Chapter 7. Additional Studies Using CFIRP Treatments: Douglas-fir Genetics and Ambrosia Beetle Log Colonization

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As highlighted in previous chapters, the primary biological objectives of CFIRP were to assess impacts of diverse silvicultural treatments on vegetation structure and growth and on the abundance and diversity of wildlife. Stand conditions resulting from implementation of the CFIRP research design, however, provided for the overlay of additional research projects that utilized subsets of CFIRP units. Described below are studies focused on genetic ramifications of the CFIRP silvicultural treatments, and chemical and biological interactions between ambrosia beetles (family Scolytidae) and aging Douglas-fir (*Pseudotsuga menziesii*) logs.

Silvicultural Treatments and the Genetics of Douglas-fir

Genetic Diversity

The potential genetic impacts of silvicultural manipulation are numerous and they may precipitate biodiversity changes not only at the stand scale but also at population and landscape scales. Although silvicultural practices can have positive or negative consequences on the genetic composition of managed forests, the research described here focused on potential negative effects.

Dysgenic selection within a stand may occur with silvicultural practices, and managed stands allowed to regenerate naturally may experience reduced genetic variability if only a few parent trees contribute seed to the next generation. Increased inbreeding is also a possibility if wide spacing of leave trees enhances self-fertilization or if mating occurs between neighboring leave trees that are close relatives (Adams and Birkes 1991; Mitton 1992). Dysgenic selection, reduced genetic variability, and increased inbreeding also may occur with artificial regeneration if seeds are collected from limited parents, from isolated trees with restricted outcrossing, from inferior phenotypes, or from trees with genotypes maladapted to the planting site. Although all of these undesirable genetic situations are possible in managed forests, careful attention to the genetic consequences of management practices can ameliorate or prevent them from occurring.

Reduction of genetic variation within tree populations is meaningful because it may lead to increased vulnerability to pest attack and climatic extremes, and to reduced ability of species to evolve in response to changing environments (Ledig 1986; Millar and Libby 1991). Despite the obvious importance of genetic variability for healthy and resilient tree populations, and the concerns expressed among forestry professionals regarding possible negative genetic impacts of forest management (Society of American Foresters 1991), little data are available to objectively assess the potential implications of diverse silvicultural practices on levels of genetic diversity (Savolainen and Kärkkäinen 1992). The research summarized below (Adams et al. 1998) helps fill this information void and adds stand genetic diversity to the varied list of topics tackled under CFIRP. The overall objective of the research was to assess the impact of the three silvicultural treatments (small patch cut, two-story, and clearcut) on the genetic composition of the overstory Douglas-fir left unharvested and of both natural- and artificial-regenerated seedlings.

Research Strategy

Allozymes are various forms of an enzyme that have the same activity but which differ slightly in amino acid sequence; they are produced by different alleles at a single genetic locus. Alleles are genes governing variations of the same trait. Because allozymes are relatively inexpensive to assay, are readily interpretable, provide genetic information at the level of individual genes, and are largely unassociated with patterns of environmental variation, they are widely applicable in genetic studies of forest trees (Adams et al. 1992b). For these reasons, allozymes were chosen to assess the impact of silvicultural treatments on the genetic diversity of seedlings and overstory trees.

Table 7-1. CFIRP stands used to assess genetic diversity of mature and naturally regenerated Douglas-fir among silvicultural treatments.

Replication (block)	Treatment	Stand number[1] Mature trees	Natural regeneration
Saddle	Control	11	—
	Small patch cut	9 & 10[2]	—
	Two-story	2	2
	Clearcut	—	1
Peavy	Control	1	—
	Small patch cut	5	—
	Two-story	4	4
Dunn	Control	5	—
	Small patch cut	7	—
	Two-story	8	8

[1] See Appendix A for stand locations.

[2] Stands were combined and treated as one sample unit.

Eleven stands across all the CFIRP location replicates were chosen for allozyme analysis; Saddle 9 and 10 were combined and treated as a single stand (Table 7-1). Near the center of each of the 10 functional stands, a rectangular sampling plot averaging 6.5 ha (16 ac) was established. In all plots except the clearcut, twigs containing dormant buds were collected in winter 1992-93 from 120 mature trees within each plot (n = 1080). The same winter, dormant buds also were collected from 120 seedlings of each of the seven planting stocks (n = 840; see Chapter 2). Because natural regeneration was poor in the three small patch cuts sampled and in the Peavy and Dunn clearcuts (Ketchum 1995), dormant buds from 120 natural seedlings per plot were collected only from the three two-story replicates and from the Saddle 1 clearcut (n = 480; Table 7-1). Seedling buds were collected during the winters of 1994-95 and 1995-96. Allozyme analyses were performed on bud tissues according to the procedures described by Adams et al. (1990). Twelve enzyme systems (ACO, PGM, PGI, SDH, GDH, GOT, G-6PD, F-EST, 6-PGD, IDH, DIA, and MDH) and 17 loci coding allele variants of these enzymes were assayed.

Three parameters were used to estimate genetic diversity: number of alleles per locus, percentage of polymorphic loci, and expected heterozygosity (the probability that two alleles sampled at random from two individuals in the same population are different). Number of alleles per locus and expected heterozygosity were calculated separately for each locus, then averaged over all 17 loci. Contingency χ^2 statistics were used to test the significance of allele-frequency differences between population samples ($\alpha < 0.01$). The degree of inbreeding was estimated with the fixation index (Nei 1987, p. 155) which equals 0 when there is no inbreeding and is > 0 and ≤ 1 when inbreeding is present. Differences between treatments in gene diversity and inbreeding were tested using t-tests ($\alpha < 0.10$; Steele and Torrie 1980), and the extent of genetic differentiation among populations was evaluated by calculating Nei's (1978) unbiased genetic distance (d). Most calculations were performed using the computer program BIOSYS-1 (Swofford and Selander 1989).

