

Mapping of Quantitative Trait Loci Controlling Adaptive Traits in Coastal Douglas Fir. III. Quantitative Trait Loci-by-Environment Interactions

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ABSTRACT

Quantitative trait loci (QTL) were mapped in the woody perennial Douglas fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco) for complex traits controlling the timing of growth initiation and growth cessation. QTL were estimated under controlled environmental conditions to identify QTL interactions with photoperiod, moisture stress, winter chilling, and spring temperatures. A three-generation mapping population of 460 cloned progeny was used for genetic mapping and phenotypic evaluations. An all-marker interval mapping method was used for scanning the genome for the presence of QTL and single-factor ANOVA was used for estimating QTL-by-environment interactions. A modest number of QTL were detected per trait, with individual QTL explaining up to 9.5% of the phenotypic variation. Two QTL-by-treatment interactions were found for growth initiation, whereas several QTL-by-treatment interactions were detected among growth cessation traits. This is the first report of QTL interactions with specific environmental signals in forest trees and will assist in the identification of candidate genes controlling these important adaptive traits in perennial plants.

DOUGLAS fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco) is the most ecologically and economically important forest tree species in the Pacific Northwest region of the United States and Canada. Like most temperate woody plants, Douglas fir is well adapted to strong seasonal cycles and attendant environmental signals. Environmental signals such as photoperiodicity, temperature, and winter chilling affect dormancy release, cell cycling, and elongation of meristematic tissue in the spring (CAMPBELL and SUGANO 1975; CAMPBELL and SORESENSEN 1978; STEINER 1979; BIGRAS and D'Aoust 1993; HÄNNINEN 1995). The signals that modulate the timing of spring bud flush are, predominately, winter chilling and spring temperatures. These signals have a synergistic effect on the release of dormancy in the spring, providing the adaptive plasticity needed to survive yearly climatic fluctuations. Winter chilling is prolonged exposure to low temperatures and the winter chill requirement is an elegant adaptation of a broad spectrum of woody plants (SORENSEN 1983), which enables them to "sense" when winter is over, so that growth resumption can occur with minimal risk of frost damage. Winter temperatures ranging from -1° to 12° are capa-

ble of releasing dormancy, with the optimal temperature being $\sim 4.5^{\circ}$ (RITCHIE 1984). In a "normal" North-western winter, Douglas fir is exposed to >2000 hr of winter chill (RITCHIE 1984). The ambient temperature of the air and soil in the spring is also important in the timing of dormancy release and rate of cell expansion in the spring. In locations or years in which the winter chilling requirement is unsatisfied, warm spring temperatures and extended day length compensate, initiating the release of dormancy (CAMPBELL and SUGANO 1975).

Photoperiod, temperature, and moisture stress affect growth cessation and hardening in the fall (ERIKSSON *et al.* 1978; MACDONALD and OWENS 1993; PARTANEN and BEUKER 1999; REPO *et al.* 2000). The key environmental signal governing timing of bud set, for most temperate woody plants, is photoperiod (VEGIS 1964; PERRY 1971). In summer, as day length begins to decrease, the shoot responds by forming a resting bud. Coastal Douglas fir, however, is also responsive to water stress (LAVENDER *et al.* 1968), reflecting its adaptation to prolonged summer droughts typical of the climate in which it grows. As soil moisture declines in mid- to late summer, shoot elongation is suspended and buds begin to form. If young Douglas fir seedlings are exposed to an increase in soil moisture during late summer, they will flush again and produce a new shoot called a "lammas" shoot, which will eventually form an overwintering bud but is less cold hardy than a normal shoot. Growth from a lammas bud is referred to as "free growth" and considered distinct from growth from the

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overwintering bud, which is referred to as “predetermined growth” (KAYA *et al.* 1994). The timing of growth initiation and cessation is associated with susceptibility to late spring and early fall frosts, respectively, and is therefore important to the long-term survival and vigor of the tree.

Controlled environments have been used to estimate the effects of winter chilling, spring heat, photoperiod, and moisture stress on Douglas fir bud phenology (CAMPBELL and SUGANO 1975; KAYA 1992; MACDONALD and OWENS 1993). CAMPBELL and SUGANO (1975) concluded that the effects of spring flushing temperature and winter chill sum on spring bud flush were highly variable, depending upon the provenance being tested. The effect of photoperiod was significant on the timing of spring bud flush only when the winter chilling requirement was not met. In reference to bud set, MACDONALD and OWENS (1993) reported that the effect of moisture on bud scale initiation was dependent on photoperiod and secondarily on temperature. Moisture stress decreased mitotic activity, reduced the size of the apical dome and the number of bud leaf primordia, and slowed the rate of bud formation. These controlled-environment experiments were valuable for multivariate dissection of environmental factors affecting bud phenology traits in Douglas fir. However, information about the number of genes controlling these traits, the degree of their individual effects, and their interactions with environmental signals could not be determined.

Growth-rhythm traits in temperate trees are typically under moderate to strong genetic control. Narrow-sense heritabilities for bud flush in Douglas fir range from 0.44 to 0.95 (reviewed in JERMSTAD *et al.* 2001a) while heritabilities for bud set range from 0.30 for seedlings to >0.80 for saplings (LI and ADAMS 1993; O'NEILL *et al.* 2001). Until recently, the inheritance of quantitative traits was studied solely by measurements on the phenotype. The development of genetic maps has now made it possible to obtain knowledge about the number of genes responsible for quantitative traits, their location within the genome, and their individual effects. Over the last two decades, quantitative trait loci (QTL) mapping of complex traits has become common in agricultural research (EDWARDS *et al.* 1987; LANDER and BOTSTEIN 1989; LIPPMAN and TANKSLEY 2001; GEORGIADY *et al.* 2002). The number of reports of QTL mapping in forest trees for economically important traits, such as growth, phenology, and development, is increasing (GROOVER *et al.* 1994; BRADSHAW and STETTLER 1995; FREWEN *et al.* 2000; HURME *et al.* 2000; SEWELL *et al.* 2000, 2002; JERMSTAD *et al.* 2001a). Nonetheless, all of these experiments were conducted under field conditions with no control over influential environmental signals such as temperature, moisture, and photoperiod. The presence of QTL-by-environment ($QTL \times E$) interactions in Douglas fir (JERMSTAD *et al.* 2001a) suggests that different suites of genes may influence growth

rhythm, depending on the environment in which the trees are grown. Therefore, it is important to know how environmental signals interact with QTL that control growth rhythm in trees.

$QTL \times E$ interactions have been mapped in angiosperm species using recombinant inbred lines (RILs), near-isogenic lines (NILs), and doubled haploids (DH). Using these materials, different treatments can be applied to replicated progeny in controlled environments (PATERSON *et al.* 1991; STUBER *et al.* 1992; JANSEN *et al.* 1995; BOREVITZ *et al.* 2002; RAUH *et al.* 2002). Thus far, $QTL \times E$ interactions have been identified in only two forest tree species, poplar (WU *et al.* 1998) and Douglas fir (JERMSTAD *et al.* 2001a). In both studies, the progeny were clonally propagated by rooted cuttings, which were then planted at multiple test sites where height or phenology traits were measured. To date, $QTL \times E$ interactions have not been estimated in forest trees under experimental application of specific environmental treatments. We have designed two experiments to estimate $QTL \times E$ interactions for growth initiation and growth cessation traits by subjecting cloned progeny from a full-sib family to different levels of key environmental signals in controlled environments. In the growth initiation (bud flush) experiment, QTL interacting with winter chill and spring flushing temperatures were mapped. In the growth cessation experiment (bud set and related growth-rhythm traits), QTL interacting with day length and moisture stress were mapped. A third experiment was performed, measuring bud flush in replicated field tests to evaluate repeated detection of QTL in multiple environments and to also map QTL interacting with site.

To facilitate these experiments, a new mapping population (cohort) was derived from the same parents and grandparents that were used in previous QTL studies (JERMSTAD *et al.* 2001a,b) and clonally replicated through rooted cuttings. The use of clones provided precision for estimating phenotypic values and enabled the estimation of QTL for multiple treatments using a single mapping population. More than 9000 plants were evaluated in this study.

MATERIALS AND METHODS

Plant materials: A clonal mapping population (cohort 2) was generated from the same parents of an earlier QTL mapping population (cohort 1; Figure 1A; JERMSTAD *et al.* 2001a,b). Seeds were sown in a greenhouse in early 1997 and grown continuously for 9 months to produce stock for rooted cutting production. Cuttings were harvested from 474 stock plants in February 1998, placed in 50:50 peat:perlite rooting mix in Multipot 104 trays, and then maintained under operational rooted cutting conditions (RITCHIE 1993). Thirty-two cuttings per clone were propagated and the stock plants were discarded. In May, rooting was assessed and rooted cuttings (hereafter “cuttings”) from 460 clones were transplanted to Styro-45 containers. Each container contained one cutting from each of 45 clones (located at random). Twenty complete

sets (11 Styro-45 containers per set) were created and placed in an outdoor growing area with overhead fertigation for the remainder of the growing season. The remaining cuttings were transplanted to nursery holding beds, or permanent clone banks, or were sacrificed for genotyping purposes.

In December 1998, all cuttings were moved to another outdoor growing area and randomly assigned, by set, to one of two experiments. The growth initiation and growth cessation experiments were performed in controlled environments in 1998–1999. Field test sites near Longview, Washington, and Springfield, Oregon, were established ($n > 400$) in 2000, using cuttings from the growth initiation and growth cessation experiment. An incomplete randomized block design was used with four blocks per site, and clones were planted in two-tree clonal plots. Test sites were fairly uniform with little microenvironmental variation. The Washington site is at 300 feet elevation and has a rocky loam soil; the Oregon site has a deep loam soil situated on a steep slope ($\sim 15^\circ$) at 650 feet elevation. The Oregon site is 160 km south of the Washington site and has a warmer, drier climate.

Treatments and phenotypic measurements: Three experiments were conducted in this study. The first two involved controlled treatments, whereas the third experiment involved field tests at two sites.

