Ethanol Production from Woody Biomass: Silvicultural Opportunities for Suppressed Western Conifers

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Abstract—The 2007 Energy Security and Independence Act (ESIA) requires 16 billion gallons of ethanol to be produced from lignocellulose biomass by 2022 in the United States. Forests can be a key source of renewable lignocellulose for ethanol production if cost and conversion efficiency barriers can be overcome. We explored opportunities for using woody biomass from thinning western conifers as source materials for conversion to biofuel. We present preliminary results using suppressed lodgepole (*Pinus contorta* Douglas ex Louden var. *latifolia* Engelm. ex S. Watson) and ponderosa pine (*Pinus ponderosa* C. Lawson var. *ponderosa)* from Pringle Falls Experimental Forest in central Oregon. We first examined growth rates of suppressed and presumably unsuppressed lodgepole pine and ponderosa pine planned for removal during thinning operations, and determined that all sampled trees were equally suppressed. We found component polysaccharides in relatively high concentrations among all sample trees. Finally, we used a new sulfite pretreatment technique for biochemical conversion of lignocellulose in wood chips from our sampled lodgepole pine and ponderosa pine to ethanol, and discuss the efficacy of the sulfite pretreatment in terms of dissolved carbohydrate composition, hydrolysis reaction rates, and sugar yield after hydrolysis. Using biomass from forest thinning to make biofuel may help mitigate the cost of fuel reduction treatments and potentially offset the cost of sustaining healthy forests and reducing the risk of catastrophic fires.

Introduction

In the United States, grain-ethanol, a biofuel, is mainly produced from the starch in kernels of field corn. Ethanol production from starches is limited by supplies of agricultural crops and costs associated with production. Currently, grain ethanol supplies 3 percent of US demand for fuel (about 4.5 billion gallons of ethanol produced annually and about 140 billion gallons of fuel used for ground transportation annually) (Somerville 2006). Using lignocellulose as biomass to produce ethanol could contribute to meeting US transportation fuel demands and help the US to achieve its goal of energy independence. Forests can be a key source of renewable biomass feedstock for ethanol production if cost and conversion efficiency barriers can be overcome. Using woody biomass in the form of small diameter trees removed during thinning projects as source materials for biofuel may mitigate the cost of thinning treatments and potentially offset the cost of sustaining healthy forests and reducing the risk of catastrophic fires.

The biochemical conversion of lignocellulose to ethanol typically consists of four major unit operations: pretreatment/size reduction, enzymatic hydrolysis, fermentation, and product separation/purification. Most pretreatment processes are designed to alter the structure of biomass cellulose by increasing the accessibility of cellulose to enzymes that convert the carbohydrate polymers into

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fermentable sugars (Lynd 1996). The pretreatment stage can account for as much as 30 percent of the total production cost in the conversion of cellulosic biomass to ethanol (Aden and others 2002). Despite extensive research and development, limited progress has been achieved in the economic development of an effective pretreatment method for woody biomass, especially conifer species.

A novel pretreatment process under development at the Forest Products Laboratory has the capability of removing woody biomass recalcitrance (the natural resistance of plant cell walls to decomposition from microbes and enzymes) for subsequent hydrolysis to glucose (Zhu and others 2009a). This process utilizes a Sulfite Pretreatment to Overcome the Recalcitrance of Lignocellulose (SPORL). The SPORL pretreatment can be applied directly to wood chips in a digester providing a low liquid to wood ratio of 3 or less in aqueous pretreatment to significantly reduce thermal energy requirements (Zhu and others 2009b). After pretreatment, size reduction by disk milling results in a coarse wood pulp at greatly reduced specific energy levels (~50 W-hr/kg) (Zhu and others 2009b). Furthermore, the resulting wood substrate has excellent digestibility in subsequent enzymatic hydrolysis. This SPORL pretreatment, however, has yet to be evaluated with western conifers.