Research Findings

Control Stands

Consistent with earlier reports (Shaw and Allard 1982a; Neale 1985; Moran and Adams 1989), mature Douglas-fir in untreated (i.e., control) stands manifest considerable variation at allozyme loci (Figure 7-1). All loci with the exception of *Gdh* were polymorphic, and up to four alleles were detected per locus. Expected heterozygosity was high, no inbreeding was detected, and genetic differentiation among control populations was small (mean d = 0.0033), with allele frequency heterogeneity significant ($P < 0.01$) at only five loci.

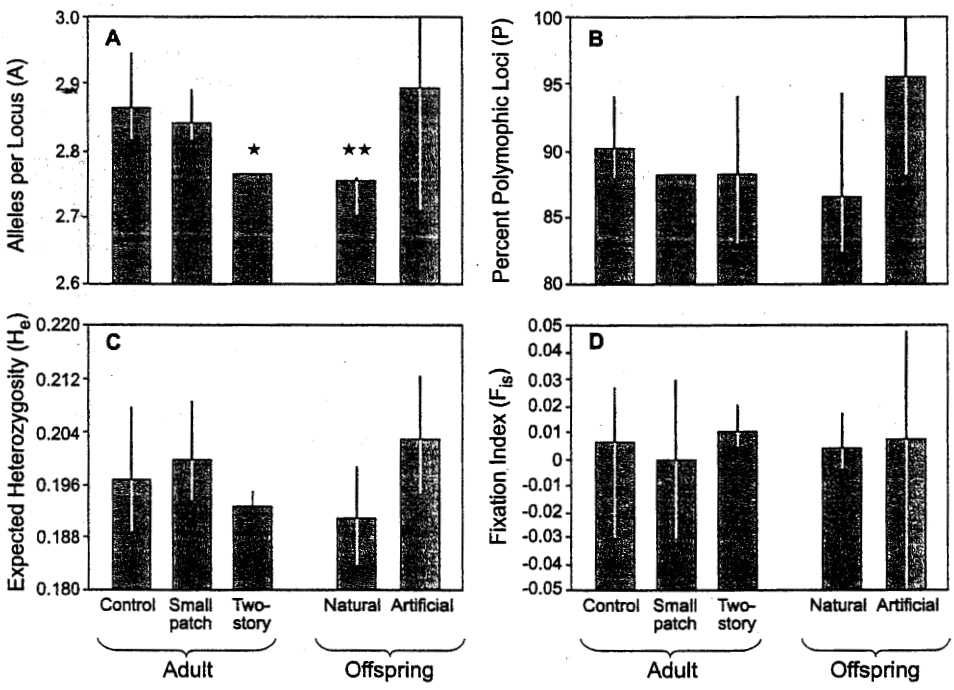

*Figure 7-1. Mean estimates of gene diversity parameters and fixation index for three adult (control, small patch cut, two-story stand) and two offspring (natural and artificial) population types of coastal Douglas-fir growing in the same vicinity. Brackets with each bar give the range over replicates (3 for each adult population type, 4 for natural offspring, 7 for artificial offspring). Based on 17 allozyme loci for gene diversity statistics and 9-11 loci for fixation index (n = 120 for each population replicate). * and ** represent significant differences from the control at P < 0.10 and P < 0.5, respectively). Modified from Adams et al. 1998.*

Post-harvest Overstory Trees

Percent polymorphic loci, expected heterozygosity, and the fixation index were comparable between control trees and residual overstory trees in small patch cut and two-story stands. Cutting the smallest trees in the two-story treatment, however, precipitated the removal of rare alleles such that significantly fewer alleles per locus

were observed among residual trees (mean = 2.76) compared with control trees (mean = 2.86). Residual small patch cut and two-story trees differed in allele frequencies from control stands in the same block at an average of only two loci. In addition, genetic distances between control stands and residual trees in the two partial-harvest treatments were very small, averaging 0.0020 for both comparisons. The results suggest that partial harvesting had minimal influence on the genetic composition of mature trees in the treated stands.

Natural Regeneration

Naturally regenerated seedlings had significantly fewer alleles per locus (mean = 2.75) than found in the overstory of the control stands (mean = 2.86), but were similar to the controls in percent polymorphic loci and expected heterozygosity. Apparently, the reduced number of alleles in natural regeneration is simply a reflection of losses in rare alleles due to harvesting, because the number of alleles per locus in naturally regenerated seedlings was nearly the same as observed in their putative parents (remaining overstory trees in the two-story plots, including the one clearcut sampled). Fixation indices were close to zero indicating that inbreeding was insignificant or that inbreds were lost prior to sampling because of poor germination or survival of inbred individuals (Sorensen and Miles 1982). Genetic distances between naturally regenerated seedlings and controls were small (mean d = 0.0028).

Artificial Regeneration

Number of alleles per locus, percentage of polymorphic loci, and expected heterozygosity all were significantly greater in artificially regenerated seedlings than in seedlings regenerated naturally. In comparison with control trees, artificial regeneration had similar numbers of alleles per locus and expected heterozygosity, but larger percentages of loci were polymorphic. Since artificial stocks generally include seeds from multiple and widely scattered stands, it was not unexpected to find greater gene diversity in artificial regeneration than in natural regeneration of individual populations. Fixation indices in artificial regeneration were not significantly different from zero, indicating again that inbreds are rare or non-existent. Genetic distances (mean d = 0.0020) between control trees and artificial regeneration were no greater than observed between controls and naturally-regenerated seedlings.