Growth initiation experiment: The growth initiation experiment was designed to identify QTL controlling growth initiation that interact with winter chill and spring flushing temperatures. Growth commences in the meristematic tissue several weeks prior to external evidence (FIELDER and OWENS 1989). To avoid destructive methods of determining growth initiation, the surrogate trait spring bud flush was used to measure growth initiation. Terminal bud flush (TBF_{GI}) was defined as the date upon which the first visible green needles emerged from the bud scales. Winter chill (WC) was defined as the number of hours at or below 4° . Flushing temperature (FT) was defined as the temperature of the greenhouses in which the cuttings were grown. The experimental design was a 2×3 factorial with two winter chill levels (750 and 1500 hr) and three flushing temperature levels (10° , 15° , and 20°), which produced six treatment combinations (Figure 1A).

Cuttings were allowed to accumulate chilling hours in outdoor ambient conditions through the fall and winter of 1998–1999. Upon accumulation of 750 hr of winter chill (late December 1998), two complete sets of replicates were moved to each of three greenhouses maintained at constant conditions of 10° , 15° , and 20° . The remaining six sets were moved into the same houses in late February, upon accumulation of 1500 hr of winter chill. Cuttings were monitored twice weekly and the Julian day (JD) of terminal bud flush was recorded for each cutting. The number of days between greenhouse entry and bud flush was determined and used for analyses (TBF_{GI}; Table 1). Clonal means were calculated for TBF_{GI} for all treatment-combinations (Table 2).

Growth cessation experiment: The growth cessation experiment was designed to evaluate the effects of moisture stress and day length on growth cessation. Growth cessation is a prolonged physiological process, beginning with the initiation of bud scales shortly following bud flush. These processes are not easily monitored except by destructive dissection and therefore are best evaluated by measuring a series of seasonal growth rhythm traits (MACDONALD and OWENS 1993). Treatments were tested in a 2×2 factorial, with two levels of moisture stress [moisture stress (MS) and no moisture stress (NMS)] and two levels of day length [natural day length (NDL) and extended day length (EDL)] producing four treatment combinations (Figure 1A).

In March 1999, unflushed cuttings were transplanted to 1.6-liter pots and randomly distributed into eight replicated sets,

two for each of four treatment combinations. Pots were placed outdoors on tables and given overhead fertigation as needed. The cuttings in two of the four treatment combinations received extended day length (supplemental light set to 16 hr) from June 21, 1999 (JD 172; Figure 1B) until September 21, 1999. The cuttings in the remaining two treatment combinations received natural daylight and were separated from those receiving extended day length by a permanent shade wall. Irrigation was withheld on one-half of the cuttings in each day-length treatment. Moisture stress was monitored by predawn pressure chamber readings, using a portable Scholander pressure chamber (RITCHIE and HINCKLEY 1975). Cuttings were watered to capacity when water potentials reached -1.0 MPa on 70% of the cuttings tested (from a sample of 20). Moisture stress was induced four times between June 21 and the end of August. Cuttings in the nonstressed moisture treatments were irrigated thoroughly and regularly.

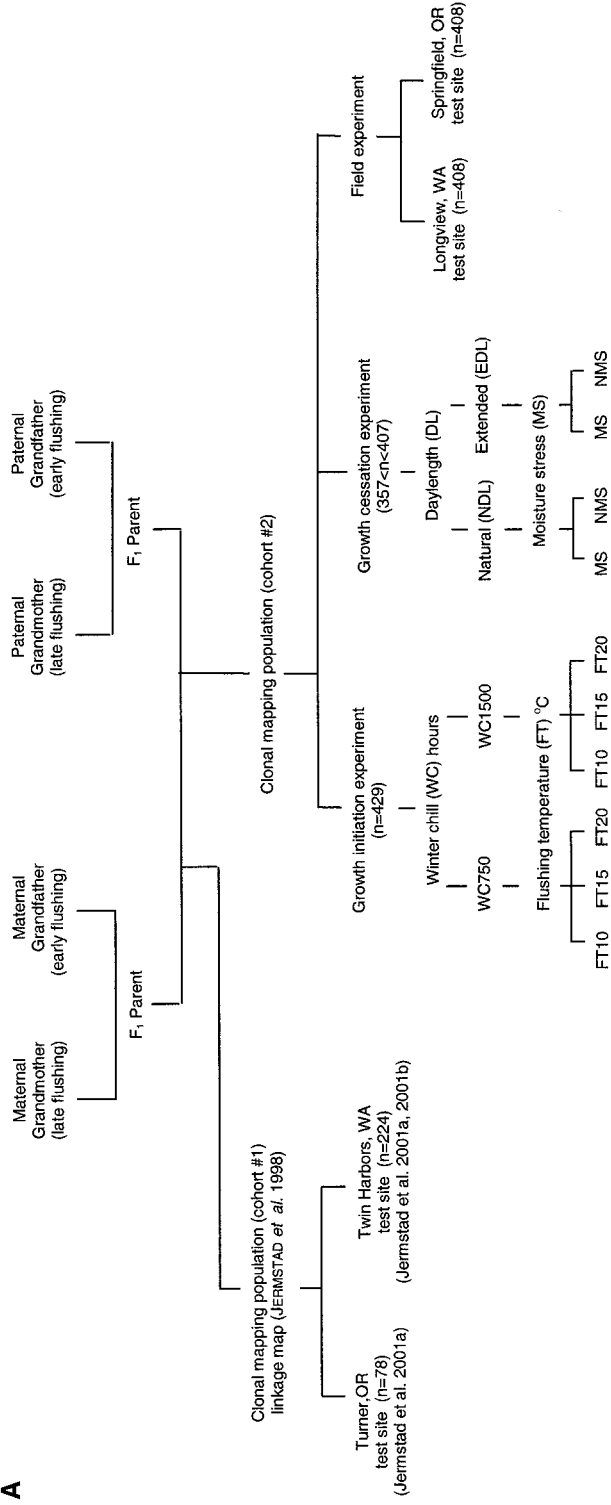
Cuttings were monitored twice weekly starting in April 1999 to obtain phenotypic data for a number of growth-rhythm traits. The JD of terminal bud flush (TBF_{GC}), the JD of observed terminal bud set (TBS), and the JD of lammas bud flush (LBF) were recorded (Table 1). The proportion of cuttings within a clone with lammas bud flush (PLF) was determined for each treatment. The duration of first flush (DFF), defined as the elapsed time between bud flush and bud set, and the elapsed time between first and second bud flushes (EBF) were determined, in days, by subtraction. Incremental height growth from spring bud flush (HT1) and incremental height growth from lammas flush (HT2) were recorded weekly for each cutting. Total incremental height growth (HTT) was determined by summing HT1 and HT2. From these data, the following variables were calculated: the duration of shoot extension (DSE), calculated as the number of days between the date of initial bud flush and the date of complete growth cessation; the JD upon which 90% of complete growth had occurred (DCG); and shoot extension intensity (SEI), or the average increase in incremental height growth between initial bud flush and DCG (millimeters per day). Clonal means were calculated for all traits measured in the growth cessation experiment for all treatment combinations (Table 2).

Field experiment: Terminal bud flush was scored in the spring of 2001 on cuttings planted at the field test sites in Longview, Washington (TBF_{FL}) and Springfield, Oregon (TBF_{FS}; Figure 1A, Table 1). Bud flush was scored on a single JD upon which it was determined, from monitoring, that $\sim 50\%$ of the cuttings in the trial had flushed. Terminal buds were scored on the basis of the stage of development as described in JERMSTAD *et al.* (2001a). Clonal means, across replications, were calculated at each site and used for QTL mapping (Table 2).

Genotyping, linkage map construction, and QTL analyses: Interval mapping and single-factor ANOVA were used to estimate QTL for traits measured in the growth initiation, growth cessation, and field experiments. Our first approach was to scan the genome for the presence of QTL using an interval mapping method and subsequently to estimate QTL $\times E$ interactions at individual markers using single-factor ANOVA. The QTL $\times E$ interactions are reported as QTL-by-treatment (QTL $\times T$) interactions for the controlled experiment and QTL-by-site (QTL $\times S$) interactions for the field experiment.

Genotypic data and linkage map construction: Seventy-two evenly spaced and informative restriction fragment length polymorphism markers used in construction of a sex-averaged genetic linkage map (cohort 1) were used to genotype 429 of the mapping population clones (cohort 2; Figure 1A). Segregation data from both cohorts were combined and linkage analysis was performed using JoinMap version 1.4 (STAM and VAN OOIJEN 1995). The Kosambi function was used to estimate map distances, and LOD thresholds of 4.0 and 0.1 were used

A



B

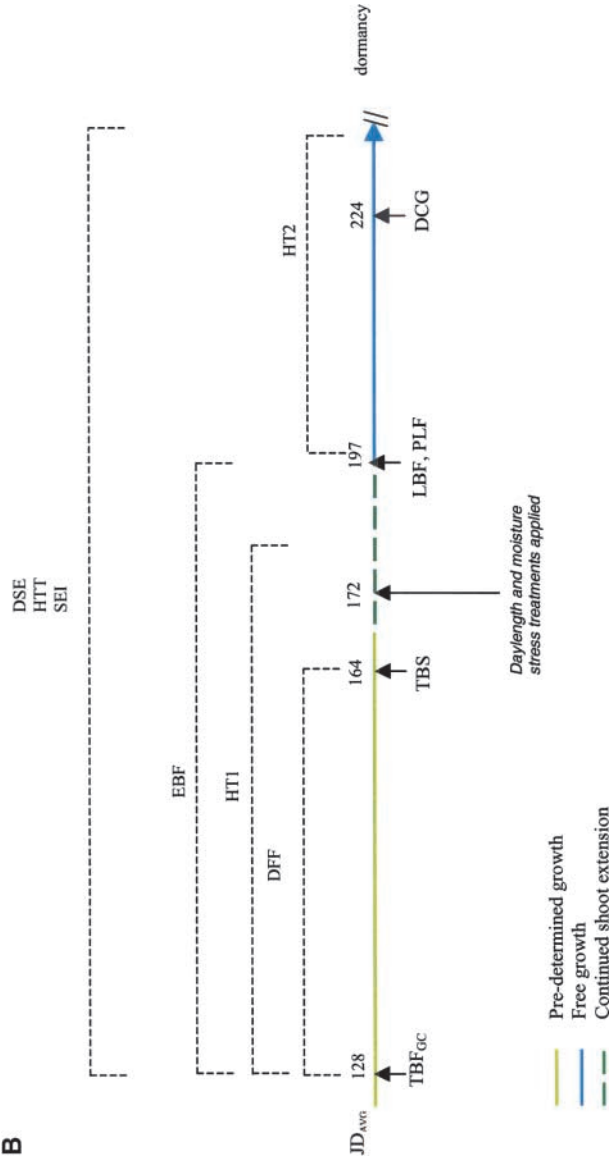


TABLE 1

Traits measured in growth initiation, growth cessation, and field experiments

Growth initiation experiment	
TBF _{GI}	Terminal bud flush (days from greenhouse entry to flush)
Growth cessation experiment	
TBF _{GC}	Terminal bud flush (Julian date)
TBS	Terminal bud set (Julian date)
DF	Duration of first flush (no. of days)
HT1	Incremental height of first flush (mm)
LBF	Lammas (second) flush (Julian date)
PLF	Proportion of trees that had lammas flush (%)
EBF	Elapsed time between first and second flush (days)
DCG	Date of 90% complete growth (Julian date)
HT2	Incremental height of second flush (mm)
HTT	Total incremental height (mm)
DSE	Duration of shoot extension (days)
SEI	Shoot extension intensity (mm/day)
Field experiment	
TBF _{FL}	Terminal bud flush at test site in Longview, Washington
TBF _{FS}	Terminal bud flush at test site in Springfield, Oregon

for grouping markers into linkage groups (LGs) and for ordering markers, respectively. The map consists of 15 LGs, rather than 17 LGs as reported in earlier analyses (JERMSTAD *et al.* 1998, 2001a,b). (In Douglas fir, the haploid number of chromosomes is 13.) The order of markers remained consistent within LGs even though, in some cases, the additional data slightly affected the estimated distances between markers. There was a negligible increase in map distance from 896.9 to 897.5 cM.