Recent work at the Forest Products Laboratory indicated that red pine (*Pinus resinosa* Aiton), grown at high stand density, produced wood with higher concentrations of glucan and xylan (both polysaccharides) compared to wood produced by red pine grown at more open stand densities. This suggests that trees grown under suppressed conditions, especially small diameter trees in dense, even-aged stands, may be useful for biofuel production. In this paper, we expand this work to further consider two conifers, lodgepole pine (*Pinus contorta* Douglas ex Louden var. *latifolia* Engelm. ex S. Watson) and ponderosa pine (*Pinus ponderosa* C. Lawson var. *ponderosa)*. Together these species represent a large proportion of the timber targeted for thinning in overstocked stands throughout the western US.

The overall goal of this study was to evaluate the potential of western conifers, particularly trees targeted for thinning, for biochemical conversion to biofuel. We used the SPORL pretreatment process, disk milling, and enzymatic hydrolysis to determine the effective yield of glucose from wood substrate samples. Specific objectives of the study were:

1. Quantify the growth rate of lodgepole pine and ponderosa pine from areas selected for thinning.
2. Relate the growth rate to the chemical composition of lodgepole pine and ponderosa pine.
3. Quantify the efficacy of the SPORL pretreatment process when combined with enzymatic hydrolysis for lodgepole pine and ponderosa pine chips.

In this paper, we introduce the study area, and present preliminary results in quantifying growth rates, relating the growth rates to chemical composition, and quantifying cellulose conversion to glucose through enzymatic hydrolysis.

Methods

Study Area

Wood was obtained from Pringle Falls Experimental Forest (lat. 43°42’ N, long. 121°37’ W). Pringle Falls Experimental Forest lies within the Deschutes National Forest in central Oregon about 48 kilometers southwest of Bend, Oregon, and was established in May 1931 as a center for silviculture, forest management, and insect and disease research in ponderosa pine forests east of the Oregon Cascade

Range. The entire experimental forest is addressed in the Deschutes National Forest Management Plan as a single management area, with Forest-level standards and guides applied when the management activities do not conflict with research.

The 1430 ha Lookout Mountain Unit of the experimental forest was added to the Pringle Butte Unit in 1936. The Lookout Mountain Unit lies on an ancient shield volcano with an upper elevation of 1900 m above a relatively flat ancient lakebed surface at 1300 m. Currently, this relatively large block of closed-canopy forest has undergone little major disturbance since stand-replacement fires occurred in 1845 and 1890, resulting in the establishment of dense lodgepole pine and ponderosa pine at lower elevations and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), grand fir (*Abies grandis* Douglas ex D. Don) Lindl.), white fir (*Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr.), sugar pine (*Pinus lambertiana* Douglas), western white pine (*Pinus monticola* Douglas ex D. Don), and mountain hemlock (*Tsuga mertensiana* (Bong.) Carrière) at higher elevations. These stands may represent some of the most productive ponderosa pine sites in central Oregon. Limited low thinning occurred in 1969 when the road network was established. Lookout Mountain is the site of limited past research, but several silvicultural studies continue as long-term projects with regional and national significance, such as the Lookout Mountain installation of the Levels-of-Growing Stock study (Oliver 2005). Compared to structural characteristics of nearby old-growth stands (at least 600 years old) (Youngblood and others 2004), ponderosa pine in the Lookout Mountain stands are relatively young, have grown exceptionally well but have declined in radial growth over the past several decades, and currently have structural characteristics that place them at imminent risk of catastrophic loss to wildfire or bark beetles. Thinning and fuel reduction treatments are planned for 1413 ha of Lookout Mountain to restore tree vigor, reduce susceptibility to insect infestation, and reduce the risk of stand-replacement wildfire (Youngblood 2009). Wood for this study came from trees targeted for removal during thinning treatments. The study area is similar in environmental characteristics and stand structures to other low elevation ponderosa pine forest-dominated landscapes throughout the pumice zone of eastern Washington, central Oregon, and northern California.

