induction of rosettes

Rosetting of buds was reversible and occurred in response to changes in physical conditions. Temperatures of 40°C and uncapped vials (lowered humidity) were both effective inducers of rosetting (Figs. 5 and 6), however, the greater effect was that of increased temperature. Induction of rosetting was most likely to occur within the first 48 h of exposure to 40°C, although some buds became rosetted between 48 and 192 h. Few buds became rosetted at the control temperature of 20°C. Temperature-induced rosette formation occurred with statistical significance ($P < 0.05$) in buds from all treatments. Following induction at 40°C, buds returned to normal when placed at 20°C in capped vials for 48 h. Rosettes were induced again by uncapping the vials and returning the buds to 40°C for 48 h. Buds did not revert to normal unless they were placed in capped vials at 20°C for at least 48 h.

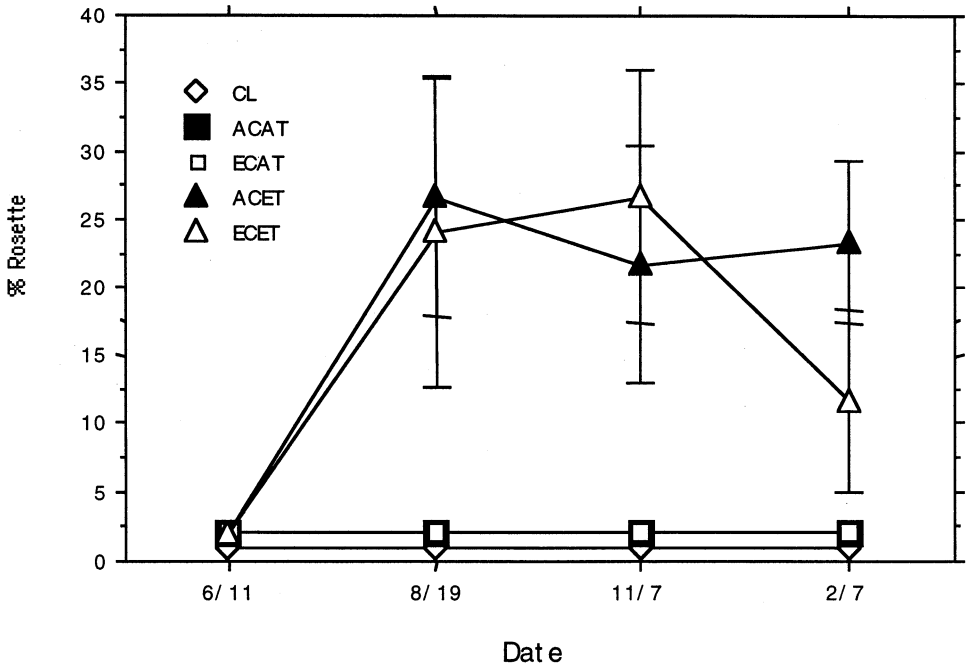

Fig. 4. Percent of dissected, rosetted buds in 1996–1997. For each climatic treatment (CL, ACAT, ECAT, ECET and ACET), $n = 60$ dissected buds per date, with $n = 40$ for the unenclosed chambers. Symbols are averages $\pm$ standard errors for six replicate chambers per temperature treatment across CO_2 levels.

4. Discussion

Elevated temperature, but not elevated CO_2, had significant effects on the morphogenesis of Douglas fir vegetative buds. At elevated CO_2, buds were most similar to buds from ambient and chamberless controls. Phenology of buds, shoot growth and needle growth were similar at elevated CO_2, ambient CO_2, and in chamberless controls (Olszyk, D., pers. commun.). One mechanism for this lack of pronounced effect on the aboveground growth and morphology of these trees may be that a greater proportion of photosynthate is allocated to their roots (Olszyk, D., pers. commun.). If that is the case, the increased carbon supply would travel away from the aerial portions of the trees and would not be as likely to affect developmental change.

Temperature influences developmental rates in plants (Jones, 1992), therefore, elevated temperature may accelerate developmental processes in buds. The earlier break of buds from elevated temperature resulted in a longer growing season, with more time for development, more time for cumulative differences in temperature to act on development, and hence more chances for developmental pathways to branch onto abberant trails.

