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ORIGINAL PAPER

Bud damage from controlled heat treatments in *Quercus garryana*

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Abstract *Quercus garryana* habitats are increasingly being managed with prescribed fire, but acorn dependent wildlife might be adversely affected if fires damage acorn crops. We examined one way that fire might affect subsequent acorn crops: through direct heating and damage of buds containing the following year's floral organs. We measured internal bud temperatures during controlled time and temperature treatments, described damage to heated buds at the tissue and cellular levels and quantified spring flowering to assess the consequences of the treatments. We found that internal bud temperature was logarithmically related to exposure time and linearly related to treatment temperature. Tissue damage was more common in bud scales, staminate and bud scale scar primordia than in leaf, pistillate, leaf axillary primordia and apical meristems. Damaged tissues were sequestered by cells with thickened cell walls. A 133°C treatment applied for 60 s produced minimal damage or mortality, but damage increased rapidly in hotter or longer treatments, culminating in 100% mortality at 273°C for 60 s. Our experiments account only for radiative, not convective heating, but suggest that fires might produce sublethal effects that affect flowering and acorn crops. *Q. garryana's* large buds possess an internal organ arrangement well suited to minimizing heat damage.

Keywords Buds· Floral prirnordia : Flowering' Heat damage . *Quercus garryana*

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Introduction

Fire is increasingly being used to manage understory and conifer competition to restore *Quercus garryana* Douglas ex. Hook.. var. *garryana* (Oregon white oak) stand structure. Oregon white oak is seral to Douglas-fir over much its range (Stein 1990; Thysell and Carey 2001). Young, rapidly growing Douglas-fir trees can surpass and overtop Oregon white oak trees, shading them out of the stand (Devine and Harrington 2006). For this reason, Oregon white oak woodlands were originally maintained with frequent fire by Native Americans (Agee 1993; Boyd 1999). Due to cessation of aboriginal burning and fire suppression, much Oregon white oak habitat has succeeded to conifer forest (Reed and Sugihara 1987; Agee 1993). Shrubs also increase over time fueling hotter, more damaging fires than occurred in historically common grassy understory (Thilenius 1968; Foster and Shaff 2003). Frequent underburning kills small conifers and decreases shrub dominance (Agee and Dunwiddie 1984; Agee 1993) while promoting Oregon white oak dominance.

Previous work suggests that fires reduce acorn crop size in the year following burning even when the trees survive (Peter and Harrington 2002). Many species of wildlife depend on acorns for food and could be affected by smaller

crops. Since white' oak buds contain the following year's reproductive organs, a better understanding of their heat tolerance would aid in developing guidelines for preserving acorn crops by reducing reproductive damage from burning. Here, we provide basic information as a first step toward such guidelines. Specifically, we examine the injurious effects of radiative heating on Oregon white oak buds.

Bud damage may result from lower temperatures over longer periods or higher temperatures over shorter periods of time (Martin et al. 1969; Plumb 1980). Because of their higher heat capacity, buds require more heating to be damaged than foliage, so buds often survive high levels of crown scorch. Temperatures of 45-55°C generally induce tissue damage in plants (Byram 1948; Levitt 1956; Larcher 1983; Seidel] 986; Colombo and Timmer 1992; Kolb and Robberecht 1996). Below 45°C, various physiologic effects have been observed including production of heat shock proteins and reduced winter bud dormancy, but mortality results only from long exposure times if at all (Vierling 1991; Shiratzi and Fuchigami 1995; Wisniewski et al. 1997).

Past work with trees has focused on seedling mortality (Snow]980; Methven 1971; Kayll 1968), or mature tree crown scorch (Byram 1948; van Wagner 1973; Peterson and Ryan 1986). These studies suggest high mortality will occur with one minute exposures to temperatures of 5l—65°C with at least some of the difference due to bud size. It is not clear, however, how different tissues, especially reproductive tissues in the bud are affected.

Even if buds survive, damage may occur. The first line of heat protection in oak buds is bud scales, but dormant tissues like oak buds also have higher thermal tolerance than active tissues (Kayll 1968). Thermal tolerance is associated with the accumulation of heat shock proteins (Vierling 1991), which accumulate to a high degree in *Quercus suber* bud tissues (PIa et al. 1998), and probably in buds of other species as well.

We examined the idea that different bud primordia and organs have different vulnerabilities to heat damage due to their positions in the bud by directly examining tissues and cellular responses to different radiative heat treatments. This study is unique in directly measuring affected tissue temperatures in a complex organ heated in air. These measurements were used to estimate bud damage in mature trees subjected to similar treatments to provide a basis for modeling bud damage and mortality.