Conclusions

With the exception of some rare alleles lost in the two-story treatment, harvesting followed by natural regeneration had little impact on the allozyme composition of Douglas-fir stands in this study. Although rare alleles may be deleterious under current environmental conditions (Bongarten et al. 1985; Strauss and Libby 1987; Bush and Smouse 1992), their importance for adaptation in future environments is unknown. Thus, in stands designated as gene conservation reserves and managed under a two-story regime, trees chosen to be left as parents of the next generation should include a range of sizes in order to maximize retention of alleles. The lack of significant changes in all measured levels of genetic diversity, with the exception

of rare alleles, suggests that overstory trees would have to be harvested more intensively than in the partially harvested stands in this study before losses in allelic diversity due to genetic drift are detectable. Previous studies reached similar conclusions (Neale 1985; Savolainen and Kärkkäinen 1992).

This study also revealed that artificial regeneration had greater levels of genetic diversity than natural regeneration. Because seeds used in artificial seedling stocks come from a variety of wild stands each with somewhat different allele frequencies, they reflect a genetic range that is greater than that found in any single stand (Adams et al. 1992a). If large numbers of parents consistently are involved in the production of planting stock, regenerated stands will likely possess considerable genetic diversity, and thus, a range of genotypes, including individuals adapted to the extreme conditions that might be experienced during the life of a stand.

Gene Dispersal

Gene dispersal via pollen and seeds has a major influence on the amount and distribution of genetic variation within populations. In addition, the extent of gene dispersal within stands has important practical implications for forest management. The degree of cross-pollination among trees influences the validity of open-pollinated seed lots used for genetic testing of parent trees and the choice of sampling strategies employed in collecting seed for reforestation and gene conservation. The extent of seed dispersal influences the spatial distribution of offspring in natural regeneration. In particular, limited seed dispersal may result in clustering of relatives and subsequent inbreeding in the next generation.

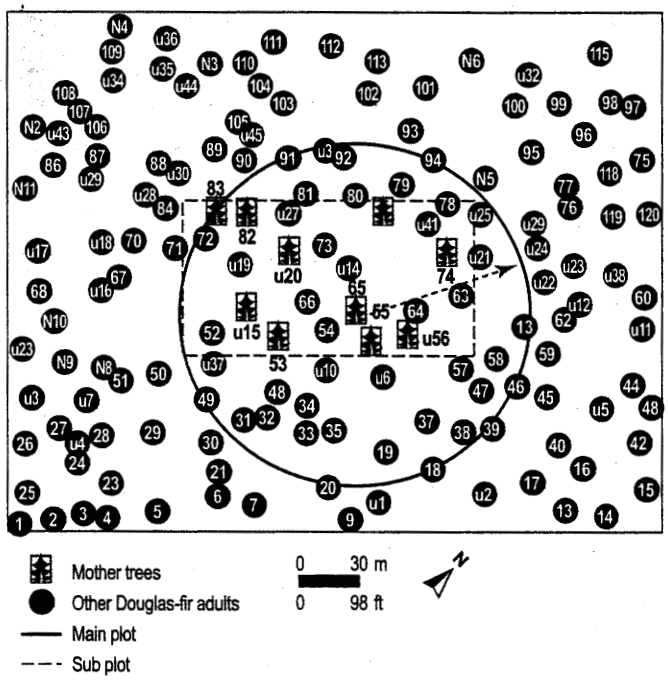

Figure 7-2. Map of the study plot in the Peavy 4 two-story stand showing the location of adult trees. Also shown is an example of a 70-m (230-ft) radius neighborhood for mother tree 65.

Research Strategy

Materials

Effective gene dispersal of Douglas-fir was investigated by applying mating models that account for the composition of allozyme genotypes observed in the offspring of individual mother trees (i.e., pollen dispersal) and seed dispersal models that account for the spatial distribution of offspring genotypes on the ground. The study focused on gene dispersal in the two-story CFIRP stands Saddle 2 and Peavy 4.

All adult trees within large rectangular plots in the two-story stands (n = 99 and 163 trees in Saddle and Peavy, respectively) were mapped according to position using surveying methods (Figure 7-2) and diameters at breast height (DBH) recorded. Density of leave trees in Peavy (29 trees/ha [12 trees/ac], 19 m [62 ft] spacing) was nearly twice that in Saddle (15 trees/ha [6 trees/ac], 26 m [85 ft] spacing). A rectangular subplot was

designated within each plot (dashed-line box in Figure 7-2), such that distances from the edge of the subplot to the boundaries of the main plot were always at least 70 m (230 ft). Cones (seeds) were collected in August 1993 from the upper crowns of eight mother trees in the Saddle subplot and 10 trees in the Peavy subplot. These seeds subsequently were used for the pollen dispersal analysis. Seed traps (0.6 m^2 [6.5 ft^2]) were placed on the ground in a grid pattern within each subplot and seed collected throughout the summer, fall, and winter of 1993. These seeds were used for the seed dispersal analysis.

To evaluate pollen and seed dispersal, a subset of seven of the 17 allozyme loci used in the genetic diversity analysis that were highly variable and could be readily scored in both dormant buds (adult trees) and in seeds were utilized. Genotypes of most of the adult trees at these seven loci were available from the genetic diversity analysis, although some additional adults (~60) not sampled previously also were scored. In total, the 7-locus genotypes of 408 seeds from mother trees at Saddle (50–54 seeds per tree), 378 seeds from the mother trees at Peavy (23–55 seeds per tree), and 130 and 157 seeds, respectively, from the Saddle and Peavy seed traps, were determined. The female gametophyte and embryo tissues of the seeds were assayed separately. The female gametophyte is haploid and has the same genetic constitution as the egg cell contributing to the embryo (i.e., female gamete). The diploid embryo has genes from both parents, and by accounting for the mother's contribution with the female gametophyte, the haploid genotype of the pollen grain contributed by the father (i.e., pollen gamete) can be inferred.