Exposure to elevated temperature during the developmentally plastic 10-week phase which follows bud initiation (Allen and Owens, 1972), may alter developmental patterns and contribute to the formation of abnormal buds. Developmental signals for bud scale and needle production may be acted on differently by the apex when they arrive through the filter of elevated temperature. It is possible that hormonal signals are perceived differently at elevated temperature. If the timing of the three phases of coniferous bud differentiation (scale initiation, a transitional phase and needle initiation; Krasowski and Owens, 1990; MacDonald and Owens, 1993) is altered, then organogenesis of buds may change and intermediate structures such as bud scale-like needle primordia

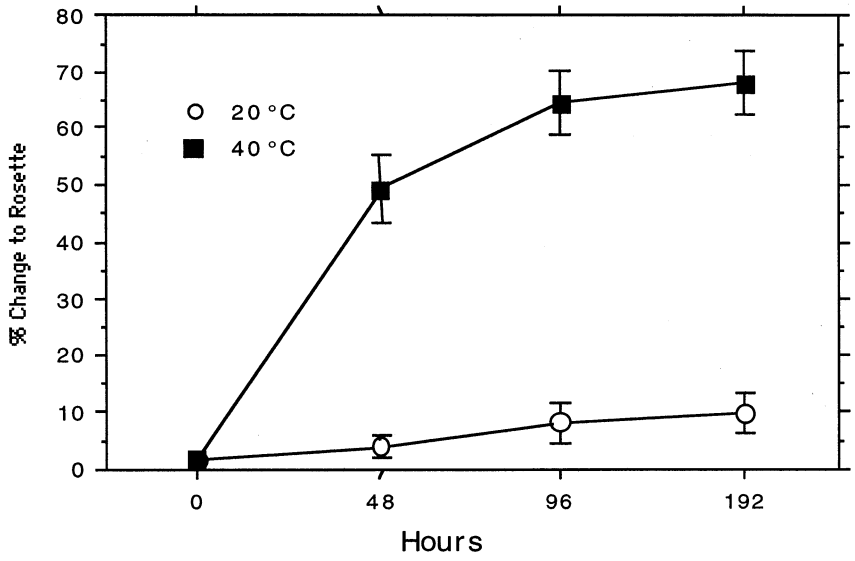

Fig. 5. Induction of rosetted buds with elevated temperature. Of the 20 buds collected from each chamber, ten were exposed to 20°C and ten were exposed to 40°C. Symbols are averages ± standard errors for six replicate chambers per temperature treatment across CO_2 levels.

may be formed. Bud development occurred earlier at elevated temperature, therefore, the white, hyaline bud scale-like apical extensions of the needle primordia may have developed because the needle primordia were older and spent more time differentiating. Apical extensions may be composed of elongated and vacuolate cells in the apical regions of needle primordia (MacDonald and Owens, 1993).

Bud scale initiation is completed by mid-summer but enlargement continues until late summer (Allen and Owens, 1972), which coincides with the appearance of convoluted scales at elevated temperature. Convolutions may develop as a result of excess lengthening or production of scales. Normal external scales and convoluted internal scales are both brown, which suggests that convoluted scales form in response to elevated temperature as a means of generating a shield against excess thermal energy. If buds form a greater proportion of scales than needle primordia, they will produce shoots with fewer needles. However, if excess scales shield the apex from high temperature, then the apex may have a greater chance of surviving to produce buds when conditions become more favorable. Buds which contain new buds with hardened, brown scales and buds without needle primordia suggest temperature-associated changes in development.

A cascade of molecular events leading to changes in bud morphology may be set in motion by elevated temperature. Although we did not investigate molecular events, elevated temperature is known to induce production of heat shock proteins (Chen et al., 1990; Vierling, 1991). Thermotolerance has been linked to low-molecular-weight chloroplast-localized heat-shock proteins in vascular plants, and plants adapted to cooler habitats accumulate smaller amounts of these proteins than plants from desert habitats (Downs et al., 1998). The lack of protection by heat-shock proteins in Douglas-fir, which is adapted to cooler habitats, suggests that these trees are not thermotolerant (Downs et al., 1998), and may be vulnerable to high temperature. Lack of thermotolerance may be manifested in the shoot apex, which tends to produce abnormal buds at elevated temperature.