Methods

Field methods

Four experiments were carried out involving the application of different timed heat treatments to buds (Peter 2006). Buds were selected from open grown sides of mature trees located at the Fort Lewis Military Installation in the state of Washington, USA. Separate sets of four trees were used for Experiments 1 and 4 and a third set for Experiments 2 and 3. All heat treatments (field and lab) were carried out with three digitally controlled Kerr® Automatic Electro-melt furnaces (Model F25725, Ken Corporation, 28200 Wick Rd, Romulus, MI, 48174-2600).

Experiment 1 was principally a laboratory experiment (see "Lab Methods" below), but the terminal bud clusters for the experiment were collected in early January from the lower and mid-portions of four tree canopies, bagged and refrigerated.

Experiment 2 assessed damage caused by field applied heat treatments with microscopic examinations of the interior of buds. Terminal bud clusters were treated in late August and early September until the total came to at least]5 buds per treatment per tree. There was nothing systematic or intentional about the sample placement in the tree canopy, so the sample was approximately random. An average treatment temperature was calculated from the furnace temperatures at the time of bud insertion and bud removal. The treatment time and average temperature over all the buds in a treatment are used in this article to identify each treatment (e.g. 60@133 is 60 s at 133°C). Thus the treatments were: 30@85, 30@107, 30@137, 60@54, 60@85, 60@111, 60@133 and an unheated control.

In Experiment 2 and later experiments, an oak branch wrapped in insulation behind the terminal cluster of buds to prevent heat girdling was inserted into the furnace in situ such that the insulative wrap sealed the opening. The treated branch tip remained on the tree for 3 weeks to allow killed tissues to dry and discolor and injured tissues to recover or die. Treated terminal bud clusters were then cut from the stem and preserved in formyl-acetic-alcohol for killing and fixation of tissues (Berlyn and Miksche 1976).

In Experiment 2, a total of 525 buds on four trees were treated with one of the 8 treatments. From this pool, 20 buds were randomly selected from each treatment to be processed for microscopic examination. Several buds damaged during processing or by insects were not used so results are based on 16-20 buds per group [Table S1 in Electronic supplementary material (ESM)].

Experiment 3 assessed sub-lethal damage caused by field applied heat treatments to the buds by counting staminate inflorescences, pistillate inflorescences and leaves produced in the following spring. Methods were the same as in Experiment 2 except for sample size and the evaluation of results. A total of 872 buds were treated so that each treatment had 105-117 buds from each of 4 trees (Table S1 in ESM). Thirteen percent of the buds fell off the

trees due to winter storm breakage, so the sample size was reduced to 762 buds. Buds that had died since treatment, but remained on the tree (22%) were noted and all organs were considered dead.

Experiment 4 assessed bud mortality from field applied heat treatments by examining buds several weeks after treatment.. This experiment had 24 treatments with 9-13 randomly selected buds from each treatment over 4 trees for a total of 279 sampled buds (Table Sl in ESM). The heat treatment target temperatures were: 60, 100, 150, 200, 250 and 300°C.. Treatment times were 15, 30, 60, and 90 s. The buds were treated on 11 September 2002 and harvested from 30 September 2002 to 1 October 2002. Buds were evaluated by cutting the bud open; dead buds were dry and brown and live buds green and moist inside, The percent of dead tissue was estimated for each of the 20 buds containing both live and dead tissue.

Lab methods

In Experiment 1, relationships for predicting internal bud temperatures given treatment time, temperature and bud size were derived from results of heat treatments applied to buds in the lab. Each sample bud was weighed, and measured for length and width. A dissecting needle was used to create a hole to insert a 32 gauge (0.2 mm) type K thermocouple through the base of each bud into the bud center.. The thermocouple wire was wrapped with insulation and the internal bud temperature prior to treatment was recorded (equilibrated at room temperature to 20-30°C). The insulated bud was placed over the furnace opening such that the bud protruded into the furnace and the insulation sealed the opening. Internal bud temperatures were monitored under the heating regimes of 40-600°C from 10 to 180 s for 9-12 randomly selected buds. Only three buds were used for treatments that produced combustion.

Prior to dehydration and embedding of the buds in Experiment 2, the tips of the bud scales were cut away to permit better infiltration of processing fluids. The buds were dehydrated using standard histologic techniques, embedded in paraffin blocks, longitudinally sectioned at 10 μm thickness with a rotary microtome, fixed to glass slides, and stained with 0.013% aqueous safranin and 0.5% fast green in 95% ethyl alcohol (Berlyn and Miksche 1976). Safranin stains tissues red, but fast green removes it from most tissues except for chromosomes, and lignified, cutinized or suberized cell walls (Berlyn and Miksche 1976). This stain combination differentiated live from dead tissue and important cell wall characteristics.