Estimation Methods

Gene dispersal models were fitted to the observed frequencies of 7-locus genotypes in the pollen gametes of seeds collected from mother trees in order to estimate levels of self-fertilization and patterns of outcrossing, including distance and direction of effective pollen dispersal. Similar models were applied to genotypic arrays of female gametes in seeds sampled on the ground to estimate effective seed dispersal. The basic model, described for pollen dispersal, is shown in Figure 7-3 (Burczyk et al. 1996). In this model, a circular area around a mother tree (M) is called a neighborhood. The probability of observing 7-locus genotype g_i in the pollen gamete of a seed from this tree is

$$p(g_i) = s\, p(g_i|M) + (1-m-s) \Sigma \phi_j p(g_i F_j) + m\, p(g_i|B),$$

where

s is the proportion of self-fertilized seeds,

m is the proportion of pollen gametes from sources outside the neighborhood,

1-m-s is the proportion of pollen gametes from males within the neighborhood,

ϕ_j is the relative mating success of the j^{th} male within the neighborhood,

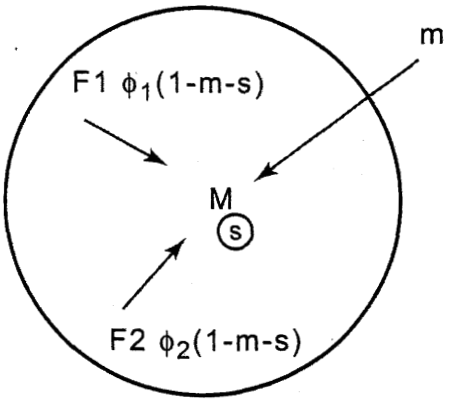

Figure 7-3. Pictorial depiction of the neighborhood model, where the male parentage of the offspring on an individual mother tree (M) is ascribed to three sources: self-fertilization (with probability s), mating with males outside the neighborhood (with probability m), and mating with specific males (F_j) within the neighborhood [with probability (1-s-m)ϕ_j]. Modified from Burczyk et al. 1996.

$p(g_i|M)$ is the probability that the mother tree produces gametes with genotype g_i,

$p(g_i|F_j)$ is the probability that the j^{th} male within the neighborhood produces this gamete, and

$p(g_i|B)$ is the probability that pollen grains produced by sources outside the neighborhood have genotype g_i.

Mating success of males within the neighborhood was assumed to be a function of one or more of the following factors: distance of the male from the mother tree, size (DBH) of the male, and the cardinal direction of the male to the mother tree. An exponential function was used to relate mating success to these factors, with one parameter for each factor (Burczyk et al. 1996). Thus, the largest model we tested for effective pollen dispersal included five parameters: s, m, and β, γ, δ. The latter three parameters related ϕ_j to distance to the mother tree (β), DBH of the male (γ), and cardinal direction of the male to the mother tree (δ). Maximum likelihood methods were used to evaluate the fit of the models (Burczyk et al. 1996). When individual parameter estimates significantly ($P < 0.05$) increased the likelihood of the model, they were retained in the model and their values reported. Otherwise, the estimated parameters were assumed to equal zero.

For seed dispersal, the center of the neighborhood is the seed trap and the model is modified such that s is deleted, m is the proportion of seeds dispersed into the trap from mother trees outside the neighborhood, and ϕ_j is the relative contribution of the j^{th} mother tree in the neighborhood to seeds in the trap. We chose a distance of 70 m (230 ft) for the radius of the neighborhoods. This radius included an average of 18 trees in the Saddle two-story stand and 45 trees in Peavy. At 70 m (230 ft), the neighborhood radius is only 1.5 to 2 times the height of the adult trees. In retrospect, we wish we had chosen somewhat larger neighborhoods (say 100 m [328 ft]), but the larger the neighborhood radius, the larger the plot size required and the more adult trees that have to be mapped and genotyped. Because the main interest in this study was to evaluate the extent to which mating in natural stands is restricted to near neighbors and the degree to which seed dispersal may be restricted, the size of the neighborhoods was adequate for this purpose.

Research Findings

Effective Pollen Dispersal

In both the Saddle and Peavy two-story stands, the great majority of effective pollen came from sources outside the 70 m (230 ft) neighborhoods (m = 0.89 and 0.74, respectively; Figure 7-4). As expected, m is smaller at Peavy ($P < 0.05$) where tree density is greater and neighborhoods included more males. In both stands, the proportion of selfed (s) offspring was estimated to be zero, which is consistent with the low levels of selfing observed previously in Douglas-fir (El-Kassaby et al. 1981; Shaw and Allard 1982b; Neale and Adams 1985). Mating success of males within neighborhoods was not significantly related to either distance from the mother