Rosetted buds have loosened and reflexed scales, which may increase exposure of the apex. The apex may produce bud scale-like needle primordia as a means of counteracting the functional

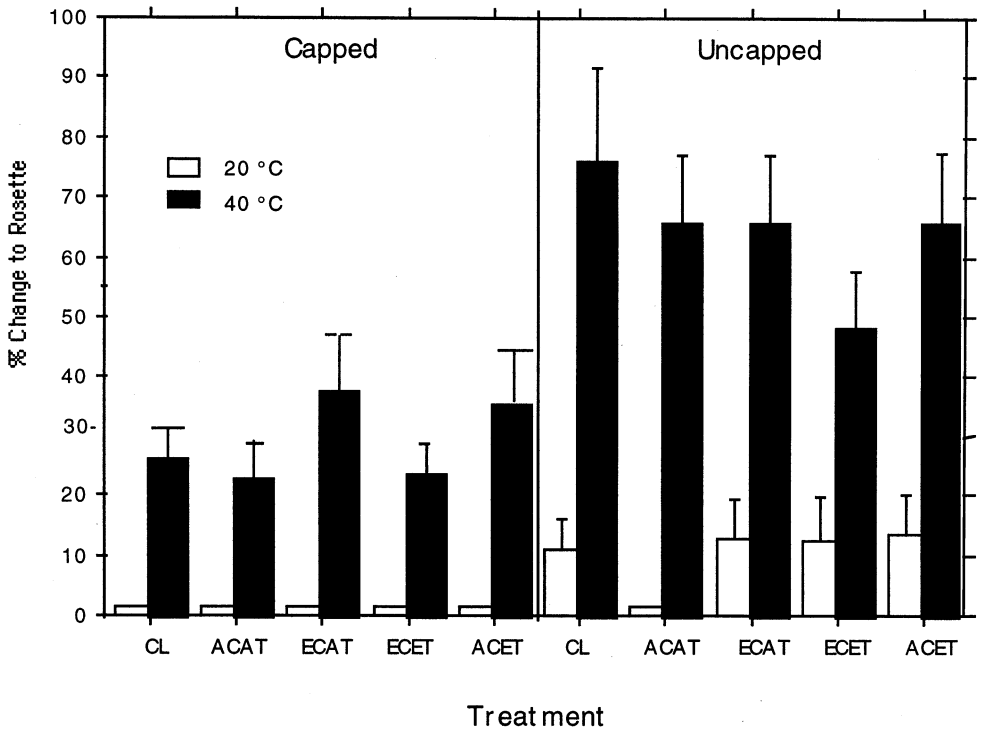

Fig. 6. The influence of capped and uncapped vials on the formation of rosettes per treatment (CL, ACAT, ECAT, ECET and ACET). Of the 20 buds collected from each chamber, ten were exposed to 20°C and ten were exposed to 40°C; five of each temperature group were in capped vials. Symbols are averages ± standard errors for six replicate chambers per temperature treatment across CO_2 levels.

loss of scales through rosetting. Thus, abnormal development may be perpetuated. Abnormalities may also be perpetuated if the buds break dormancy at unseasonal times when environmental conditions may not be optimal for development of the next generation of buds (Owens et al., 1986). By late fall, rosettes often broke in elevated temperature chambers where buds had been exposed to elevated temperatures since bud initiation. Elevated temperature may contribute to unseasonal breaking of rosettes by interfering with their perception of hormonal signals that maintain dormancy. Heat stress has been demonstrated to release buds from dormancy (Shirazi and Fuchigami, 1995; Wisniewski et al., 1997). If buds break dormancy earlier than usual, they may be subject to spring frost damage which could eventually result in altered tree architecture.

Induction of rosettes was not treatment-specific; therefore, buds from any climatic treatment had a similar predisposal to rosetting if exposed to high temperature and low humidity. Outer surfaces of scales may shrink and cause the scales to reflex if they become less hydrated upon exposure to high temperature and low relative humidity, or, the outer surfaces may expand and cause scales to deflex upon a return to ambient temperature and humidity. The reversibility of induced rosetting implies that rosette formation is a physical process that occurs in response to high temperature and low humidity. Soil moisture fell below the wilting point in elevated temperature chambers at mid-summer (Johnson, M., pers. commun.), which coincided with the appearance of rosettes. This suggests that rosetting is related to soil moisture.

Lack of chilling may also play a role in unusual development (Guak et al., in press). Formation of

small buds on elongated stalks with brown, reduced needles may be a response to lack of chilling. In Corvallis, OR, the threshold for chilling degree days in Douglas fir is not met by dramatically low temperatures but is met by the moderate temperatures of November. A constant elevation in temperature of 4°C during this crucial time may be sufficient to trigger changes in bud development, as the apex would not receive the cue that it has met its chilling requirements (Lavender and Stafford, 1985), and would not be vernalized. The timing of the breaking of dormancy and shoot expansion may then be altered (Kozlowski et al., 1991).

Abnormal development involved not only morphological change, but also deviations from the synchronous bud break of spring. Unusually shaped buds often had correspondingly unusual phenologies characterized by unseasonal, delayed or absent bud break. Distinct phenological changes at elevated versus ambient temperatures were found in leader buds (Olszyk D., pers. commun). Therefore, abnormal and normal bud breaks were often temporally separated. Terminal branch buds that we examined exhibited differences in phenology comparable to those of the leader buds.