At least five slides with five serial sections each, distributed through the inner 2/3 of each bud were observed. Damage observations were made for the shoot apex, pistillate primordia, leaf axial primordia, leaf primordia, scale axial primordia, soft (inner) scales, and hard (outer) scales (Fig. S1 in ESM). Observations included: (1) Presence or absence of damage on a bud basis for each organ type from which the percentage of buds with any kind of damage was calculated. (2) The number of organs with or without any kind of damage was recorded so the percent of damaged organs could be calculated. For soft scales, layers of scales in the two central-most sections were counted and the larger count was used. (3) The shortest distance from the outside of the bud to the first live tissue was recorded in mm. If the bud was killed to the center, the distance to the center was recorded.

Analysis

Statistical analysis was done with SAS version 8.01 software (2000) (significance declared at $P \leq 0.05$). Transformations were sought for data that deviated from assumptions of normality or equal error Valiance for use in parametric analysis, otherwise non-parametric tests were used (Zar 1999). Transformations were sometimes used to linearize the response variable for regression analysis.

In Experiment 1, treatment times of 10-120 s were used in multiple regression models to bracket the experimental field treatments and model internal bud temperatures below 100°C. Internal bud temperature was the response variable and treatment time (s), treatment temperature, starting (ambient) temperature, and bud weight, length or width were independent variables.

Null hypotheses tested for in Experiment 2 were: (1) there is no difference by treatment in the frequency of damage in the different organ types (tested with KW ANOVA and Dunn tests). (2) There is no difference by organ type in the frequency of damage in the different treatments (tested with KW ANOVA and Dunn tests). (3) Organ damage from the hottest treatment (60@133) did not differ from the control (tested with Students t test).

The relationship of mean percent damage to treatment temperature for 60 s treatments was investigated with stepwise multiple regression (entry and removal limits were $P = 0.05$). Bud diameter, treatment temperature, and ambient temperature at treatment time were independent variables, The percent damaged individual primordia types per bud, percent damaged inner (apical meristem, leaves, pistillate and leaf axial meristems) or outer group primordia (staminate and scale axial primordia) per bud, and the distance to live tissue from the outside of the bud (mm) were response variables.

Linear regression analysis was used to explore relationships between mean treatment damage indexes for 60-s treatments and the calculated internal bud temperature

Table 1 Internal mean temperature (°C) from Experiment 1 time/temperature treatments

Time (s)	Treatment Temperature											
	40	**60**	**80**	**100**	**120**	**150**	**200**	**250**	**300**	**400**	**500**	**600**
10	**23.9**	**27.6**	**31.7**	**31.2**	**40.4**	**40.5**	**47.1**	**55.4**	**72.4**	**96.1**	***107.4***	***123.8***
15	**25.0**	**30.0**	**37.9**	**38.3**	**40.1**	**48.8**	**57.3**	**66.2**	**94.4**	107.0	***121.1***	184.3
30	**28.4**	**34.1**	**45.8**	**49.1**	**56.0**	**64.6**	**77.6**	**96.8**	106.3	***133.6***	234.5	*301.3*
60	**30.0**	**39.0**	**50.5**	**62.3**	**67.2**	**83.2**	**97.9**	117.4	139.4	274.5	*498.5*	
90	**30.3**	**43.4**	**59.2**	**68.4**	**77.6**	**87.5**	102.2	123.9	***191.9***	*382.7*		
120	**32.2**	**46.9**	**58.9**	**72.0**	**84.2**	**95.1**	116.2	147.2	248.0			
150	34.0	48.6	61.9	75.7	80.3	100.3	125.2	157.4	276.3			
180	33.2	51.9	63.9	76.4	84.9	104.8	144.2	***215.1***				

Bold italic text 1-50% charring, *underlined text* 50-99% charring, *italics* only combustion. Values in bold face type (except bold italic) were treatments used for internal bud temperature relationships

(derived in Experiment 1). Response variables included the distance to live tissue, and the percent damaged primordia by type, and by inner and outer primordia groups.

For Experiment 3, the null hypotheses that (1) the number of organs produced in the spring was not different between heat treatments, and (2) the distribution of dead buds was not different between treatments, were investigated with ANOV A.

In Experiment 4, a spline mesh plot of the raw data using x = time, y = temperature, z = % mortality suggested a non-linear relationship. A logistic relationship was fit to the data describing the arcsine of the square root of percent mortality as a function of treatment time and treatment temperature and evaluated with an F test for significance and R^2 for strength.

Results

Experiment 1: internal bud temperature response

From the laboratory heat treatment results (Table 1), four linear regression models were produced that describe the way buds heat up, incorporating treatment time, temperature and the starting or ambient temperature (Table 2). Internal bud temperature was linearly related to treatment temperature, but logarithmically related to exposure time. Models 1-3 each incorporate a different size variable (bud diameter, length or weight), all of which performed equally well and were inversely related to the internal bud temperature. Model 4 (Table 2) performed almost as well as models 1-3, but without a size variable. Model 4 was selected to model internal bud temperature for Experiment 2 since the preserved bud dimensions in Experiment 2 may differ from fresh bud dimensions.