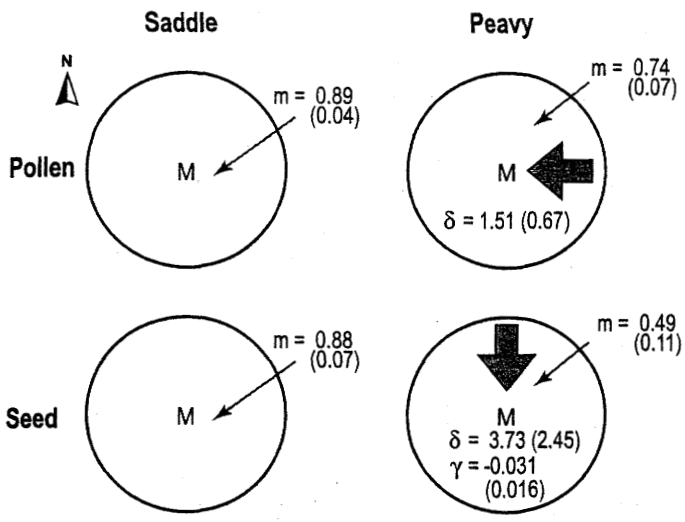

Figure 7-4. Neighborhood model parameter estimates (standard errors in parentheses) for effective pollen and seed dispersal in the Saddle (15 trees/ha; 6 trees/ac) and Peavy (29 trees/ha; 12 trees/ac) two-story stands. m = proportion of pollen gametes in the offspring of mother trees (or seeds in a seed trap) from sources outside the 70-m (230-ft) radius neighborhood. γ and δ are parameters that relate the relative contributions of males (or females) within neighborhoods to pollen gametes (or seeds) depending on their size (DBH) and the cardinal direction to mother trees (or seed traps).

tree or DBH of males (i.e., $\beta = \gamma = 0$). The only deviation from random mating detected within neighborhoods was a tendency at Peavy for males in an easterly direction from mother trees to be more successful in mating than those from other directions (estimated $\sigma = 1.51$). A σ of 1.51 indicates that neighborhood males east of mother trees are 20 times more successful in mating than those west of mother trees. Perhaps this is due to wind direction within the stand during pollen shed, but the standard error on the estimate is large.

Effective Seed Dispersal

Most seed also appears to have come from trees outside the neighborhoods in the Saddle two-story stand (m = 0.88), while about one-half of the seeds sampled at any one location at Peavy came from trees more than 70 m (230 ft) away. The greater density and smaller size of trees at Peavy (mean height = 38 m [125 ft], versus 46 m [151 ft] in Saddle) probably resulted in more limited seed dispersal in this stand. Topography of the two sites may also be a factor. Saddle is on a north-facing slope, while Peavy is on flat terrain. Interestingly, both directionality and size of mother trees significantly influenced seed dispersal at Peavy (i.e., estimates of both γ and σ are significantly different from zero). More seed came from trees north of traps than elsewhere, and from smaller trees than larger trees (because estimated γ is negative). The latter finding is the reverse of expectation because fecundity is expected to be positively associated with tree size. Perhaps smaller trees are more vigorous and responded more readily to release following harvesting.

Conclusions

It is evident that individual mother trees mate with large numbers of males in the two-story stands that were studied. This is consistent with the large genetic diversity and low levels of inbreeding observed in the natural regeneration of these stands (Figure 7-1). Seeds from relatively few females capture much of the genetic diversity in a local population. This means that seed from a relatively small number of trees is adequate to characterize the genetic makeup of a stand in provenance studies or in collections made for gene conservation purposes. In addition, the large number of males mating with each female means that open-pollinated seed lots are adequate for evaluating the breeding value of individual mother trees in progeny tests (i.e., breeding values are not biased because only a few, perhaps unrepresentative, male parents are involved in mating). Broad seed distribution within the two-story stands indicates that natural regeneration under this system does not lead to tight spatial clustering of close relatives and potentially increased inbreeding in the next generation.

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Interactions between Ethanol and Ambrosia Beetles in Aging Douglas-fir Logs

Ambrosia beetles (family Scolytidae) help initiate log decomposition by boring into the sapwood where they rear young in excavated egg galleries and cultivate fungi (ambrosia) for food (Dowding 1984; Carpenter et al. 1988; Schowalter et al. 1992). Their pin-hole-size tunnels stained by fungus can cause considerable damage to the wood and decrease economic values of commercial logs and lumber (McLean 1985). Conifer logs in the Pacific Northwest are attacked most heavily by ambrosia beetles in spring, with Douglas-fir and western hemlock (*Tsuga heterophylla*) their preferred hosts (Johnson 1958; Chapman 1961; Zhong and Schowalter 1989).

Ethanol is synthesized in plant tissues by anaerobic respiration when the O_2 supply is inhibited (Kimmerer and MacDonald 1987; Kimmerer and Stringer 1988; Harry and Kimmerer 1991; MacDonald and Kimmerer 1991). Ethanol was found in tissues of aging conifer logs under attack by ambrosia beetles (Cade et al. 1970; Moeck 1970), and subsequent experiments demonstrated ethanol would attract ambrosia beetles to artificial traps (Moeck 1970; Nijholt and Shönherr 1976; Klimetzek et al. 1986; Liu and McLean 1989; Schroeder and Lindelöw 1989). In addition, these beetles may respond synergistically to traps releasing their pheromones in combination with ethanol, or ethanol plus α-pinene, one of the monoterpenes found in most conifer tissues (Vité and Bakke 1979; Borden et al. 1980; Shore and McLean 1983).

Temporal Variability in Ethanol Concentrations and Densities of Ambrosia Beetle Galleries

Despite the economic and ecological impacts of ambrosia beetles and their known response to ethanol, the process of ethanol synthesis and accumulation in logs under field conditions was poorly understood when CFIRP was initiated. Thus, research consistent with the CFIRP design was implemented to determine relative concentrations of ethanol in Douglas-fir logs felled during fall, winter, and spring, and to confirm a relationship between log ethanol concentrations and subsequent densities of ambrosia beetle gallery holes.