Genome scan for the presence of QTL: The all-marker multiple regression method of KNOTT *et al.* (1997) was used to scan the genome for the presence of QTL at 5-cM intervals following both a one- and two-QTL model (KNOTT *et al.* 1997; JERMSTAD *et al.* 2001a,b). For each model, the mapping software provided *F*-statistics for the most likely QTL per LG, as well as the sum of squares (SS); degrees of freedom; and the effects for the paternal, maternal, and paternal $\times$ maternal interaction components. Critical thresholds of the *F*-distribution, *P*(*F*), for suggestive and significant QTL were established at $P \leq 0.01$ and $P \leq 0.005$, respectively. These *P*-value thresholds were found to be comparable to chromosome-wide experimental thresholds obtained from permutation tests (MapQTL v. 4.0, VAN OOIJEN *et al.* 2002) conducted on several traits in the current study and were the same as those used in previous QTL studies (KNOTT *et al.* 1997; SEWELL *et al.* 2000; JERMSTAD *et al.* 2001a,b). In some regions of the genome, and in some cases whole LGs (discussed below), marker informativeness

was suboptimal and thus the statistical model was not “full rank.” In such cases, the numerator degrees of freedom were reduced and the *P*(*F*) was determined according to KNOTT *et al.* (1997).

The proportion of phenotypic variance explained (PPVE) by each QTL was calculated as

$$\sigma_p^2 = [(\text{reduced model SS/d.f.}) - (\text{full model SS/d.f.})]/[\text{reduced model SS/d.f.}].$$

LGs based solely on markers segregating in only one parent, as is the case in LGs 9, 10, and 15, failed to meet full rank criteria, and *P*(*F*) and PPVE were calculated on the basis of the reduced degrees of freedom for the full model.

A QTL scan was performed for each treatment combination [*e.g.*, six separate genome scans for the growth initiation experiment ($n = 429$) and four separate genome scans for the growth cessation experiment ($n = 406$)] for each trait. For the field experiment, genome scans were performed using the clonal mean of replicates within each test site. Although >440 clones were planted at the test sites, only 408 clones were genotyped and common to both sites (Figure 1A).

Single-factor ANOVA for detection of QTL $\times$ E interactions: ANOVA (PROC GLM, SAS 8.02; SAS Institute) was used to estimate QTL $\times$ T or QTL $\times$ S interactions for all traits measured in the growth initiation, growth cessation, and field experiments. The same 72 markers that were used for interval mapping were also used in the ANOVAs. The experimental unit used in the ANOVA for traits measured in the controlled experiments was the individual cutting. For the field experiment, clonal means for each site were used in the ANOVA. An approximate chromosome-wide experimental threshold of $P(F) \leq 0.005$ was used for reporting QTL $\times$ E interactions, which is comparable to the chromosome-wide experimental threshold used for interval mapping.

The model for estimation of QTL $\times$ T interactions in the growth initiation experiment was

$$Y_{ijkml} = \mu + R_i + W_j + T_k + G_l + C_{m(l)} + (WT)_{jk} + (WG)_{jl} + (TG)_{kl} + (WTG)_{jkl} + (WC)_{jm(l)} + (TC)_{km/(l)} + (WTC)_{jkm(l)} + Y_{ijkml},$$

where

μ is the trait mean;

R_i is the replication effect, $i = 1, 2$;

W_j is the winter chill effect, $j = 1, 2$;

T_k is the flushing temperature effect, $k = 1, 2, 3$;

G_l is the genotype effect, $l = 1 \dots n$, where n equals number of genotypic classes;

$C_{m(l)}$ is the random clone within genotype effect, $m(l) = 1 \dots x$, where x equals the number of observations per genotypic class;

$(WT)_{jk}$ is the winter chill $\times$ flushing temperature interaction effect;

$(WG)_{jl}$ is the winter chill $\times$ genotype interaction effect;

FIGURE 1.—(A) Three-generation pedigree of Douglas fir showing two mapping populations (cohorts), treatment combinations for the growth initiation and growth cessation experiments, and sample sizes used in QTL analyses. Two levels of winter chill (WC) and three levels of flushing temperature (FT) produced six treatment combinations in the growth initiation experiment. Two levels of day length (NDL, natural daylength; EDL, extended daylength) and two levels of moisture stress (MS, moisture stress; NMS, no moisture stress) produce four treatment combinations in the growth cessation experiment. Two test sites were used in the field experiment. (B) Temporal relationships among traits that were evaluated during the seasonal growth cycle. The average Julian date (JD_{AVG}), calculated from clonal means, is shown for TBF_{GC}, TBS, LBF, and DCG, and the JD that growth cessation treatments commenced is shown.

TABLE 2

Trait means and standard errors estimated in the growth initiation, growth cessation, and field experiments

Trait ^a	WC750_FT10 ^b	WC750_FT15	WC750_FT20	WC1500_FT10	WC1500_FT15	WC1500_FT20
Growth initiation experiment						
TBF _{GI}	106.4 (0.4) ^c	101.0 (0.7)	82.8 (1.2)	53.1 (0.2)	39.9 (0.2)	23.3 (0.2)
Trait	EDL_MS	NDL_MS	EDL_NMS	NDL_NMS		
Growth cessation experiment						
TBF _{GC}	128.5 (0.2)	126.5 (0.2)	130.2 (0.2)	129.0 (0.2)		
TBS	163.0 (0.3)	163.9 (0.3)	163.0 (0.3)	165.5 (0.4)		
DFF	34.5 (0.3)	37.4 (0.3)	32.9 (0.4)	36.5 (0.4)		
HT1	377.8 (3.6)	379.8 (3.7)	368.6 (3.6)	384.1 (3.9)		
LBF	195.8 (0.6)	195.4 (0.4)	198.7 (0.5)	197.7 (0.5)		
PLF	72.5 (NA) ^d	75.2 (NA)	84.4 (NA)	83.9 (NA)		
EBF	68.2 (0.6)	69.4 (0.4)	68.8 (0.5)	68.9 (0.5)		
DGC	220.9 (0.5)	220.1 (0.4)	225.8 (0.6)	228.8 (0.4)		
HT2	122.8 (3.1)	132.2 (2.6)	163.3 (3.9)	174.8 (3.4)		
HTT	500.6 (4.9)	512.0 (4.6)	531.9 (5.7)	557.8 (5.4)		
DSE	92.4 (0.6)	93.6 (0.5)	95.6 (0.6)	99.8 (0.5)		
SEI	4.89 (0.0)	4.96 (0.0)	5.01 (0.1)	5.04 (0.0)		
Trait	Springfield, Oregon site	Longview, Washington site				
Field experiment						
TBF _V	3.26 (0.0)	3.51 (0.0)				

^a See Table 1 for trait descriptions.^b See Figure 1 for treatment-combination nomenclature.^c Standard errors are in parentheses.^d NA, not available. PLF is the proportion of cuttings within clone with lammas flush and thus standard errors could not be estimated.

$(TG)_{kl}$ is the flushing temperature $\times$ genotype interaction effect;

$(WTG)_{jkl}$ is the winter chill $\times$ flushing temperature $\times$ genotype interaction effect;

$(WC)_{jm(l)}$ is the winter chill $\times$ clone within genotype effect;

$(TC)_{km(l)}$ is the flushing temperature $\times$ clone within genotype effect;

$(WTC)_{jkm(l)}$ is the winter chill $\times$ flushing temperature $\times$ clone within genotype effect; and

$Y_{ijkm(l)}$ is the sampling error.

Genotype, winter chill, and flushing temperature were fixed effects, along with their two- and three-way interactions. Terms involving clone were considered random and the SAS Random statement was used to obtain correct F -tests for the fixed effects.

The model for estimation of QTL $\times$ T interactions in the growth cessation experiment was

$$Y_{ijkm(l)} = \mu + R_i + D_j + M_k + G_l + C_{m(l)} + (DM)_{jk} + (DG)_{jl} \\ + (MG)_{kl} + (DMG)_{jkl} + (DC)_{jm(l)} + (MC)_{km(l)} \\ + (DMC)_{jkm(l)} + Y_{ijkm(l)},$$

where

μ is the trait mean;

R_i is the replication effect, $i = 1, 2$;

D_j is the day length effect, $j = 1, 2$;

M_k is the moisture stress effect, $k = 1, 2, 3$;

G_l is the genotype effect, $l = 1 \dots n$, where n equals number of genotypic classes;

$C_{m(l)}$ is the random clone within genotype effect, $m(l) = 1 \dots x$, where x equals the number of observations per genotypic class;

$(DM)_{jk}$ is the day length $\times$ moisture stress interaction effect;

$(DG)_{jl}$ is the day length $\times$ genotype interaction effect;

$(MG)_{kl}$ is the moisture stress $\times$ genotype interaction effect;

$(DMG)_{jkl}$ is the day length $\times$ moisture stress $\times$ genotype interaction effect;

$(DC)_{jm(l)}$ is the day length $\times$ clone within genotype effect;

$(MC)_{km(l)}$ is the moisture stress $\times$ clone within genotype effect;

$(DMC)_{jkm(l)}$ is the day length $\times$ moisture stress $\times$ clone within genotype effect; and

$Y_{ijkm(l)}$ is the sampling error.