Experiment 2: microscopic examination of primordia damage from heat treatments

Description of heat damage

Heat damage was usually located in upper or outer parts of the bud. Light to moderate damage appeared as thickened, safranin-stained cell walls suggesting a cell wall hardening response with lignin or suberin. In heavily damaged areas, similar safranin-stained cells compartmentalized predominantly necrotic tissues (Fig. 1). Abscission or sequestration zones sometimes formed an irregular layer of thick walled, safranin-stained cells adjacent to or intercalated with necrotic tissues. Subtending this layer was a zone of dead or dying cells without safranin staining, which was subtended by live cells that showed a limited amount of cell wall thickening and safranin staining (Fig. 1). Damage to hard scales was not obvious, because much of the tissue was already hardened. In lightly damaged soft scales, the outer cells typically stained with safranin, except near the base where abscission zones sometimes formed across the scale (Fig. 2). These apparently suberized zones may form anywhere, but the typical kill pattern suggested heat was conducted down the bud tissues from the tip or inward from the side. Scales insulated deeper live tissues from this kind of heat movement.

Damage to leaf and staminate primordia was manifested by safranin staining and by tissue collapse in heavily damaged meristematic tissues producing regions of dense and discolored tissue (Fig. 1). Damage to the apical meristem, pistillate primordia and leaf axial buds was usually manifested as tissue collapse as opposed to cell wall thickening and safranin staining suggesting greater heat sensitivity.

Bud scale scar primordia are complex organs with both meristematic and non-meristernatic tissues (Fontaine et al. 1998; Peter 2006). The most frequent kind of damage

Table 2 Empirical models for internal bud temperature and beta values associated with each term from Experiment 1

	Time (s)	Model *P*	Model r^2	Model (where $\log_{10}$ of the ending internal bud temperature in °C =)	*P* inter.	*P* var. 1	*P* log (temp)	*P* ambient	*P* size
1	10–120	<0.01	0.90	−0.262 + 0.0024**time* + 0.556**logtemp* + 0.441**logambient* + 0.423**invdiam*	<0.01	<0.01	<0.01	<0.01	<0.01
2	10–120	<0.01	0.90	−0.160 + 0.0024**time* + 0.569**logtemp* + 0.507**logambient* − 0.018**length*	0.03	<0.01	<0.01	<0.01	<0.01
3	10–120	<0.01	0.90	−0.264 + 0.0024**time* + 0.568**logtemp* + 0.487**logambient* + 0.0011**invweight*	<0.01	<0.01	<0.01	<0.01	<0.01
4	10–120	<0.01	0.88	−0.395 + 0.0024**time* + 0.554**logtemp* + 0.632**logambient*	<0.01	<0.01	<0.01		<0.01

These relationships were derived from data with treatment times of 10–120 s that produced mean treatment internal bud temperatures at or below 100°C

logtemp log 10 of the treatment temperature in °C, *logambient* log 10 of the starting (ambient) bud temperature in °C, *invdiam* inverse of the mean of 2 bud diam. meas.(mm) at 90° to each other, *time* duration of the treatment in seconds, *length* bud length in mm, *invweight* inverse of the bud weight in gm

observed was cell wall thickening and safranin staining of the soft scales that enclose the meristematic tissue. In two cases, when the apical meristem of the bud was dead and the bud scale scar primordia had not been damaged, the bud scale scar primordia were much enlarged.

Assessment of damage per bud at the organ level (presence or absence of damage)

Soft scales tended to have the highest levels of damage among organ groups (Table 3). According to Dunn tests, in the 60@85 treatment damage was higher in soft scales than in the leaf axial buds, and in the 60@133 treatment, damage was more frequent in soft scales and staminate primordia than in the apical meristem, pistillate primordia, leaf axial buds or leaf primordia. In direct comparisons of the 60@133 and control treatments, only the frequency of damage to staminate primordia was not significantly different (Table 3).

Assessment of damage at the organ group level

The results related in the previous section and Table 3 suggest three organ groups distinguished by ANOVA and Dunn tests over all treatments for presence of damage. The apical meristem, pistillate primordia, leaf primordia and leaf axial buds formed an inner group of centrally located, less frequently damaged organs. Staminate primordia and vegetative bud primordia lie directly under soft scales and thus formed an outer group of more frequently damaged organs. Soft scales formed a third outermost and most frequently damaged group. These three groups were analyzed separately, but iri parallel fashion to the analysis of individual organ types. Thus, two new hypotheses were tested with Kruskal-Wallis ANOVA and Dunn tests: (1) there is no difference by treatment in the percent of damaged primordia observed across the different organ groups, and (2) there is no difference by organ group in the percent of damaged primordia observed across the different heat treatments.