Field Methods

Lodgepole pine and ponderosa pine wood samples were collected from two stands near the lower elevation of Lookout Mountain (1370 meters above sea level). In each case, an initial assessment was conducted to determine the diameter distribution of live trees. The plant community at both sample sites is best described as belonging to the ponderosa pine/bitterbrush-snowbrush/sedge plant association (CPS312) (Volland 1985). Soils are well-drained, relatively undeveloped, and derived from aerial deposits of pumice or scoria flow that exceed 2 m in depth after the explosion of ancient Mount Mazama. Surface horizons are loamy coarse sand to fine sandy loam. The closest weather station is 40 m lower in elevation at Wickiup Dam, OR (latitude 43°41'N, longitude 121°41'W), about 11 km to the southwest, with continuous records since 1941. The mean annual temperature at Wickiup Dam is 6.3 °C, and the mean annual precipitation totals 534 mm, mostly as snow during winter or rain during early summer convection storms.

Measurements of stand structure were conducted in circular 0.04-ha plots, with two plots at each sampling site. Trees selected for growth analysis and wood chemistry sampling were between about 10 and 36 cm diameter at breast height (1.37 m), were representative of the lower 75 percent of the stand diameter distribution, had low crown ratios and low to mid canopy positions, and were presumed to be suppressed. In addition, a single tree of both species with large diameter, high crown ratio, and dominant or codominant crown position was selected to represent relatively unsuppressed growth. A total of 13 lodgepole pines and 16 ponderosa pines were felled by hand using Deschutes National Forest fire crews during the last half of June, 2008. Trees were delimbed, total bole length measured, and then the bole was bucked into sections. From each tree, a 10-cm thick cross-sectional disk was cut from immediately below breast height, and a 71-cm short bolt was cut from below this cross-sectional disk. Additional 71-cm short bolts with minimum small-end diameter greater than 10 cm were cut at 6.1, 12.2, 18.3, and 24.4 m on all trees. All short bolts and cross-sectional disks were marked with numbered metal tags for identification. All short bolts were wrapped in plastic to minimize drying, and were shipped to the Forest Products Laboratory in Madison, WI, for analysis.

Stand Structure and Tree Growth

Stand structure was characterized by basal area, stem density, diameter class distribution, stand density index, and tree height. Basal area was computed separately by live and dead trees for each species as a summation of the cross-sectional areas of all trees equal to or greater than 1.37 m in height. Trees were grouped into 10 cm diameter size classes to give size-frequency distributions for each species. Stand density index (SDI) is a relative density measure based on the relationship between mean tree size and number of trees per unit area in a stand (Reineke 1933). This statistic has proved useful for quantifying relative density across a wide variety of stand conditions because it is independent of site quality and stand age (Long and Daniel 1990). We used the individual tree summation approach rather than the more easily applied but biased approach based on uniform diameter classes (Woodall and others 2003). Because the diameter distribution was unknown or was not normal, SDI was calculated as a summation of individual tree values as:

$$SDI = \sum (T/A)(dbh_i/10)^b,$$

where SDI is stand density index, T/A is trees per acre, dbh_i is diameter in inches at breast height of the i_{th} tree in the plot, and the exponent b is a species-specific value (Shaw 2000). Values of the exponent b were 1.77 for ponderosa pine and 1.74 for lodgepole pine (Cochran and others 1994). For comparison, SDI at full stocking is 365 for ponderosa pine and 277 for lodgepole pine (Cochran and others 1994).

The cross-sectional disks cut at breast height were sanded with increasingly fine grit sandpaper (#150, 220, and 320) to clarify ring structure, and annual ring widths measured by two people under a binocular microscope by using a Velmex measuring stage with an AccuRite linear encoder (resolution 0.001 mm) with a Metronics digital readout unit coupled to a computer with MEASURE J2X software (version 4.1.2). On each cross-sectional disk, ring widths along the largest radii and the radii opposite were measured. Crossdating and measurement quality control were completed for each radii by using the computer program COFECHA version 6.06 (Holmes 1986) (accessed at the Tree-Ring Lab, Lamont-Doherty Earth Observatory, Columbia University). Undated ring width series were correlated with a master dated series (Lookout Mountain Lower, OR046; downloaded from the International Tree-ring Data Bank, World Data Center for Paleoclimatology) collected in 1995 from an adjacent ponderosa pine old-growth stand for identifying pandora moth outbreak frequency (Speer and others 2000) with emphasis on the most recent century. For example, the OR046 chronology documented exceptionally low radial growth in 1930 and 1995 associated with insect herbivory, and also low radial growth in 1977.