Research Strategy

This study was conducted in the Dunn 4 strip cut stand harvested in August 1991. Eighteen Douglas-fir trees destined for snag creation (see Chapter 2) were topped at 15–18 m (50–60 ft) above ground; six trees were topped in November 1991, six in January, and six in March 1992. A 2.4-m (8.0-ft) log was cut and delimbed from the base of each crown the day after felling. Phloem and sapwood samples were then collected with an increment borer along the top, sides, and bottom of each log. These samples were analyzed by gas chromatography to determine ethanol concentrations (see Kelsey 1994a for detailed methodologies). November-felled logs were resampled in January and March, and January-felled logs were resampled in March for ethanol analysis. Densities of *Trypodendron lineatum* and *Gnathotrichus retusus* gallery holes in the sapwood were determined for each log in August 1992. Their entrance holes were separated based on size (Kinghorn 1957).

Research Findings

Ethanol concentrations in tissues from freshly felled logs were similar among dates, but concentrations in the phloem were consistently higher than in sapwood (Figure 7-5; Kelsey 1994a). By January, sapwood ethanol concentrations had increased significantly in November-felled logs with quantities similar to the phloem. After four months on the ground, ethanol concentrations had increased four times in the phloem and 83 times in the sapwood. In contrast, ethanol concentrations in January-felled logs during their first two months on the ground remained unchanged on the tops, and decreased on the bottoms and sides.

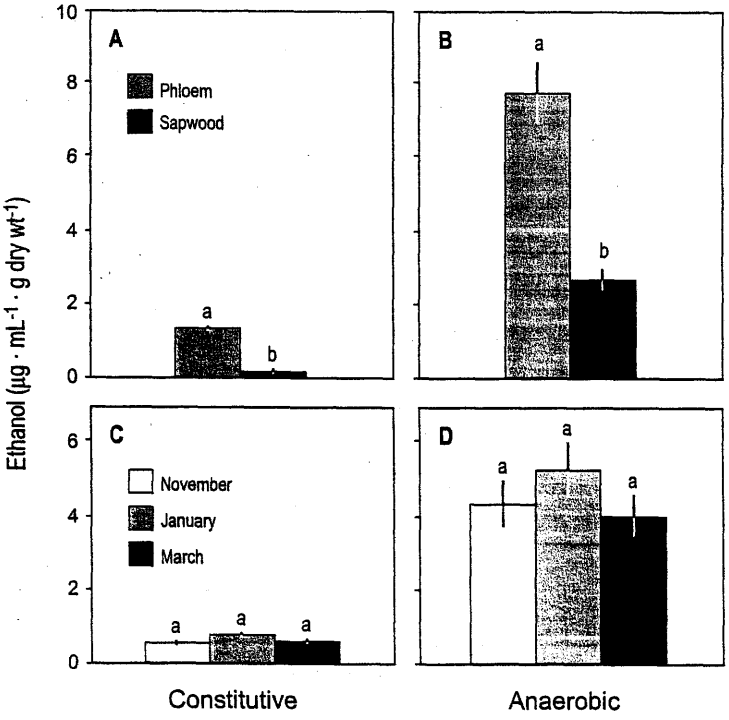

Figure 7-5. Constitutive and anaerobic ethanol concentrations (mean ± standard error) among tissues (A and B, n = 18) and felling dates (C and D, n = 6) from freshly felled logs. Anaerobic concentrations were measured after inducing the tissues to synthesize ethanol by incubating in a N_2 atmosphere for 24 hours at 30°C (86° F). Within each graph, bars with the same lowercase letter are not significantly different (P < 0.5). Modified from Kelsey 1994a.

Ethanol accumulation in November-felled logs indicates their tissues were or had been respiring anaerobically. Because January-felled logs did not accumulate ethanol, but had the potential to do so as indicated by laboratory experiments (Figure 7-5B and C; Kelsey 1994a), something in the environment caused these logs to respond differently. A review of the timing and quantity of rainfall from November to March revealed that November-felled logs received rainfall sooner, more consistently, and in greater quantities than January-felled logs. This most likely contributed to the generation of hypoxic conditions and prolonged periods of anaerobic respiration in November-felled logs. Hypoxic conditions probably were never established in January-felled logs; consequently, they had much lower ethanol concentrations.

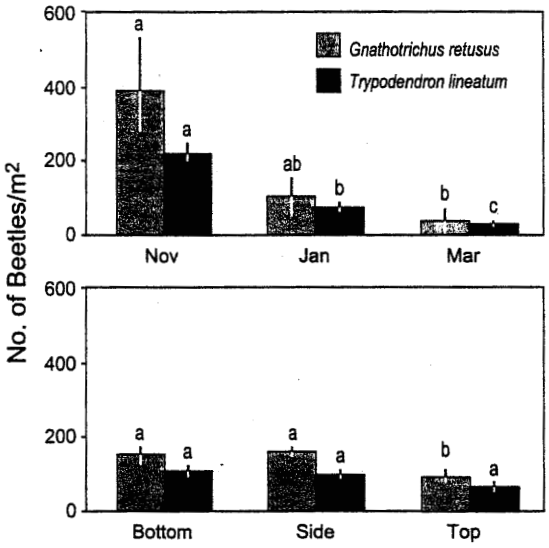

Figure 7-6. August densities of ambrosia beetles (mean ± standard error) in Douglas-fir logs felled the previous November, January, or March (n = 6) and at various positions on the logs (n = 18). Within each graph, bars with the same lowercase letters within species are not significantly different (P < 0.05). Modified from Kelsey 1994a.

When α-pinene is released simultaneously with ethanol, there can be a synergistic attraction of *T. lineatum*, provided α-pinene release rates are not too high (Schroeder and Lindelöw 1989). In March when beetles began to attack, the three age groups of logs differed in their ratio of ethanol/α-pinene concentrations. Phloem and sapwood ratios were 6.5 and 6.1, respectively, for November-felled logs; 0.4 and 0.2 for January-felled logs; and 0.9 and 0.02 for March-felled logs. Thus, November logs had ethanol/α-pinene ratios more attractive to ambrosia beetles.