Soft scales had significantly more damage than the inner group in all treatments except the control and more than the outer group over all treatments combined (Table 4). The outer group had more damage than the inner group in all 60 s treatments, but not in any 30 s treatment or the control..

Relationship of mean damage to treatment and internal bud temperature

The relative importance of damage factors was investigated with stepwise regression in the *60-s* treatments for each primordia type, organ group, and the distance to live tissue in the bud. No models at the individual bud primordia level were significant.. Four models showed treatment effects

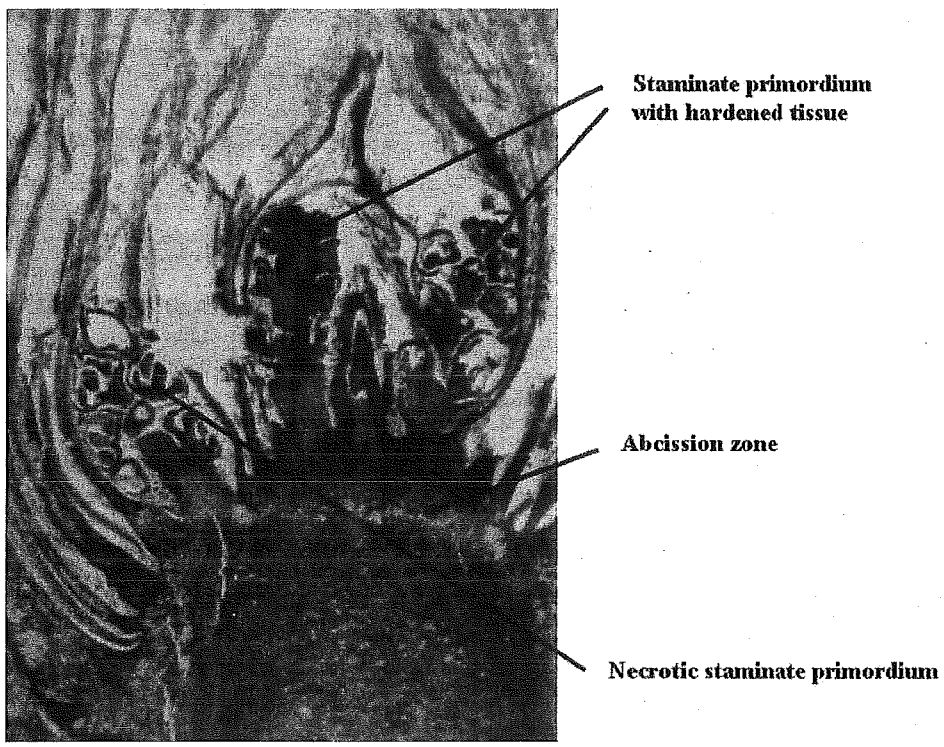

Fig. 1 A heat-killed bud treated for 60 s at 133°C. The staminate primordium on the left was killed outright. Note the shrunken tissues (cell collapse). The two staminate primordia in center and right survived for a period and responded with heavy cell wall thickening (stained red with safranin). An abscission or sequestration zone is forming below the primordia in the pith. Apparently damage was too severe to repair and the entire bud is being sealed off