Trees growing at a constant rate under uniform environmental conditions will increase in diameter by producing increasingly smaller growth rings as the circumference of the bole increases, and this pattern of ring widths is easily modeled as a negative exponential growth curve. All tree ring width series were standardized in the R statistical programming environment (dplR) (Bunn 2008) by first fitting each ring width series to the model:

$$G_t = ae^{-bt} + k,$$

where the growth trend G_t is estimated as a function of time t with coefficients a, b, and k. Annual values from each ring width series were then divided by the corresponding value of the growth trend G_t. The resulting ring width indices have no residual age effect, a mean of 1.0, and equal variances. Finally, ring-width indices were averaged across trees from each site to build a common chronology. Deviations above or below this standardized index represent periods when growth was influenced by exogenous factors including climate, insect herbivory, and density-influenced suppression. While climate and insect herbivory may cause short-term changes in radial growth, suppression is more chronic. Ring width indices were examined for periods when growth indices were ≤ 0.9 for at least 5 years. Descriptive statistics were used to compare suppressed and reputed unsuppressed chronologies. These statistics include the mean ring width, mean sensitivity (MS), the first-order serial autocorrelation (AC) to detect eventual persistence retained after the standardization, the mean correlation between trees ($\overline{r}_{wt}$) and within trees ($\overline{r}_{wt}$), and the expressed population signal (EPS) to estimate the amount of year-to-year growth variations shared among trees of the same chronology (Briffa and Jones 1990; Wigley and others 1984). The EPS is based on the mean correlation between all tree-ring series included in the chronology and increases from zero to one with sample size and with the strength of the mean correlation.

Growth Analysis and Wood Chemistry

Thin disk samples were cut from each short bolt, oven dried, and sanded to expose the ring structure. A representative pith-to-bark wedge sample with companion strip (fig. 1) was then split from each disk for analysis of chemical composition and growth characteristics, respectively. Strips were then progressively sanded with 600-, 800-, 1000-, 1200-, and 1500-grit sandpaper and polished on clean copier paper. To determine the growth characteristics of each strip, portions were viewed under a stereo-microscope at various magnifications to measure rings, and in some cases, individual tracheids. Once the growth characteristics were known, the wedge samples were split into sections of relatively uniform growth by first scoring the row of earlywood cells with a razor blade at the beginning of the section of interest and then fracturing the section from the remainder of the wedge (fig. 2). This technique resulted in a very clean fracture between the latewood band of the previous section and the earlywood band of the next section. The fractured sections were then chipped into smaller fragments with a sharp chisel and chopped into 20-mesh particles in a Wiley Mill in preparation for chemical analysis. The samples were first disintegrated in concentrated sulfuric acid and then centrifuged to produce an aliquot that could be injected into a Dionex ion chromatograph with pulsed amperometric detection to determine polysaccharide composition (Davis 1998).

To date, analysis of growth and chemical composition has been conducted on only a subset of short bolts selected to represent extreme differences between trees. For lodgepole pine, three short bolts with the smallest breast-height diameters were analyzed in addition to the reputed "unsuppressed" tree. For ponderosa pine, three samples with the smallest breast-height diameters, one additional sample with larger diameter yet a discrete and extreme period of suppressed growth, and the short bolt from the reputed "unsuppressed" tree were analyzed.