In August 1992, densities of *T. lineatum* and *G. retusus* were highest in November-felled logs and progressively lower in January- and March-felled logs (Figure 7-6; Kelsey 1994a). Densities of *G. retusus* gallery holes were correlated with ethanol concentrations in the phloem (r^2 = 0.6735) and sapwood (r^2 = 0.731). Densities of *T. lineatum* gallery holes increased with increasing ethanol concentrations up to a maximum and then decreased, suggesting repellency at high ethanol concentrations.

Conclusions

The relationships between log ethanol concentrations and densities of ambrosia beetle galleries are consistent with ethanol functioning as an attack stimulant (McLean and Borden 1977) and as a key factor in ambrosia beetle preference for aging logs. Post-harvest log treatments that minimize ethanol accumulation could reduce attack densities of ambrosia beetles.

Ethanol Accumulation and Densities of Ambrosia Beetle Galleries in Logs with and Without Branches

Observations during the study summarized above, and with logs similarly cut in a second stand nearby, indicated that branched crowns of November-felled logs were only lightly attacked by ambrosia beetles compared to delimbed logs. It was hypothesized that branch retention restricts ethanol accumulation in logs and reduces their attraction of ambrosia beetles. This hypothesis was tested by measuring ethanol concentrations in branched and delimbed logs while they were being attacked and colonized by beetles in May.

Research Strategy

This study was conducted in the Dunn 2 (large patch cut) and Dunn 4 (strip cut) CFIRP stands. In November 1991, the fall after harvest, six Douglas-fir trees within the residual portions of each stand were selected to become wildlife snags, and their crowns felled at 15–18 m (50–60 ft) above ground. The day after felling, a 2.4-m (8.0-ft) log was cut from the base

of each crown and delimbed. The remaining portion of crown was left as one long piece with branches attached [mean crown length ± SE was 15.6 ± 1.5 m (51.2 ± 4.9 ft)]. Diameters of delimbed [53.8 ± 3.8 cm (21.2 ± 1.5 in.)] and branched [49.3 ± 3.6 cm (19.4 ± 1.4 in.)] logs were not significantly different. Tissues from all logs were sampled in May to ascertain ethanol, acetaldehyde (the metabolic precursor to ethanol during anaerobic respiration), α-pinene, and water concentrations (see Kelsey 1994b for detailed methodologies). Densities of ambrosia beetle gallery holes in the sapwood were determined for each log in August 1992.

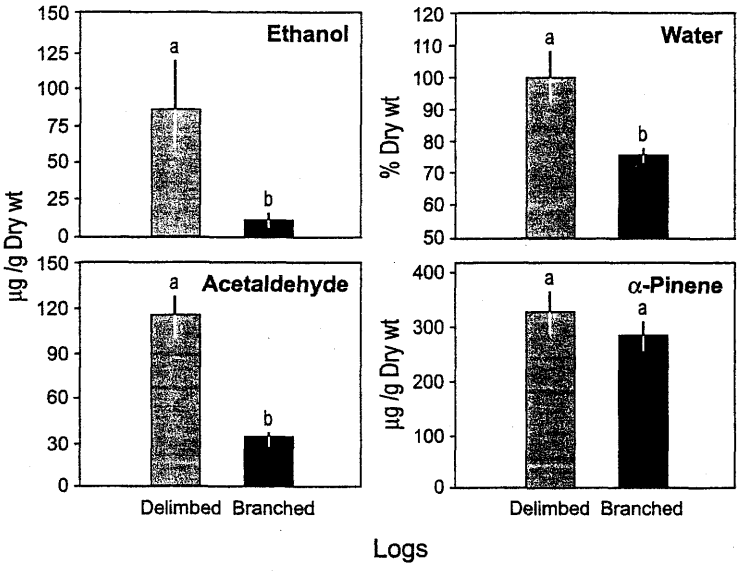

Figure 7-7. Ethanol, acetaldehyde, water, and α-pinene concentrations (mean ± standard error, n = 10) in delimbed and branched logs when sampled in May. Modified from Kelsey 1994b.

Research Findings

Ethanol, acetaldehyde, and water concentrations in delimbed logs were significantly higher than in branched logs, but α-pinene was not (Figure 7-7; Kelsey 1994b). Delimbed logs were attacked by ambrosia beetles in March, whereas attacks on branched logs did not occur until much later. Densities of *T. lineatum* and *G. retusus* galleries in August were 16 and nine times greater, respectively, in delimbed logs than in branched logs (Figure 7-8; Kelsey 1994b). *Trypodendron lineatum* showed no preference for log positions, whereas *G. retusus* attacked the sides more heavily than tops, despite greater quantities of ethanol in log tops than sides. These position preferences are not always consistent across studies (Prebble and Graham 1957; Dyer 1963; Lindgren et al. 1982) and may be related to levels of sunlight logs receive. Multiple regression analyses revealed that densities of *T. lineatum* galleries were best explained by the interaction between ethanol concentrations and log type (branched or unbranched), while densities of *G. retusus* galleries were explained by an interaction between acetaldehyde concentrations and position on the log.