If unusual buds opened in spring, they often did not produce normal shoots. Clusters of small buds enclosed in a single set of scales had the appearance of a single large bud, but each bud in the cluster was too small to contain many needles. The appearance of a cluster in the place of a single bud suggests that the main shoot apex within the bud was damaged and/or that hormonal activity resulted in the growth of lateral bud primordia. However, we did not assess the interactions between hormonal activity and temperature in terms of their effects on bud morphogenesis.

Smaller buds produce shorter shoots than larger buds (Kozlowski et al., 1973). If all buds in a cluster produced new and surviving shoots, a tree would have a multiplicity of small shoots. When more shoots exist, there are more potential points and directions of growth which can change the branching pattern and architecture of a tree. Stems are modular, segmented, and produce reserve buds (Stafstrom, 1995). If buds that were destined to remain dormant are released from dormany and produce shoots, then the morphology, and possibly the function, of a tree will change. For example, all branching geometries become less efficient light-gatherers as they continue to branch (Niklas, 1992).

Trees from elevated temperature chambers tended to be shorter (Olszyk D., pers. commun.). Although trees from the terracosms were still in the sapling stage at harvest in summer, 1997, elevated temperature trees were recognizable by their oddly branched and stunted appearance. This contrasted with the typical excurrent and monopodial growth form found at ambient temperature. Continual disruption of a monopodial growth pattern can contribute to an overall sympodial growth form (Romberger et al., 1993).

In a related experiment, trees that are adapted to the Willamette Valley's present climate would be placed in a habitat with temperatures that are naturally 3–4°C higher than those of the Willamette Valley. Bud development at elevated temperature could then be assessed in a natural environment and compared to bud development in the chamber experiment. These trees could remain in place over several decades to assess possible changes in tree architecture wrought by changes in bud development. One caveat is that a habitat that is naturally 3–4°C higher in temperature may itself be subject to climatic change over time.

5. Conclusion

Morphogenesis of buds is influenced by elevated temperature, but not by elevated CO_2. Unusual morphological and phenological traits in buds at elevated temperature may be perpetuated as the trees continually attempt to counter the effects of constant exposure to elevated temperature. However, bud abnormalities may appear solely because it becomes more difficult for trees to produce normal buds with synchronous phenology at elevated temperatures. The temper-

ature increase imposed by the terracosms is an example of potential climatic change in Oregon. Morphological changes occurring with changing climates may influence the survival of Douglas-fir trees.

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References

Allen, G.S., Owens, J.N., 1972. The Life History of Douglas-fir. Information Canada, OT. Cat No.: Fo42-4972.

Beuker, E., 1994. Adaptation to climatic changes of the timing of bud burst in populations of *Pinus sylvestris* L. and *Picea abies* (L.) Karst. Tree Physiol. 14, 961–970.

Billington, H.L., Pelham, J., 1991. Genetic variation in the date of budburst in Scottish birch populations: implications for climate change. Funct. Ecol. 5, 403–409.

Cannell, M.G.R., Smith, R.I., 1986. Climatic warming, spring bud burst and frost damage on trees. J. Appl. Ecol. 23, 177–191.

Chen, Q.L., Lauzon, M., Derocher, A.E., Vierling, E., 1990. Accumulation, stability, and localization of a major chloroplast heat-shock protein. J. Cell Biol. 110, 1873–1883.

Chomba, B.M., Guy, R.D., Weger, H.G., 1993. Carbohydrate reserve accumulation and depletion in Engelmann spruce (*Picea engelmannii* Parry): effects of cold storage and prestorage CO_2 enrichment. Tree Physiol. 13, 351–364.

Downs, C.A., Heckathorn, S.A., Bryan, J.K., Coleman, J.S., 1998. The methionine-rich low-molecular-weight chloroplast heat-shock protein: evolutionary conservation and accumulation in relation to thermotolerance. Am. J. Bot. 85, 175–183.

Fielder, P., Owens, J.N., 1989. A comparative study of shoot and root development of interior and coastal Douglas-fir seedlings. Can. J. For. Res. 19, 539–549.

Fielder, P., Owens, J.N., 1992. Shoot-tip respiration of 1st-year interior and coastal Douglas-fir seedlings during bud development. Can. J. For. Res. 22, 765–768.