Genotype, day length, and moisture stress were fixed effects, along with their two- and three-way interactions. Terms involving clone were considered random, and the SAS Random statement was used to obtain correct F -tests for the fixed effects.

To test for QTL $\times$ S interactions affecting bud flush in the field experiment, ANOVA was performed using PROC MIXED with the SAS Repeat statement to account for clonal replication at two test sites (TBF_{FL} and TBF_{FS}).

The model for estimation of QTL $\times$ S interactions in the field experiment was

$$Y_{ijk} = \mu + G_i + S_j + (G \times S)_{ij} + C_k + \gamma_{ijk},$$

where

μ is the trait mean;

G_i is the genotype effect, $i = 1 \dots n$, where n equals the number of genotypic classes;
 S_j is the site effect, $j = 1, 2$;
 $G \times S_{ij}$ is the genotype $\times$ site interaction effect;
 C_k is the clone effect, $k = 1 \dots n$, where n equals the number of clones for the given marker m_i ; and
 γ_{ijk} is the sampling error.

Genotype, site, and genotype $\times$ site interaction were fixed effects, and the clone term was considered random.

RESULTS

The effect of environment on phenotypic variance in growth rhythm traits: The treatments applied in the growth initiation experiment had significant effects on the timing of bud flush ($P \leq 0.0001$). On average, cuttings receiving 750 hr of winter chill took >60 days longer to flush than cuttings that received 1500 hr of winter chill. As anticipated, increased flushing temperatures accelerated bud flush, with cuttings brought into the warmest houses (20°) flushing nearly 40 days earlier than those in the coolest houses (10°). Treatments applied in the growth cessation experiment had significant effects on the phenotype for many of the traits measured ($P \leq 0.01$). The phenotypic responses to day length and moisture stress in the growth cessation experiment were typically less pronounced than those observed for winter chill and flushing temperature in the growth initiation experiment. However, large sample sizes (<800 cuttings per treatment combination) made even very small differences statistically significant. The effects of day length on phenotype were unexpected in some growth-rhythm traits. Day length treatments may have been confounded by unintentional temperature effects caused by the shade wall that was used to separate the day length environments. Nonetheless, sufficient variation was found in the traits measured in the growth cessation experiment to enable QTL mapping. Although trait data appeared to approximate normal distributions, 7 of 11 tests for nonnormality were significant (Martinez-Iglewicz test, Number Cruncher Statistical Software, v. 2001). Past experience has shown that transformation of such data has not substantially influenced QTL detection. Consequently, no adjustments to data were made in these analyses. Analyses of the phenotypic data, including phenotypic correlations and phenotypic distributions, can be viewed at <http://dendrome.ucdavis.edu/NealeLab/publications.html>.

QTL detection and QTL $\times$ T interactions: A modest number of QTL (4–11) were detected per individual trait analyzed in this study. QTL were found on 14 of the 15 linkage groups; no QTL were detected on LG 13. QTL detected within 20 cM of each other were counted as a single QTL for a given trait. Genome scan profiles from the one-QTL model interval mapping analyses and QTL $\times$ E interactions for the growth initiation, growth cessation, and field experiments are shown in Figures 2–15. For the sake of simplicity and also due to space constraints, the results of the two-QTL model are

not presented here. Tabulated results from both the one- and two-QTL models, including F -values, F -distribution probabilities, parental effects, parent-interaction effects, and PPVE, are presented at <http://dendrome.ucdavis.edu/NealeLab/publications.html>. Critical thresholds of the F -distribution probabilities $P(F)$ for suggestive and significant QTL were established at $P \leq 0.01$ and $P \leq 0.005$, respectively. The critical F -value threshold for suggestive QTL ($P \leq 0.01$) is shown by a dotted horizontal line and the critical F -value threshold for significant QTL ($P \leq 0.005$) is shown by a dashed horizontal line. The critical F -value thresholds for suggestive and significant QTL were higher for LGs 9, 10, and 15 because the regression model was not full rank. The range in PPVE for individual QTL in this study was 0.7–9.5%. QTL were largely additive in effect with the exception of the incremental height growth traits.

A small number of QTL $\times$ E interactions (0–5) were found per individual trait (Figures 2–15). Markers that mapped close to one another and detected interactions with the same treatment were inferred as a single QTL interaction. Also, only interactions found near QTL peaks identified by interval mapping were considered relevant. A tabulated summary of the ANOVA results for each marker is available at <http://dendrome.ucdavis.edu/NealeLab/publications.html>.

DISCUSSION

It is of basic scientific interest to understand the genetic control of the seasonal growth cycle in perennial plants. Genetic control of seasonal growth rhythm is complex (quantitative), and thus we have used a QTL mapping approach to begin to dissect the quantitative inheritance of perennial growth. In this study, we have identified sets of QTL that control different phases of growth, beginning with bud flush in the spring and ending with growth cessation in mid- to late summer. Furthermore, we have identified interactions between several QTL and some of the environmental signals influencing perennial growth phases. To our knowledge, this is the first time QTL have been mapped in forest trees under experimental treatment of environmental signals that affect perennial growth.

Our main objective was to discover QTL for growth initiation and growth cessation traits that interact with specific environmental signals, *e.g.*, winter chill, flushing temperature, day length, and moisture stress. The rationale for studying these QTL is twofold. First, QTL governed by environmental signals are fundamentally important and will promote understanding of the physiological and biochemical processes that govern patterns of seasonal growth. The knowledge derived from these comprehensive QTL mapping experiments will be invaluable in our future efforts to identify candidate genes controlling the annual growth cycle in conifers. Second, knowledge of QTL controlling these growth-rhythm traits and their interaction with the environment may

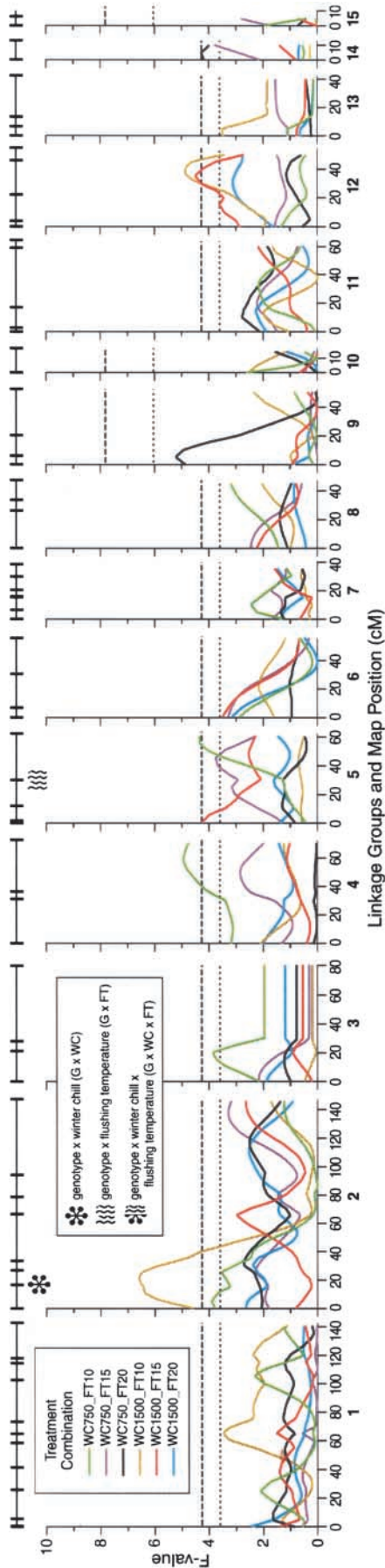


FIGURE 2.—Terminal bud flush (TBF_{CL}) was used as a surrogate trait to measure growth initiation in the spring. The overwintering bud is released from dormancy and growth is initiated. Seven QTL for terminal bud flush were detected in the growth initiation experiment (TBF_{CL}). QTL were found on six LGs (2, 3, 4, 5, 12, and 14) and were detected in five of the six treatment combinations. Only two QTL $\times$ T interactions were found, one for winter chill on LG 2 and one for flushing temperature on LG 5. The interaction detected on LG 5 is located at a marker that is intermediate between two QTL detected by interval mapping.

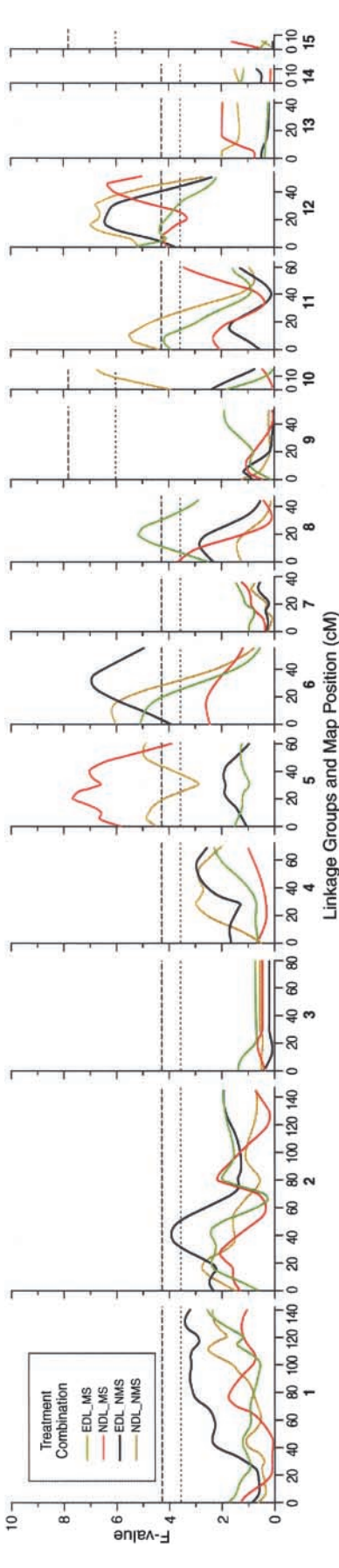


FIGURE 3.—Terminal bud flush (TBF_{CL}) was also measured in the growth cessation experiment (TBF_{CC}). Cuttings were measured 2 months prior to the application of treatments (Figure 1B); thus treatment interactions were not estimated. Eleven QTL were detected on seven LGs (2, 5, 6, 8, 10, 11, and 12). The differences of QTL scans among treatment combinations are due to block effect and most notable on LGs 5, 6, 8, and 11.