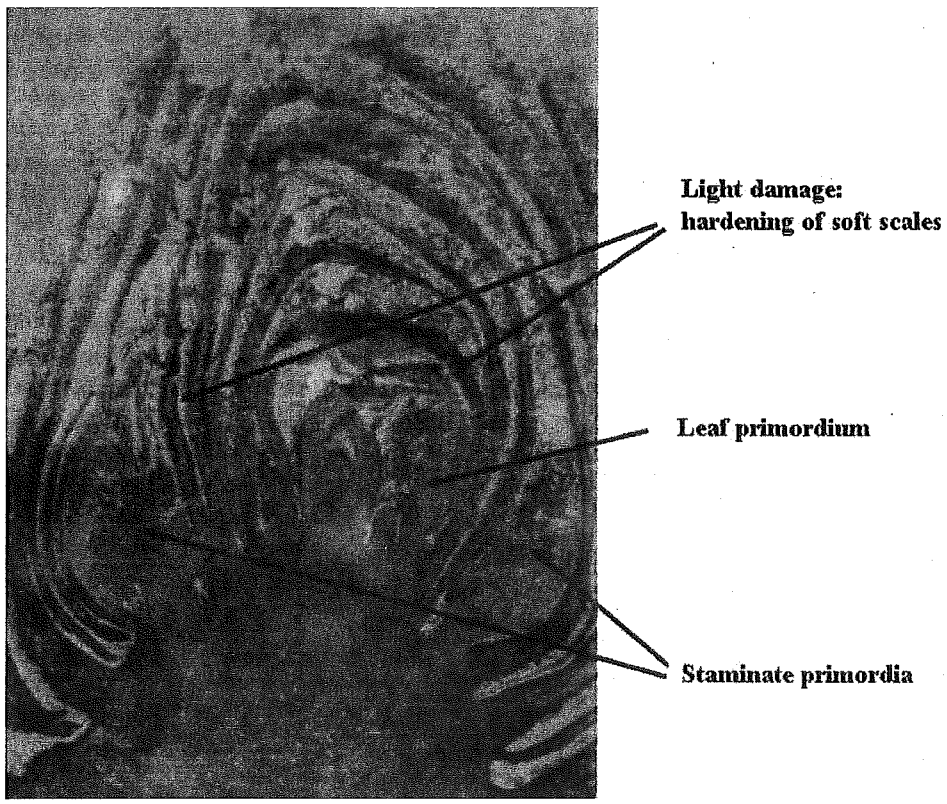

Fig. 2 Light damage manifested as hardened soft scales (safranin staining). This bud was treated at 60@133°C. It appears that heat penetrated in from the sides and down from the top to the level of the staminate and leaf primordia

with a data set of mean values by treatment, and treatment temperature was the only significant explanatory variable (Table 5, Models 1–4).

Relationships of bud damage to calculated internal bud temperature were also investigated with linear regression for the 60-s treatments (Table 5, Models 5-6). Internal bud

Table 3 Percent buds from Experiment 2 with damage of each organ type

Organ	All	Control	30@85	30@107	30@137	60@54	60@85	60@111	60@133	KW *P*	KW *P* 60@133 versus control
All		4.7a	26.7bc	20.7ab	23.4abc	22.4abc	27.5bc	22.4abc	39.2c	<0.01	
Apical mer.	8.5a	0.0	33.3	0.0	10.0	10.0	0.0	7.7	7.7	0.21	0.01
Pistillate	8.0a	0.0	14.3	12.5	16.7	0.0	0.0	0.0	12.5	0.90	0.02
Leaf	10.2a	0.0	7.7	17.6	10.5	6.3	14.3	7.1	15.8	0.77	0.03
Leaf axial bud	5.8a	0.0	0.0	0.0	7.7	0.0	0.0	12.5	20.0	0.24	0.02
Scale scar bud	31.1b	0.0	40.0	16.7	31.3	33.3	44.4	33.3	43.8	0.17	<0.01
Staminate	31.7b	14.3	25.0	23.1	20.0	36.4	31.3	35.7	72.7	0.10	0.07
Soft scale	47.1c	12.5a	45.0ab	45.0ab	50.0ab	44.4ab	52.6ab	35.0ab	85.0b	<0.01	0.04
KW *P*	<0.01	0.25	0.08	0.06	0.04	0.02	0.04	0.17	<0.01		

Also shown are Kruskal-Wallis ANOYA *P* values and Dunn test results ($P = 0.05$). Letters by row (except in the "all" column) indicate significantly different groups among the eight treatments. Letters in the "all" column indicate significantly different groups among the seven organ types, suggesting three distinct organ groups

Table 4 Percent of damaged primordia by organ group and treatment

Treatment	Inner group		Outer group		Soft scales		Dunn test	KW *P*
	n	Damage (%)	*n*	Damage (%)	*n*	Damage (%)		
Control	44	0	26	8a	16	13a	a, a, a	0.09
30@85	39	13	27	33ab	20	45ab	a, a, b	0.02
30@107	41	10	31	19ab	20	45ab	a, ab, b	0.01
30@137	48	10	26	27ab	20	50ab	a, ab, b	<0.01
60@54	41	5	26	35ab	18	44ab	a, b, b	<0.01
60@85	38	5	34	38ab	19	53ab	a, b, b	<0.01
60@111	39	8	26	35ab	20	35a	a, b, b	0.01
60@133	55	15	27	56b	20	85b	a, b, b	<0.01
Overall	345	8	223	31	153	47	a, b, c	<0.01
KW *P*		0.24		0.02		<0.01		

Kruskal-Wallis ANOYA *P* values and Dunn test results ($P = 0.05$) comparing damage within each treatment are indicated to the right. Dunn test results comparing treatments within groups are indicated by letters in the columns of data where significant differences were found

temperatures were calculated with model 4 (Table 2) for each bud. In the 60-s treatments, the relationships for the percent damaged inner and outer group primordia per bud were significant, but not with percent damaged soft scales.