Gates, W.L., Henderson-Sellers, A., Boer, G.J., Folland, C.K., Kitoh, A., McAvaney, B.J., Semazzi, F., Smith, N., Weaver, A.J., Zeng, Q.-C., 1995. Climate models—evaluation. In: Houghton, J.T., Meira Filho, L.G., Callander, B.A., Harris, N., Kattenberg, A., Maskell, K. (Eds.), Climate Change 1995. Supplementary Report IPCC Scientific Assessment. Cambridge University Press, Cambridge, pp. 229–284.

Guak, S.-H., Olszyk, D.M., Fuchigami, L.H., Tingey, D.T., in press. Effects of elevated CO_2 and temperature on cold hardiness and bud burst in Douglas-fir (*Pseudotsuga menziesii*). Tree Physiol. (in press).

Jones, H.G., 1992. Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology, 2nd ed. Cambridge University Press, Cambridge.

Kozlowski, T.T., Torrie, J.H., Marshall, P.E., 1973. Predictability of shoot length from bud size in *Pinus resinosa* Ait. Can. J. For. Res. 3, 34–38.

Kozlowski, T.T., Kramer, P.J., Pallardy, S.G., 1991. The Physiological Ecology of Woody Plants. Academic Press, New York.

Krasowski, M.J., Owens, J.N., 1990. Seasonal changes in the apical zonation and ultrastructure of coastal Douglas-fir seedlings (*Pseudotsuga menziesii*). Am. J. Bot. 77, 245–260.

Kupila-Ahvenniemi, S., Pihakaski, S., Pikakaski, K., 1978. Wintertime changes in the ultrastructure and metabolism of the microsporangiate strobili of the Scots pine. Planta 144, 19–29.

Lavender, D.P., Stafford, S.G., 1985. Douglas-fir seedlings: some factors affecting chilling requirement, bud activity, and new foliage production. Can. J. For. Res. 15, 309–312.

MacDonald, J.E., Owens, J.N., 1993. Bud development in coastal Douglas-fir seedlings under controlled-environment conditions. Can. J. For. Res. 23, 1203–1212.

Matile, P., 1975. The Lytic Compartment of Plant Cells. Springer–Verlag, Berlin.

Murray, M.B., Smith, R.I., Leith, I.D., Fowler, D., Lee, H.S.J., Friend, A.D., Jarvis, P.G., 1994. Effects of elevated CO_2, nutrition and climatic warming on bud phenology in Sitka spruce (*Picea sitchensis*) and their impact on the risk of frost damage. Tree Physiol. 14, 691–706.

Myking, T., Heide, O.M., 1995. Dormancy release and chilling requirement of buds of latitudinal ecotypes of *Betula pendula* and *B. pubescens*. Tree Physiol. 15, 697–704.

Niklas, K.J., 1992. Plant Biomechanics: An Engineering Approach to Plant Form and Function, 1st ed. University of Chicago Press, Chicago, IL.

Owens, J.N., Molder, M., 1973. A study of DNA and mitotic activity in the vegetative apex of Douglas-fir during the annual growth cycle. Can. J. Bot. 51, 1395–1409.

Owens, J.N., Webber, J.E., Ross, S.D., Pharis, R.P., 1986. Interaction between gibberellin A4/7 and root-pruning on the reproductive and vegetative process in Douglas-fir. IV. Effects on lateral bud development. Can. J. For. Res. 16, 211–221.

Romberger, J.A., Hejnowicz, Z., Hill, J.F., 1993. Plant Structure: Function and Development. Springer–Verlag, Berlin.

Shirazi, A.M., Fuchigami, L.H., 1995. Effects of "near-lethal" stress on bud dormancy and stem cold hardiness in red-osier dogwood. Tree Physiol. 15, 275–279.

Stafstrom, J.P., 1995. Developmental Potential of Shoot Buds. In: Gartner, B.L. (Ed.), Plant Stems: Physiology and Functional Morphology. Academic Press, San Diego, CA, pp. 257–276.

Tingey, D.T., McVeety, B.D., Waschmann, R., Johnson, M.G., Phillips, D.L., Rygiewicz, P.T., Olszyk, D.M., 1996. A versatile sun-lit controlled-environment facility for studying plant and soil processes. J. Environ. Qual. 25, 614–625.

Vierling, E., 1991. The roles of heat shock proteins in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42, 579–620.

Wisniewski, M., Sauter, J., Fuchigami, L., Stephen, V., 1997. Effects of near-lethal heat stress on bud break, heat-shock proteins and ubiquitin in dormant poplar (*Populus nigra Charkowiensis* × *P. nigra incrassata*). Tree Physiol. 17, 453–460.