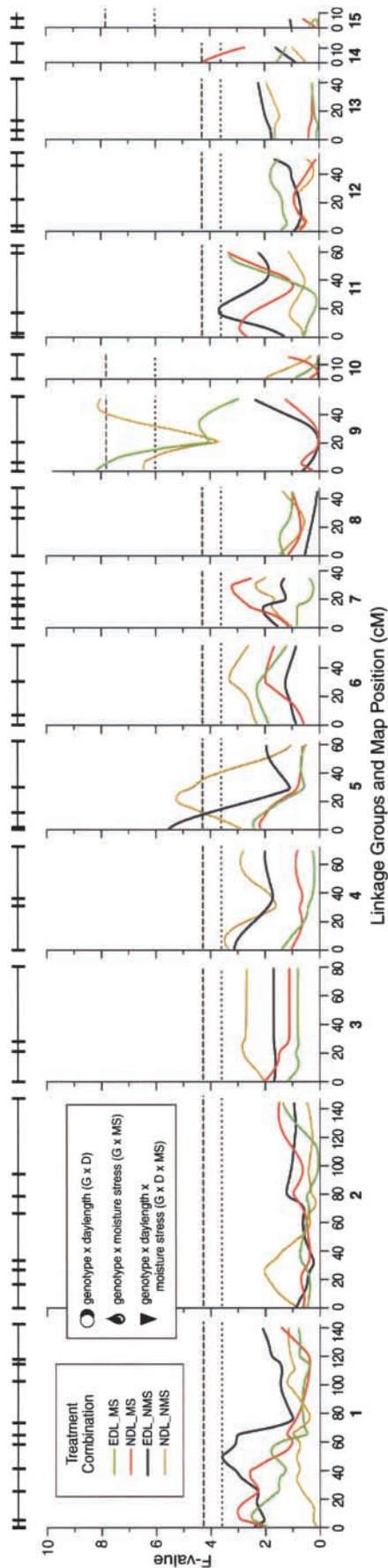


FIGURE 4.—Terminal bud set (TBS) marks the transition from primary leaf initiation to bud scale initiation and ultimately to embryonic shoot formation (preformed buds). Seven QTL for terminal bud set were detected on five LGs (1, 5, 9, 11, and 14). LGs 5 and 9 each contain QTL that were detected in two treatment combinations. No QTL $\times$ T interactions were detected.

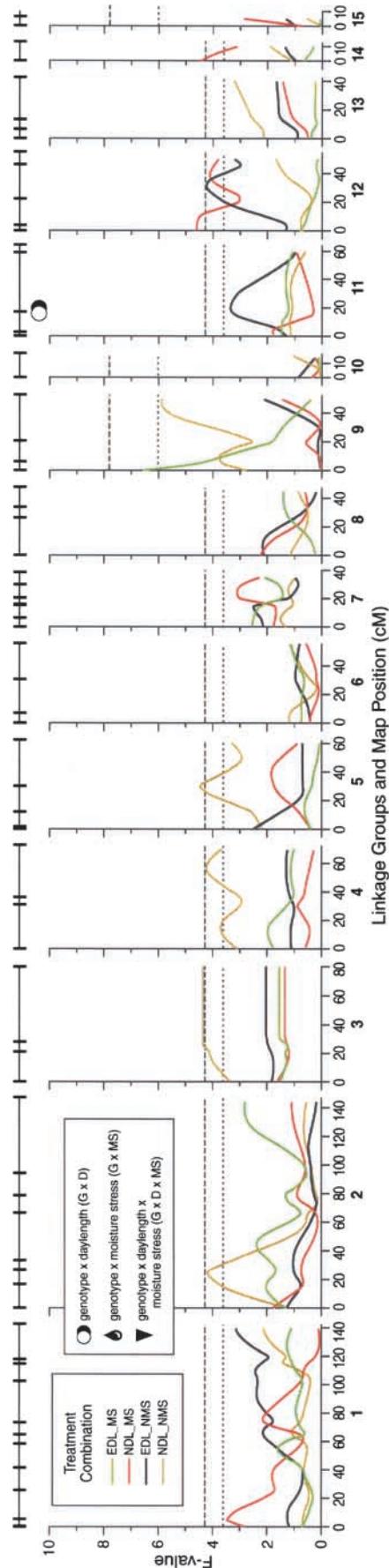


FIGURE 5.—Duration of the first flush (DFF). QTL for the duration of the first flush may be a reflection of genes segregating for growth-rhythm events from the previous growing season (preformed buds dictating how many cells are active in first flush) or for the initiation of bud scales. Nine QTL were detected for the DFF on seven LGs (2, 3, 4, 5, 9, 12, and 14), of which nearly all were found also in either TBS_{EC} or TBS, but not both. QTL detected for DFF on LGs 5, 9, and 14 were also found for TBS (Figure 4), while the QTL on LGs 2, 5, and 12 were detected for TBS_{EC} (Figure 3). The genome scan profiles for DFF and TBS are remarkably similar, even though F-values were generally higher for TBS. The interaction with day length detected on LG 11 was at a marker position where the interval mapping F-value was just below the suggestive level.

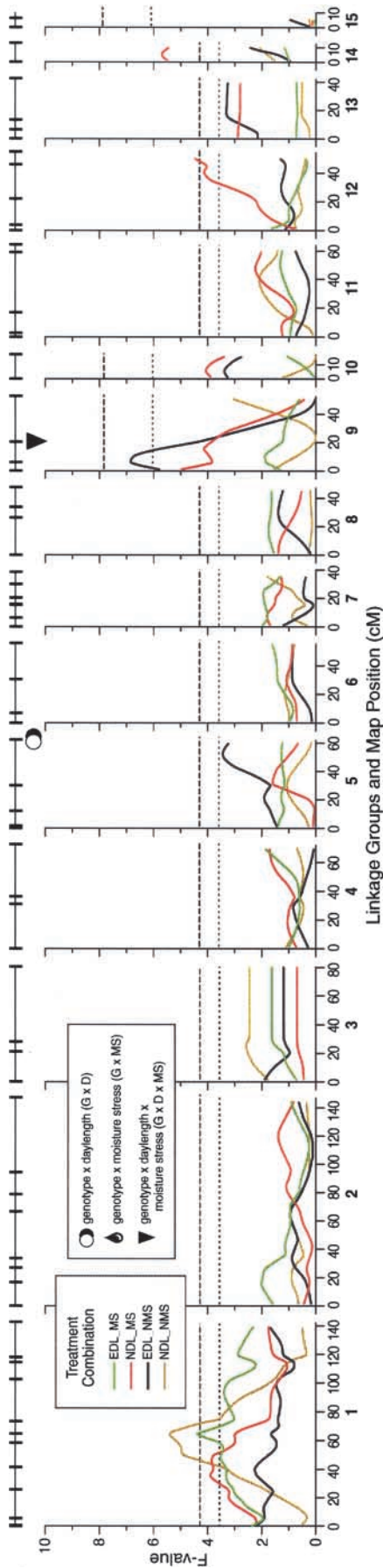


FIGURE 6.—Incremental height growth from the first flush (HT1) is essentially growth determined by the preformed bud, which is formed in the previous year. Genes contributing to growth, through cell expansion and or subapical cell division, may possibly be influenced by environmental signals. Four QTL for HT1 were detected on four LGs (1, 9, 12, and 14). QTL $\times$ T interactions were detected on two LGs (5 and 9); however, only one (LG 9) was at a position where a QTL was detected by interval mapping. This QTL showed a three-way interaction (genotype $\times$ daylength $\times$ moisture stress). Similarities in QTL scans were found among HT1, TBS (Figure 4), and DFF (Figure 5).

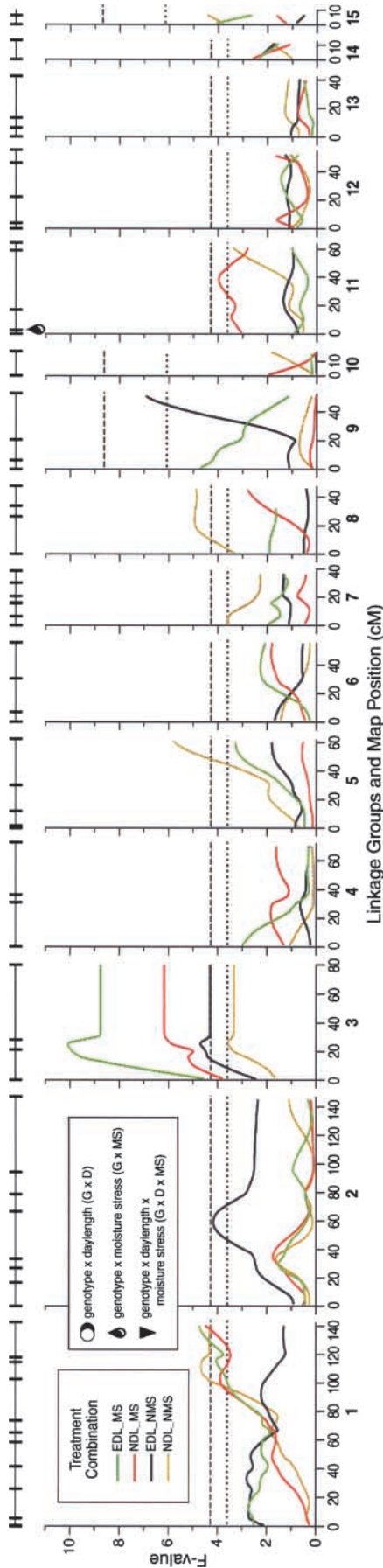


FIGURE 7.—Lammas bud flush (LBF) signals the initiation and expansion of neo-formed cells that have not undergone a hardening phase. Eight QTL were detected for lammas bud flush on LGs 1, 2, 3, 5, 7, 8, 9, and 11. A QTL $\times$ T interaction was detected on LG 11 proximal to a suggestive QTL detected by interval mapping.