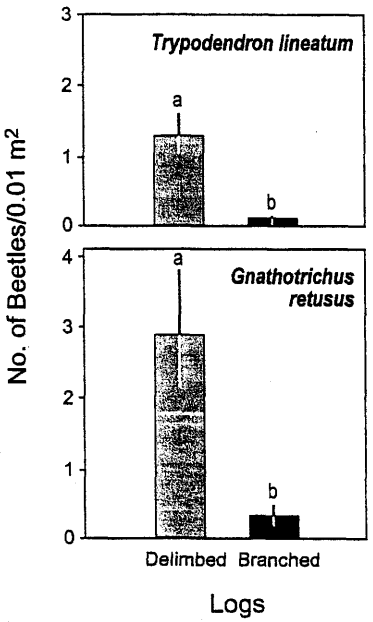

Figure 7-8. August densities of ambrosia beetles (mean ± standard error, n = 10) in delimbed and branched logs felled the previous November. From Kelsey 1994b.

As determined in the temporal variability study described above, rainfall seemed to play an important role in creation and maintenance of hypoxic conditions necessary for ethanol synthesis. Evaporation from needles and twigs probably caused absorbed rain to move through log tissues by capillary action, similar to transpirational water movement in live trees. This lowered the tissue water content in branched logs and apparently interfered with establishment or duration of hypoxic conditions. This limited ethanol accumulation and reduced the densities of ambrosia beetle galleries, in contrast to delimbed logs with higher tissue water and ethanol contents.

A relationship between *G. retusus* attack densities and acetaldehyde concentrations is notable because acetaldehyde has received only minor attention relative to many studies with ethanol. Thus, the response of ambrosia beetles to acetaldehyde released alone, or in combination with ethanol and α-pinene warrants further study.

Conclusions

Differences in log chemistry measured during the initial attack and early colonization of delimbed and branched logs influenced subsequent levels of ambrosia beetle attack. Ethanol appears to be the most important compound, but acetaldehyde also may be involved, particularly for *G. retusus*. In branched logs, a lower tissue water content probably results from translocation and evaporation of absorbed rainwater through the foliage, which apparently limits hypoxia and restricts synthesis of acetaldehyde and ethanol. Branch retention on logs can decrease subsequent ethanol concentrations and densities of ambrosia beetle galleries.

Related Studies on Ethanol Synthesis and Accumulation in Logs and Stumps

The CFIRP studies described above initiated a series of closely related experiments examining ethanol synthesis and accumulation in fall cut logs and stumps, but the more recent studies were not conducted on CFIRP sites. Key results from these experiments are briefly described below because of their relevance to the initial CFIRP work and their implications to forest health, ecology, and management.

Ambrosia beetles prefer to attack and colonize logs of aging Douglas-fir and western hemlock over logs of western redcedar (*Thuja plicata*) and Pacific silver fir (*Abies amabilis*) (Prebble and Graham 1957; Johnson 1958; Chapman 1961, 1963; Zhong and Schowalter 1989), but an explanation for this preference has never been provided. To determine whether ethanol was influencing host species selection by ambrosia beetles, fall cut logs of Douglas-fir, western hemlock, and western redcedar were left in the forest through winter. By early June, logs of Douglas-fir contained 2.1 times more ethanol than western hemlock, and 3.3 to 4.0 times more ethanol than western redcedar (Kelsey and Joseph 1997). Also, densities of ambrosia beetle (*T. lineatum*, *Gnathotrichus retusus*, and *G. sulcatus*) gallery holes were significantly higher in logs of Douglas-fir and western hemlock than in western redcedar. Douglas-fir and western hemlock had similar numbers of beetle galleries even though Douglas-fir tissues contained significantly more ethanol. Beetles seemed unable to discriminate between logs with different ethanol concentrations when ethanol exceeded some threshold concentration. Ethanol and α-pinene accounted for 61% of the variation in gallery densities for *T. lineatum* and 52% of the variation in *Gnathotrichus* species. Ethanol was positively related to densities of beetle galleries, while α-pinene was negatively related. None of the variation in ambrosia beetle attack densities was explained by acetaldehyde concentrations (Kelsey and Joseph 1997).

Various forest insects colonize stumps or roots of stumps following harvest. Some of these insects vector root diseases to healthy trees or stands, and others may damage seedling and sapling regeneration. Stumps of Douglas-fir in western Oregon and ponderosa pine (*Pinus ponderosa*) in central Oregon were created in the fall by various forest management practices (Kelsey and Joseph 1999a). The trees cut varied in age and size, and their stumps were exposed to different environmental conditions through winter and spring. Regardless of these differences, all

stumps showed similarities in their synthesis and accumulation of ethanol. By spring, tissues remaining above ground produced three to 116 times more ethanol than tissues in their roots. Above-ground tissues were probably more hypoxic and produced greater quantities of ethanol than roots because of their direct exposure to precipitation and warmer temperature. Ponderosa pine stumps contained two to six times more ethanol than Douglas-fir. Insects that colonize stumps very likely use ethanol as a primary host attractant.

Although precipitation was suspected as an environmental parameter strongly influencing ethanol accumulation in logs and stumps during winter, as discussed above, its function had never been tested directly. To evaluate the role of precipitation in ethanol synthesis, an experiment was initiated with one group of fall cut, delimbed Douglas-fir logs protected from rain (dry logs), and another group of similar logs exposed to rain (wet logs). The following spring, ethanol concentrations in tissues of wet logs were significantly higher than in dry logs (Kelsey and Joseph 1999b). A third group of logs with branches was also exposed to rain. They contained low ethanol concentrations in the spring, similar to dry logs. Again, water evaporating from foliage reduced tissue water content and ethanol concentrations in branched logs. Densities of *Gnathothrichus* species gallery holes in late summer were highest in wet logs where ethanol concentrations had been greatest during spring. These findings confirm that rain is an important environmental factor influencing ethanol accumulation in logs, and further shows that attacks from ambrosia beetles can be manipulated by controlling ethanol production in aging log tissues.

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