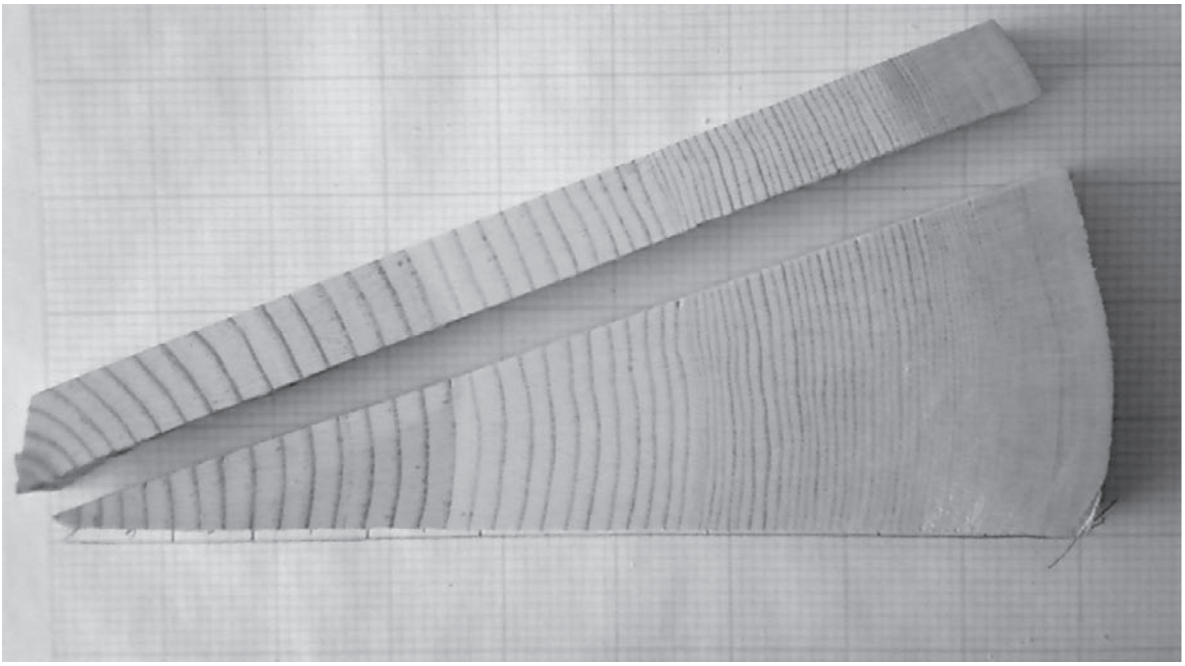

Figure 1—Breast height wedge section with companion strip removed from the reputed unsuppressed tree in a ponderosa-dominated stand sampled for biofuel potential at Lookout Mountain, Pringle Falls Experimental Forest, central Oregon.