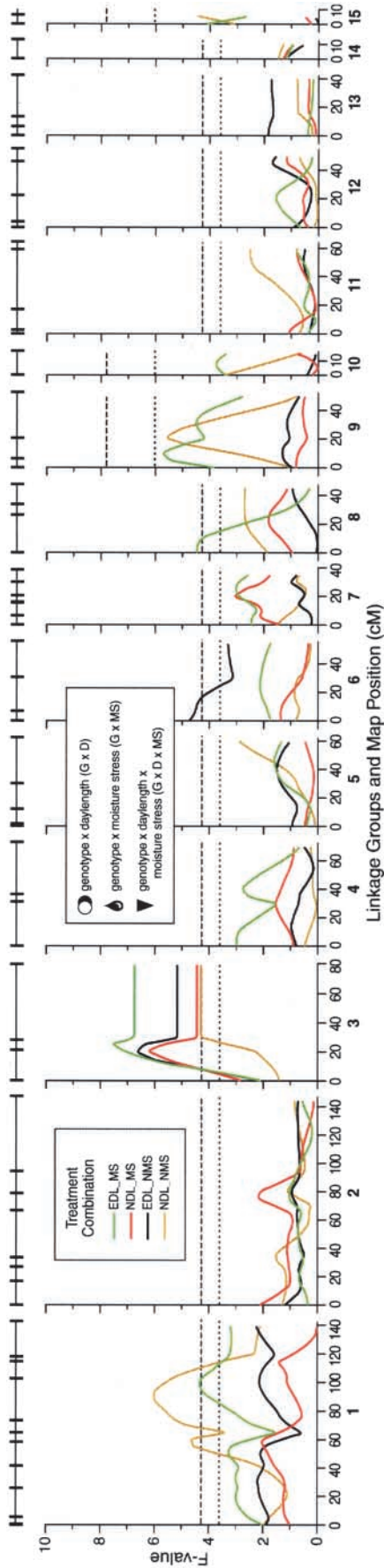


FIGURE 8.—Proportion of lammas flush (PLF). QTL for the proportion of lammas bud flush may represent genes segregating for cell initiation and expansion or for competency to initiate hardening and dormancy (or lack thereof). Five QTL for proportion of lammas flush were detected on four LGs (1, 3, 6, and 8). A QTL was detected in all four treatment combinations on LG 3, showing a profile very similar to LBF (Figure 7). Some correlation is expected since PLF is derived directly from LBF. Similar genome scan profiles were also observed for PLF and HT2 (Figure 11). No QTL $\times$ T interactions were detected for this trait.

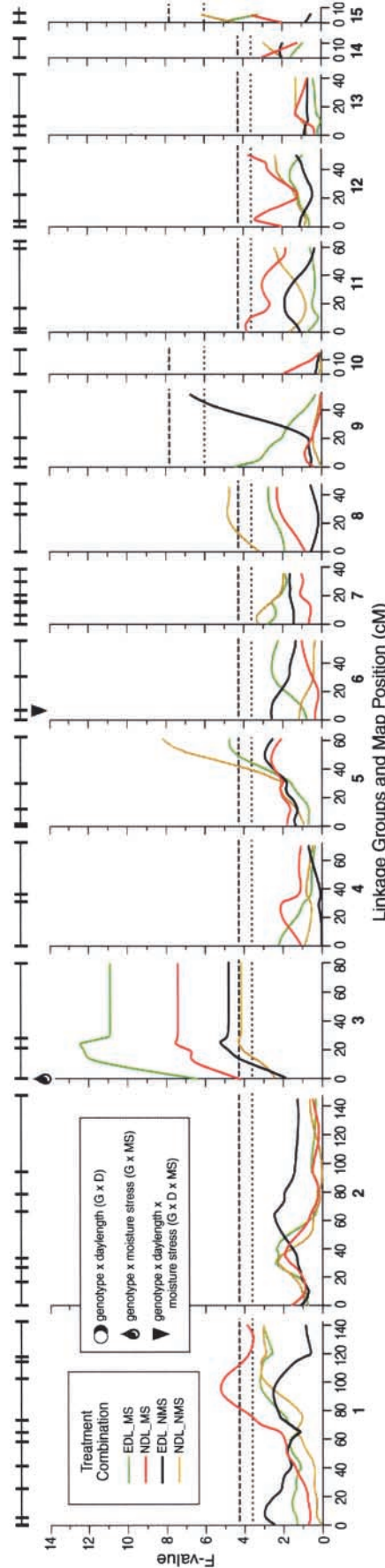


FIGURE 9.—The elapsed time between flushes (EBF) is dependent on the timing of lammas flush, as it is the number of days between flushing events. Eight QTL were detected for EBF on eight LGs (1, 3, 5, 8, 9, 11, 12, and 15), with many of the QTL located in the same genomic region as the QTL detected for LBF (LGs 3, 5, 8, and 9). This finding is congruent with the high phenotypic correlation ($r_{\text{AVG}} = 0.86$) between these two traits. A QTL was detected on LG 3 in all four treatment combinations, not only for LBF, but also for PLF and HT2. A QTL $\times$ T interaction for moisture stress was detected on LG 3 and a three-way interaction with both moisture stress and day length was detected on LG 6, although a QTL was not detected on LG 6 on the basis of interval analysis.

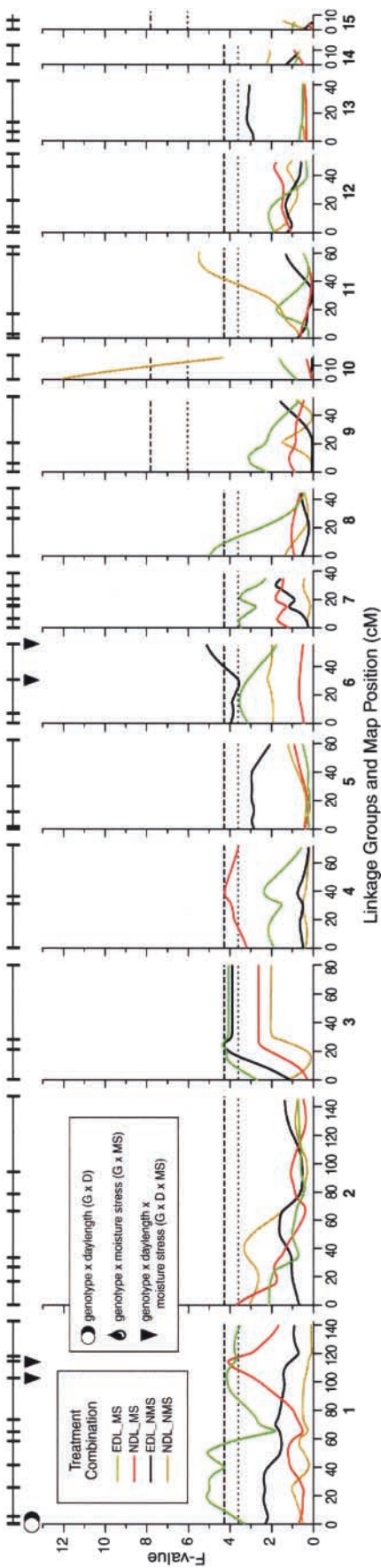


FIGURE 10.—Date of 90% complete growth (bcg). The timing of growth cessation is an important adaptation to ensure that the tree is hardy for winter. Ten QTL were detected for bcg on LGs 1, 2, 3, 4, 6, 7, 8, 10, and 11. A QTL $\times$ T interaction for day length was found on LG 1, and three-way interactions were found on LGs 1 and 6. These three-way interactions were also detected in DSE (Figure 13).

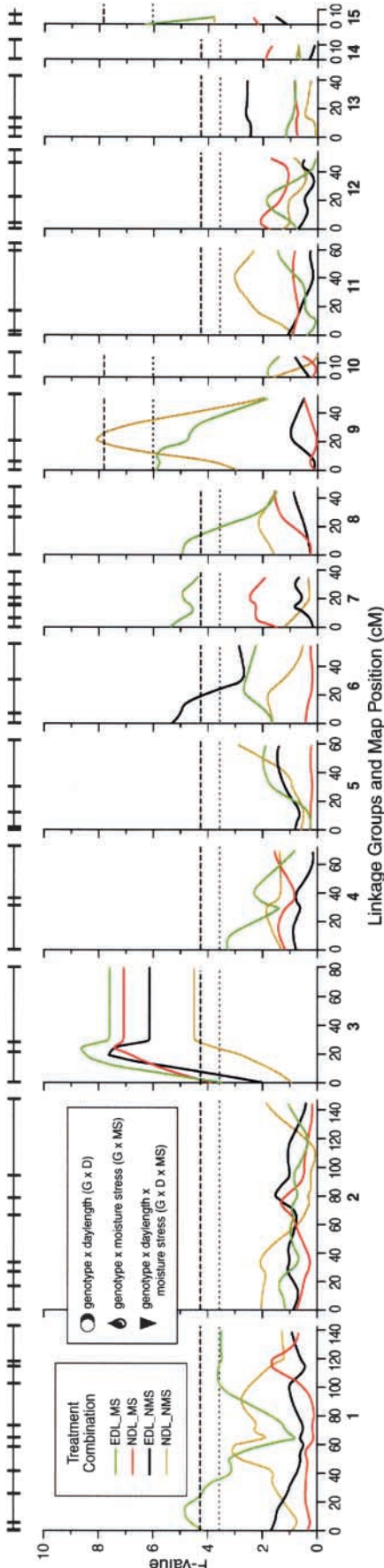


FIGURE 11.—Incremental height growth from lammas flush (HT2) is essentially a function of the growth program of the neo-formed bud (lammas bud). Similar to HT1, cell expansion and subapical cell cycling may be influenced by treatments during growth. Eight QTL were detected for HT2 on seven LGs (1, 3, 6, 7, 8, 9, and 15). A QTL was detected in the same region on LG 3 in all four treatment combinations and had a similar genome scan profile as those for LBF (Figure 7) and PLF (Figure 8). The genome scan profile for HT2 was more similar to that of PLF and less similar to that of LBF, reflective of the phenotypic correlations for these traits ($r_{\text{avg}} = 0.81$ and -0.24 , respectively). HT1 and HT2 appear to be unrelated and controlled by unique suites of QTL. This was unexpected given that the mechanics of subapical cell cycling and cell expansion are similar for both first flush and lammas flush. No QTL $\times$ T interactions were detected for this trait.