Experiment 3: damage evaluation from spring organ emergence

Neither the number of organs emerging from each bud in the spring nor the number of buds that died over the winter differed between the eight treatments suggesting that these treatments had little lasting effect..

Experiment 4: assessment of bud mortality

The relationship of percent killed buds to treatment time and temperature followed a logistic curve (Fig. 3). This model predicts that the most severe treatments used in Experiments 2 and 3 were sublethal, although the 60@ 133 treatment lies close to the mortality threshold (Fig. 3).

Discussion

Bud damage ranged from massive to spotty, and was sometimes mixed with apparently healthy tissues, but was only detectable by observing the interior of the bud with prepared specimens under a microscope. A zone of secondarily thickened and apparently suberized cells compartmentalized damaged tissue from the rest of the bud. Heat shock proteins, which are associated with control and repair of heat damage, and with the suberization process, are known to accumulate in bud tissues (PIa et al. 1998) suggesting a mechanism for this response. Heat shock proteins can be produced within minutes of heat exposure (Sachs and Ho 1986) to stabilize or repair

Table 5 Models 1-4: stepwise regression models for mean damage by organ groups, live distance and total damaged bud tissue in the 60 s treatments

	Model	*P*	r^2	*P* intercept
1	Livedist = 0.59087 + 0.00248(*temp*)	0.05	0.76	<0.01
2	pd soft scales = −0.013 + 0.00202(*temp*)	0.01	0.92	0.71
3	pd outgroup = 0.02583 + 0.00212(*temp*)	0.04	0.80	0.67
4	pd innergroup = −0.0340 + 0.00133(*temp*)	0.04	0.81	0.38
5	pd innergroup = −0.0708 + 0.0034* *intemp*	0.05	0.78	0.07
6	pd outergroup = −0.031 + 0.0052* *intemp*	0.05	0.78	0.77

Models 5-6 relate inner and outer organ group damage to internal bud temperature for the 60 second treatments

livedist distance (mm) from the outside of the bud to the first live tissue, *pd soft scales* percent damaged soft scales, *pd males* percent damaged staminate primordia, *pd outgroup* percent damage outer group primordia, *pd innergroup* percent damaged inner group primordia, temp treatment. temperature, *intemp* calculated internal bud temperature

proteins and enzymes (Vierling 1991). Sequestration of damaged tissue suggests the treatments administered in Experiment 2 were not lethal to buds, which was borne out by the low bud mortality associated with similar treatments in Experiments 3 and 4.

Colombo and Timmer (1992) divided heat damage to *Picea mariana* foliage into direct damage (manifested within minutes of exposure) and indirect damage (manifested over days to weeks). Direct damage in oak buds is typified by tissue necrosis. Undamaged tissue incidentally sequestered with directly damaged tissue by the formation of a suberization or abscission zone is probably what Colombo and Timmer (1992) referred to as indirect damage, because the live tissues were gradually cut off and died as the zone formed. Thus, Colombo and Timmer's (1992) indirect damage might be an abscission or sequestration response. In heavily damaged oak buds, the abscission or sequestration zone formed at the bud-stem contact, cutting the entire bud off. Thus the stimulus determining where in a damaged organ this zone forms is of interest.

Colombo and Timmer (1992) noted that both kinds of damage increased from low to high values over small gradients of temperature. This has been observed by others too (Methven 1971), and appears to be true for oak buds. The slope in the heat-mortality response surface above the 1% level became very steep (Fig. 3).

Bud size is important to bud survival in fires (Byram 1948; Peterson and Ryan 1986) and Q. *garryana* buds are larger than Douglas-fir buds-the principal competitor for space in much of Q. *garryana's* range, suggesting one reason why oaks might be favored in an underburning fire regime. Byram's (1948) finding that the susceptibility of a bud to heat damage is proportional to the reciprocal of the bud diameter was born out by this study (Modell, Table 2). Model 1 (Table 5) suggests that depth of damaged tissue depends on treatment temperature-not bud size. Thus, larger buds should experience proportionately

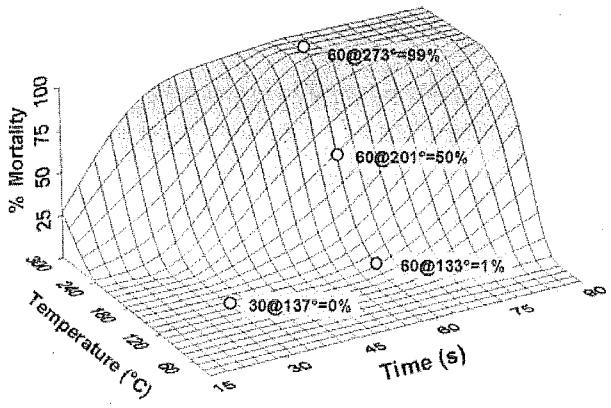

Fig. 3 Response surface from Experiment 4 showing the relationship of bud mortality to temperature and time. Also shown are the locations of the highest 30 and 60 s heat treatments from Experiment 2 and two other calculated mortality values. % mortality = 1/[1 + *e* - (-22.909 + 0.1651 x time + 0.0648 x temperature)] 'p = < 0.01, ' r^2. = 0.87

less tissue damage than smaller buds as predicted by Byram (1948) and the relationships of heat penetration from Experiment 1.