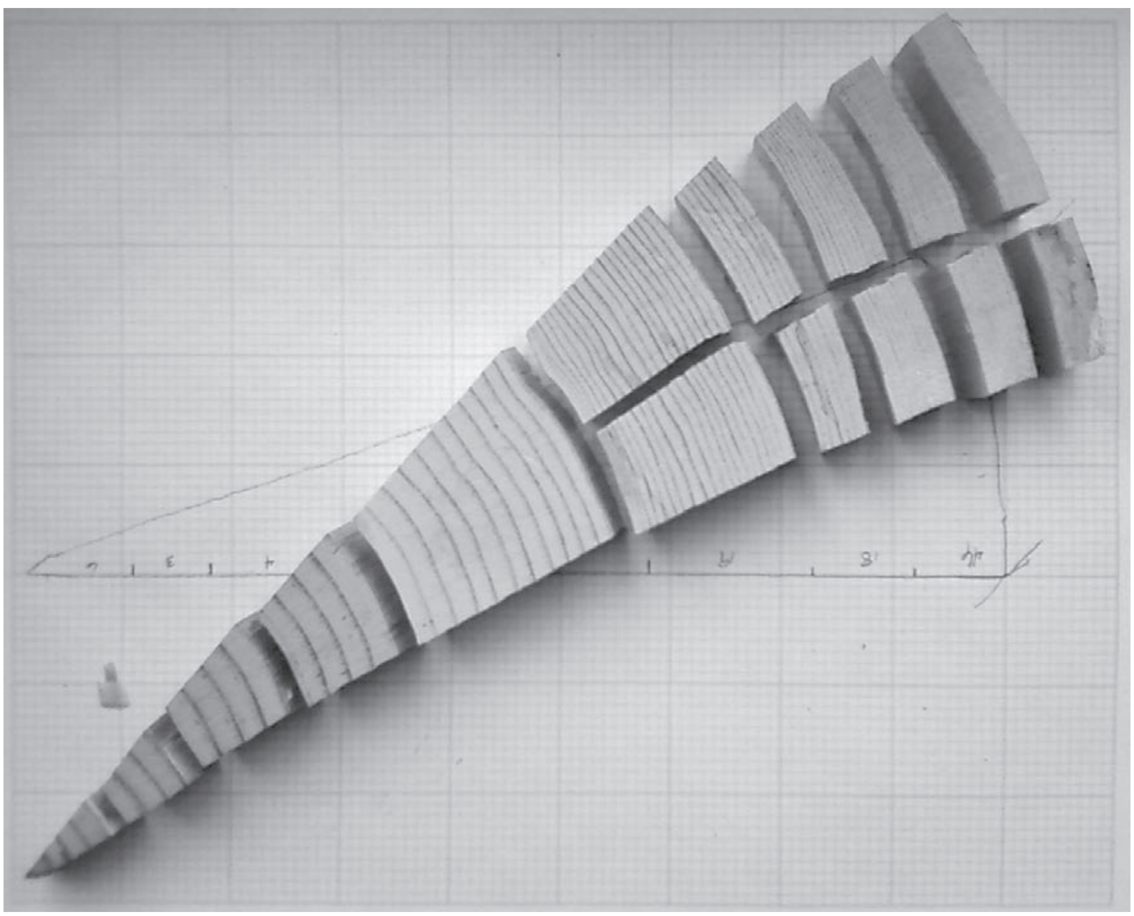

Figure 2— Breast height wedge section, divided into separate parts based on radial growth patterns, in preparation for polysaccharide composition analysis of a reputed unsuppressed tree in a ponderosa-dominated stand sampled for biofuel potential at Lookout Mountain, Pringle Falls Experimental Forest, central Oregon.

Enzymatic Cellulose Conversion

Short bolts were hand debarked and chipped. The wood chips were screened, retaining only particles greater than 6 mm and less than 38 mm in length to ensure smooth operation in disk milling. The thickness of the chips ranged from 3 to 8 mm.

The wood chips were conditioned to 30 wt% solids, then loaded into a laboratory batch digester (capacity 23 liters) and subjected to a SPORL pretreatment at 180 °C for 30 minutes. An 8 percent sodium sulfite charge on wood (dry basis) was added to the pretreatment liquor for an initial sodium sulfite concentration of 2.67 percent. A second pretreatment was conducted in more acidic conditions (pH 1.9) by adding sulfuric acid to the liquor at a dosage of 2.21 percent on wood (dry base) or acid concentration of 0.4 percent (volume-to-volume). After the SPORL pretreatments, the chips were directly fed into a 305 mm atmospheric disk refiner (plate pattern D2B-505, Andritz Sprout-Bauer, Springfield, OH) for fiberization. The chemical compositions of the untreated wood and pretreated wood substrates were analyzed using an improved high-performance anion exchange chromatograph with pulsed amperometric detection (HPAEC-PAD) (Davis 1998). Reported data averages of duplicate measurements conducted three weeks apart.

Enzymatic hydrolysis was conducted using commercial enzymes at 2 percent of substrate solid (weight-to-volume percent) in 50-mL sodium acetate buffer using a shaker/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 200 rpm. The pH and temperature were adjusted to 4.8 and 50 °C, respectively. A mixture of Novozyme Celluclast 1.5 L cellulose with an activity loading of approximately 15 filter-paper units per gram (FPU/g) substrate and Novozyme 188 (β-glucosidase) cellobiase with an activity loading of approximately 22.5 cellulose-binding module per gram (CBU/g) substrate were used for enzymatic hydrolysis[1]. Hydrolysates were sampled periodically for glucose analysis using a Glucose Analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH).