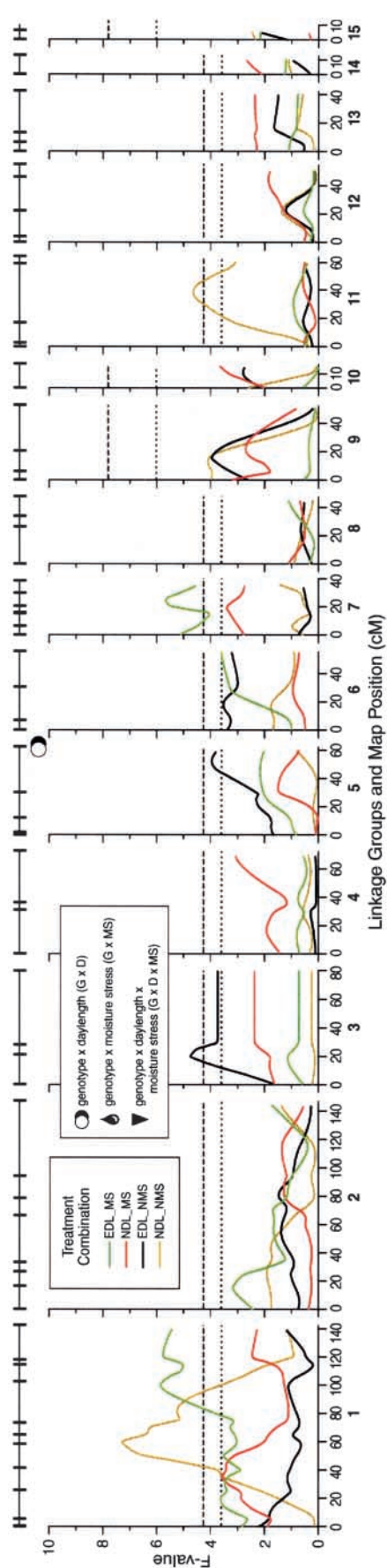


FIGURE 12.—Total incremental height (HTT) growth is a summation of first flush and lammas flush growth. Eight QTL were detected for HTT on six LGs (1, 3, 5, 6, 7, and 11). The QTL detected on LG 1 was also detected in HT1 and SE1, while the two QTL on LG 7 were also detected in HT2, but not HT1. The phenotypic correlation found between HTT and HT1 is high ($r_{\text{AVG}} = 0.78$), moderately high between HTT and HT2 ($r_{\text{AVG}} = 0.67$), and low between HT1 and HT2 ($r_{\text{AVG}} = 0.06$). One QTL $\times$ T interaction with day length was detected on LG 5 near a QTL detected by interval mapping.

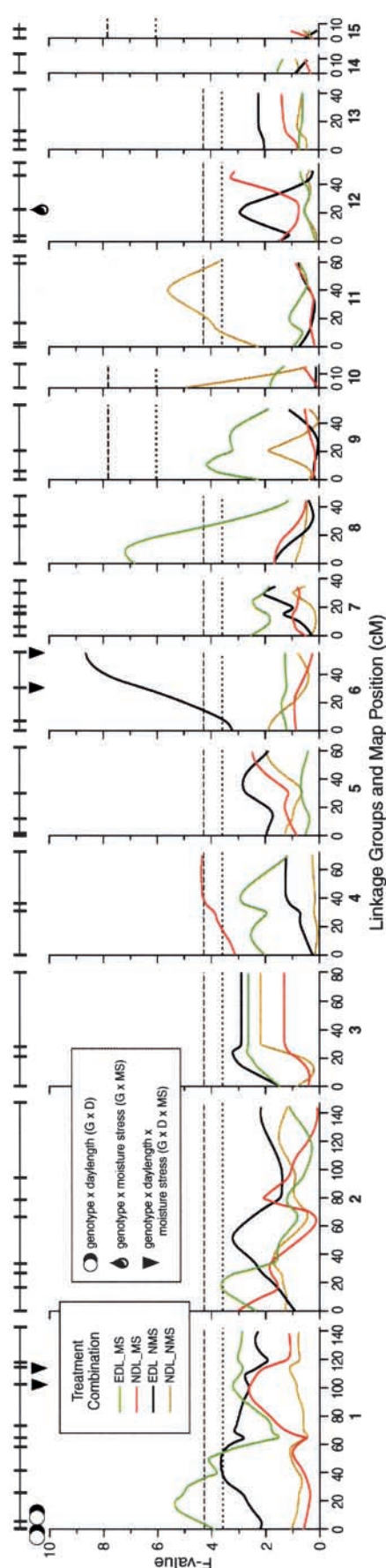


FIGURE 13.—QTL for the duration of shoot extension (DSE) likely represent genes segregating for cell expansion or for the hardening transition. Six QTL were detected for DSE on LGs 1, 2, 4, 6, 8, and 11. The genome scan profile for DSE was notably similar to those for HT2 (Figure 11) and DCG (Figure 10). This is not unexpected since all three traits are measured at the end of the growing cycle (Figure 1B). A QTL $\times$ T interaction with day length was found on LG 1 and a three-way interaction was detected on LG 6. Additional interactions were detected (LGs 1 and 12) but were not supported by interval mapping.

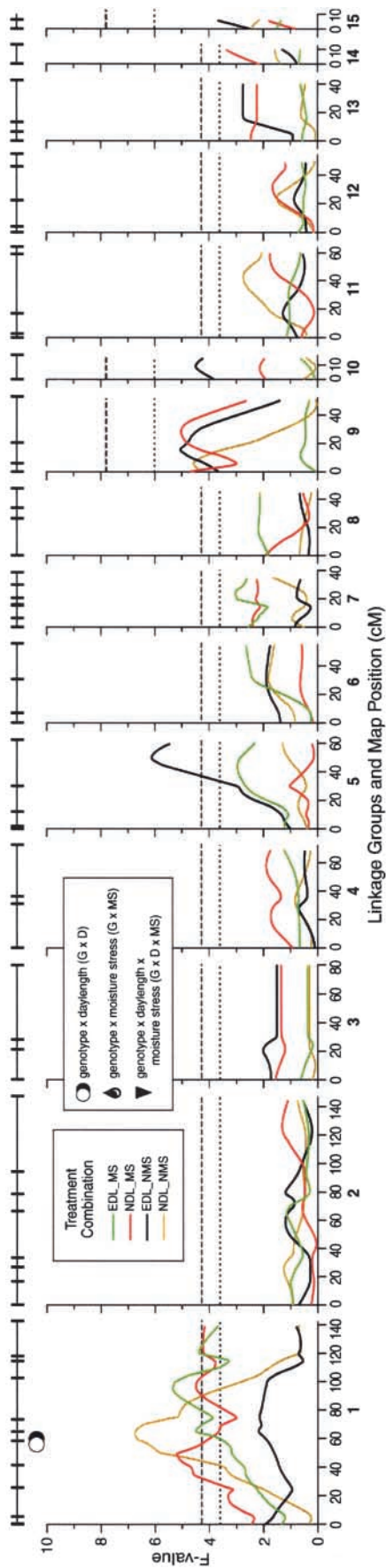


FIGURE 14.—Shoot extension intensity (SEI) is a measure of the average daily growth rate over the entire growth cycle. QTL for SEI were detected on LGs 1 and 5. The genome scan profiles for SEI on LG 1 are remarkably similar to those for PLF, HTL, and HTT. A QTL $\times$ T interaction with day length was detected on LG 1.

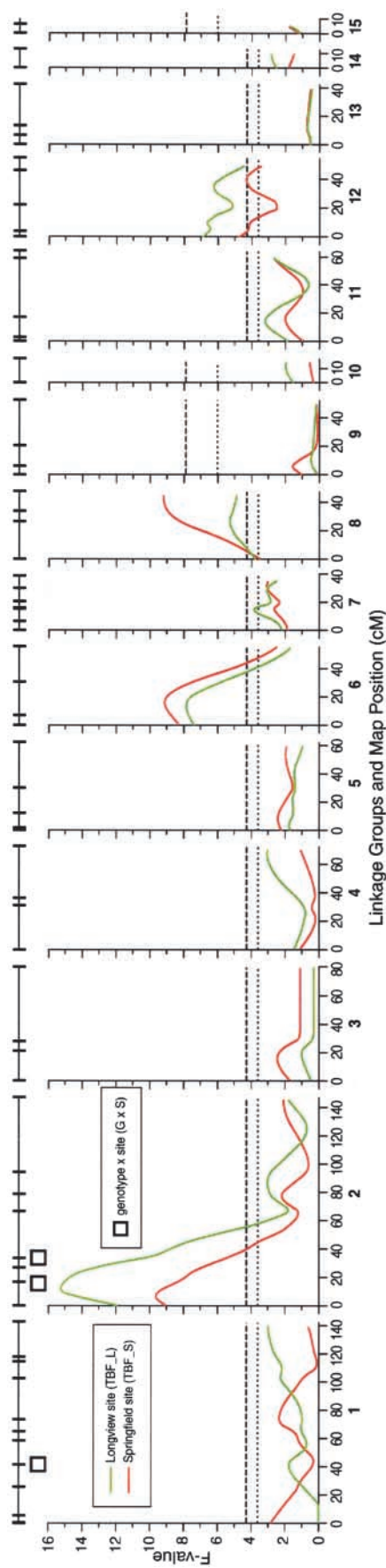


FIGURE 15.—Terminal bud flush at test sites (TBF_L and TBF_S). The repeated detection of QTL in multiple environments is paramount for realizing the utility of QTL mapping for breeding purposes. QTL interacting with the environment are elusive and unpredictable, depending upon plantation site and yearly fluctuations in climate. Six QTL for terminal bud flush were detected in the field test experiment (LGs 2, 6, 7, 8, and 12), five of which were detected at both sites. The genome scan profiles for the two test sites were remarkably similar. Moreover, the QTL for bud flush on LGs 2 and 12 were also found in the growth initiation experiment (TBF_G; Figure 2) and in the growth cessation experiment (TBF_C; Figure 3). The two closely positioned QTL $\times$ S interactions on LG 2 were detected at a location supported by interval mapping, whereas the site interaction detected on LG 1 was not supported by interval mapping.

be useful for marker-aided breeding. Moreover, the repeated detection of QTL controlling growth-rhythm traits in multiple environments lays a foundation for tree breeders to develop a better understanding of quantitative trait architecture and the underlying molecular basis of genotype-by-environment interactions.

Number of QTL controlling growth initiation and growth cessation in Douglas fir: Initially, the genome was scanned for the presence of QTL controlling growth initiation and growth cessation using an interval mapping method. A total of 90 QTL were detected at the suggestive level among all traits, 55 of which were detected at the significant level (Figures 2–15). However, because some QTL were repeatedly detected in correlated traits, the number of unique QTL is <90.