Several models were proposed to calculate internal bud temperature from radiative heating. Internal bud temperature represents the minimum temperature that all bud tissues experienced in the treatment and the highest temperature that the innermost tissues experienced. Models 1-3 (Table 2) each incorporated a different bud size measurement-all of which worked about equally well. Interestingly, these models did not perform substantially better than a similar model without a bud size variable. However, if buds were drawn from a different population of trees with different bud size or shape characteristics, there might be more advantage to models with size variables.

The bud heating experiments showed that oak buds are well insulated, but, comparisons with other species are

difficult since other studies have not evaluated buds in this way. Kayll (1968) found that 60 s treatments at 51-65°C killed several species of conifer seedlings. If it is assumed that tissue damage is initiated at approximately 50°C (Levitt 1956; Larcher 1983; Seidel 1986; Colombo and Timmer 1992; Kolb and Robberecht 1996), then oak bud damage should begin in inner primordia with treatments of 10 s at 179°C, 30 s at 146°C, 60 s at 109°C, or 120 s at 60°C (Table 2, Model 4). In fact, only 15% of buds in Experiment 2 had inner organ damage with the 60@133 treatment (internal temperature of 56°C according to Table 2, Model 4) and only 5% of all buds were killed. This is probably because these treatments only brought the center of the bud to the threshold of damage production- more time would be required for more damage. In an actual fire, however, bud heating occurs by both radiative and convective heating, so might occur more rapidly due to convective reduction of the boundary layer around the bud. Thus, our experiments capture only one of the factors in play during a fire.

None of the 60-s treatments produced detectable spring damage in Experiment 3. Winter damage may have partially obscured the results (13% of the buds were lost), but it is likely that much of the heat induced damage was repairable or did not prevent undamaged organs from functioning. Presumably at bud burst, healthy tissues expanded normally and damaged sequestered tissues were sloughed. Closer inspection of leaves and flowers might have revealed an asymmetry or necrotic tissues, but pistillate inflorescences expand 40-50 times from their primordial size, so a small amount of necrotic tissue would not be highly visible. It is not known if the organ regenerates damaged tissue or only expands what is left. In two cases, microscopic evaluation revealed that scale scar buds enlarged, apparently assuming some of the function of killed apical meristems, suggesting a redundant mitigation mechanism. Reproductive organs were never observed in scale scar buds, and reproductive organs were usually (but not always) present in spring buds.

Soft scales were the most vulnerable organ in the bud, but it is not clear what consequence, if any, results from damaging them. There is probably little difference in the functional purpose of hard and soft scales-both protect the meristematic tissues, and whether they are alive or dead may matter little in this regard.

Floral arrangements are quite variable in the plant world-many combinations are possible (staminate flowers above pistillate flowers, separate sexes or mixed sexes, etc.). Flower structure has traditionally been interpreted in terms of pollination mechanism.. However, for a large perennial plant that is frequently underburned, it may be important to have the organs arranged such that those that are involved with photosynthesis or require considerable reproductive investment are well protected. This is the arrangement found in oak buds. Hard, then soft scales densely covered in trichomes are the first lines of heat defense. Staminate inflorescences are located just inside the bud scales in the next most vulnerable location possibly because there are fewer consequences to their loss than the loss of pistillate primordia since pollination can be achieved from other trees. Scale axial buds can also be lost with little harmful effect as many of them accumulate in the bark as epicormic buds over periods of years. If leaf primordia are lost, leaves might be replaced by epicormic sprouting allowing the tree to survive, but even if pistillate primordia survived, the cost in resources to produce new foliage may forfeit the tree's ability to carry an acorn crop. Frequent loss of pistillate primordia precludes reproduction since pistillate primordia are not present in epicormic buds.

Q. *garryana* buds are well adapted to withstand surface fires. The buds are large and well insulated with pubescent scales that wrap around the foliar and reproductive organs and extend above them. There is an internal arrangement that favors foliage and pistillate inflorescences over the more expendable staminate inflorescences. Buds with the most foliage and pistillate inflorescences tend to be bom in the upper canopy away from the heat of fire (peter 2006). It is possible that this suite of characteristics is partly an evolved response to a long association of this species with fire prone habitats.

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