The estimation of the number of QTL and the PPVE by each QTL is heavily dependent upon the number of progeny evaluated (BEAVIS 1995). The PPVE among all traits and all treatments ranged from 0.7 to 9.5%. Early studies reported small numbers of QTL controlling traits, often with overestimated PPVE (KEARSEY and FARQUHAR 1998). In most cases, these results have been shown to be due to small sample size (BEAVIS 1995; WILCOX *et al.* 1997; FREWEN *et al.* 2000) and to the lack of statistical power to detect all the QTL present on a chromosome (HYNE *et al.* 1995; KEARSEY and FARQUHAR 1998). Sample sizes >300 are recommended to accurately estimate the number and effects of QTL controlling a trait (HYNE *et al.* 1995). In this study, we increased the sample size from our previous QTL study by nearly twofold ($n > 400$; Figure 1A), while retaining the precision of phenotypic evaluation provided by clonal replication of the mapping population. Nonetheless, the number of QTL for bud flush detected in this experiment and the estimated size of effects did not show substantial differences from those found in the previous study (cohort 1; JERMSTAD *et al.* 2001a). In both studies, a modest number of QTL (5–10) of modest effect (5–10% PPVE) were found to be controlling adaptive traits in Douglas fir.

The amount of phenotypic variation explained by an individual QTL was generally small, but sometimes fluctuated depending upon the treatment. For example, a QTL for EBF was detected on LG 3 at 25 cM in all four treatment combinations and the PPVE varied almost threefold among treatments, *e.g.*, 7.5% (EDL_MS), 5.1% (NDL_MS), 3.5% (EDL_NMS), and 2.7% (NDL_NMS). In this example, where large and balanced samples were used, differences in the PPVE for the same QTL illustrate that sample size is not the only important criterion for accurate estimation of QTL effects, but that environment also plays an important role.

Seasonal growth in this study is marked by four phenological events: spring bud flush, bud set, lammas bud flush, and growth cessation (TBF_{CG}, TBS, LBF, and DCG; Figures 3, 4, 7, and 10). Although a small number of QTL were found in common among the four traits,

the genome scan profiles were notably dissimilar and support the hypothesis that these traits are controlled by unique suites of genes (REHFELDT 1983; CAMPBELL 1986), especially in young trees (LI and ADAMS 1993; ADAMS and BASTIEN 1994).

Comparisons of genome scan profiles among the traits can help identify QTL affecting more than one trait. Such QTL were mostly found within three groups of traits: (1) predetermined growth from an overwintering preformed bud (TBF_{CG}, TBS, DFF, and HT1), (2) free growth from a neoformed or lammas bud (LBF, PLF, DCG, and HT2), or (3) traits that represent a capitulation of both (EBF, HTT, DSE, and SEI; Figure 1B). For example, the genome scan profiles among TBS, DFF, and HT1 (Figures 4, 5 and 6, respectively) were similar and the genome scan profiles for LBF, PLF and HT2 (Figures 7, 8 and 11, respectively) were similar. Likewise, QTL for growth traits that capitulated the growth cycle (HTT, DSE, and SEI), showed strong relationships to one another, and most likely represent similar functionality (subapical cell expansion or elongation). The QTL for incremental height growth from an overwintering bud (HT1) were notably dissimilar from the QTL for incremental height growth from a lammas bud (HT2), suggesting different genetic control for these two forms of growth. KAYA *et al.* (1989) found low genetic correlations between predetermined growth and free growth in open-pollinated Douglas fir seedlings grown in two environments. The same study also showed that the amount and duration of predetermined growth was highly correlated to the size of the preformed bud, which in turn was highly correlated to the timing of bud set in the previous year. The pattern of QTL reported here strongly suggests separate genetic control for growth from the two bud types.

Resolving whether QTL that are detected in multiple traits represent pleiotropy or tightly linked QTL is difficult in large genomes such as conifers, where hundreds of genes may be encoded per centimorgan. Furthermore, repeated detection of QTL could be the result of autocorrelation between traits. For example, EBF is highly correlated with LBF (phenotypic $r_{\text{AVG}} = 0.86$); the measurement of both traits relies on the date of lammas flush, so it is not surprising that the genome scans for these two traits are similar.

QTL for growth initiation interacting with winter chill and flushing temperature: The timing of spring bud flush in Douglas fir varies depending on geographic origin. Elevation and latitude, which largely determine winter and spring temperatures, have been shown to play a critical role in adaptation to the timing of dormancy release and the initiation of shoot growth (CAMPBELL and SORENSEN 1978; CAMPBELL 1986). Because QTL $\times$ E interactions were identified for bud flush in a previous Douglas fir study (JERMSTAD *et al.* 2001a), we expected to find QTL interacting with key environmental signals (*i.e.*, winter chill and flushing tempera-

ture) that influence this trait. We detected one QTL $\times$ T interaction with winter chill ($P \leq 0.005$) and one QTL $\times$ T interaction with flushing temperature ($P \leq 0.005$). The QTL interacting with winter chill on LG 2 is at the same map location as a QTL interacting with site at the Longview and Springfield test sites (Figure 15). It is plausible that the interaction with site detected in the field experiment is actually a QTL interaction with winter chill.

QTL for growth cessation interacting with day length and moisture stress: In the same manner that winter and spring temperatures vary at different elevations and latitudes and affect the timing of growth initiation, photoperiod and moisture stress also vary and play a critical role in determining when a tree ceases annual growth and prepares for winter dormancy. QTL interacting with light quality and moisture stress have been successfully mapped in other plant species. QTL for growth interact with moisture stress in barley (TEULAT *et al.* 2001) and in *Arabidopsis thaliana*, QTL for germination, hypocotyl length, rosette leaf number, and date of flowering interact with light quality (VAN DER SCHAAR *et al.* 1997; STRATTON 1998; BOREVITZ *et al.* 2002). In this experiment, we tested whether QTL controlling growth cessation and growth-rhythm traits interact with day length or moisture stress. A small number of QTL that interact exclusively with each environmental signal were detected. The only traits for which QTL $\times$ T interactions were not detected were TBS, PLF, and HT2. In addition, we found QTL for several traits (HT1, EBF, DCG, and DSE) that interacted with both environmental signals. Twice as many QTL $\times$ T interactions with day length (seven) as those with moisture stress (three) were detected among all traits evaluated in the growth cessation experiment. Because of the possible confounding effect of temperature associated with the day length treatments, some of the QTL $\times$ T interactions with day length may actually be due to an interaction with temperature. In some populations of Douglas fir, temperature interacts with day length to optimize growth processes (CAMPBELL and SORENSEN 1978; MACDONALD and OWENS 1993). A future experiment might include temperature as an additional treatment.

QTL-by-site interactions for bud flush at the field test sites: Replicated tests have been used in a small number of angiosperm species for identifying QTL-by-environment interactions (STUBER *et al.* 1992; WU *et al.* 1998; SOURDILLE *et al.* 2000; SARANGA *et al.* 2001; TEULAT *et al.* 2001). We previously evaluated QTL for bud flush in replicated test environments (JERMSTAD *et al.* 2001a) and found only a small fraction of QTL repeatedly detected at both sites. This result was possibly due to the small number of clones ($n = 78$) established at one of the test sites (Figure 1A). In the current experiment, we used the same three-generation pedigree as in the previous study, but planted a large and equal number of clones (progeny) at each of two sites. Genome scans

for each of the two sites were compared and QTL $\times$ S interactions were estimated. Five of the six QTL were detected at both sites (LGs 2, 6, 8, and 12; Figure 15), one of which (LG 2) showed an interaction with site. Furthermore, genome scan profiles for both sites were remarkably similar, regardless of whether the critical F -value threshold was met. This finding is encouraging, given that so few QTL were found in common between previously replicated field tests. It appears that the large number of progeny used in the current field study contributed to the repeatability of detection. Although the test sites differed in elevation, latitude, and climate, nearly all of the QTL detected in this experiment were detected at each site. This finding supports the assertion that bud flush is a highly heritable trait (REHFELDT 1983; LI and ADAMS 1993; AITKEN and ADAMS 1997).

It is important to evaluate the reliability of QTL detection, but this is rarely done in forest trees due to a variety of constraints. Replication of QTL experiments is rarely performed and comparisons of QTL detected in different mapping populations are confounded by either genetic background or environment (BROWN *et al.* 2003). In this study, the same clonal mapping population was tested for the timing of bud flush at each of two field sites. In addition, the QTL detected in the field sites were compared to QTL for bud flush detected in the growth initiation and growth cessation experiments (Figures 2, 3, and 15). In spite of the environmental variation introduced in this study, repeated detection of QTL was observed on LGs 2, 5, 6, 8, and 12, affirming the reliability of detection methods used and the spatial stability of QTL governing bud flush. QTL on LGs 2 and 12 were detected in the growth initiation, growth cessation, and field experiments. By comparative mapping, QTL for bud flush reported in this article were compared with those detected in the earlier study (JERMSTAD *et al.* 2001a); the QTL on LG 2 was detected in all genetic tests evaluated.

CONCLUSION

QTL interacting with the environment are of great interest to plant physiologists and geneticists wishing to understand the effects of specific environmental signals and the genetic and biochemical responses they induce. Phenology of flowering time (SHELDON *et al.* 2000), vernalization requirement (JOHANSON *et al.* 2000; OKA *et al.* 2001), dormancy release (LOPEZ-MOLINA *et al.* 2001), cell cycling (DEN BOER and MURRAY 2000), and the response to environmental signaling, including plant hormones (FANKHAUSER 2002; GARCÍA-MARTINEZ and GIL 2002), have been studied in depth in angiosperm species. Genotype-by-environment interactions for growth and phenology traits in Douglas fir were known to exist on the basis of findings from previous genetic research, but the loci responding to key environmental signals were not known. We have identified several QTL that

interact with environmental signals governing the growth cycle in Douglas fir. We have also discovered that the genes governing predetermined growth are unique from those governing free growth, or lammass growth, which frequently occurs in young trees. Several QTL for terminal bud flush have been identified in multiple genetic tests, indicating that a subset of QTL controlling this trait are spatially stable and can be detected in diverse environments. The knowledge gained from this study will prove invaluable in current efforts to identify growth cycle candidate genes for use in association studies and detection of allelic variation contributing to patterns of phenology and growth.

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