Results

Stand Structure and Tree Growth

Both the lodgepole pine stand and the ponderosa pine stand were overstocked, with high density, high SDI, and high basal area (table 1). These values are consistent with the outcome of more extensive field sampling of ponderosa pine stand structure throughout the Lookout Mountain Unit[2], indicating that basal area generally ranged from 34.4 to 41.3 m^2 ha^{-1} and SDI generally ranged from 223 to 257. Cochran and Barrett (1999) showed that continued mortality in ponderosa pine from mountain pine beetle occurred when the SDI was above 240, and set an upper management zone (UMZ) for SDI to sustain healthy stand conditions for forest stands based on the dominant plant association. Upper management zones defined specifically for the Deschutes National Forest represent the level of stocking or relative density beyond which there is imminent risk of catastrophic loss of overstory trees to bark beetles[3]. Values for the UMZ for the lower portion of Lookout Mountain containing the lodgepole pine and ponderosa pine stands

1 Enzymes were generously donated by Novozymes (Franklinton, NC).

2 Data on file, LaGrande Forestry Sciences Laboratory, LaGrande, OR.

3 Booser, J, and J. White. Calculating maximum stand density indexes (SDI) for Deschutes National Forest plant associations. Unpublished paper, on file, Deschutes National Forest.

Table 1—Structural attributes of density, mean diameter at breast height, stand density index, and basal area by species in two stands sampled for biofuel potential at Lookout Mountain, Pringle Falls Experimental Forest, central Oregon.

Site	Density	Diameter	Stand density index	Basal area
	stems ha^{-1}	*cm*		*m^2 ha^{-1}*
Lodgepole stand				
Live lodgepole pine	375	20.0	109.2	13.72
Dead lodgepole pine	50	12.6	21.2	2.74
Live ponderosa pine	538	14.6	132.5	18.58
Dead ponderosa pine	87	2.8	2.7	0.26
Total	1050	16.7	265.6	35.28
Ponderosa stand				
Live lodgepole pine	112	8.2	15.8	2.02
Dead lodgepole pine	0	0	0	0
Live ponderosa pine	938	18.3	293.5	39.97
Dead ponderosa pine	100	6.6	4.5	0.44
Total	1150	16.3	313.8	42.44

sampled in this study fall between an SDI of 102 and 156, thus these two stands are 225 percent above the UMZ and are in imminent risk of complete overstory mortality from bark beetles.

Sampled diameter distributions for both stands had steeply decreasing or reverse J shapes, indicating that these stands were relatively young and individual trees were experiencing strong competition for space and resources (fig. 3). Diameter distributions and stem densities for both stands did not resemble diameter distributions and stem densities of nearby old-growth ponderosa pine stands (Youngblood and others 2004).

Diameter-total tree height relations were consistent with relatively young stand structure; lodgepole pine showed steeper height growth with diameter compared to ponderosa pine (fig. 4). Total height of lodgepole pine likely is near the upper limit for this region, while ponderosa pine on similar relatively productive sites will reach 45 m in total height (Youngblood and others 2004).

As expected, breast-height ages in both the lodgepole pine- and ponderosa pine-dominated stands were consistent with single-cohort structure originating from a single stand-replacement event (fig. 5). Lodgepole pine breast-height ages ranged from 91 to 107 years. Assuming 10 years to reach breast height, this suggests a stand replacement disturbance such as a fire and a re-establishment date of 1890. Ponderosa pine breast-height ages ranged from 73 to 107 years. This wider period of re-establishment is not unusual, especially since ponderosa pine seedlings are known to be more sensitive to low temperature than lodgepole pine in this region (Cochran 1972).

Both the lodgepole pine and ponderosa pine ring-width analysis indicated that the reputed "unsuppressed" trees were actually suppressed. Reputed unsuppressed trees had similar establishment dates, sensitivity, and first-order autocorrelation as suppressed trees at the same location (table 2). In addition, the chronology developed for the reputed unsuppressed trees showed little difference from the chronology developed for all other suppressed trees, as shown for lodgepole pine in figure 6. For lodgepole, all chronologies contained periods with less than expected growth from 1944 through 1957, from 1994 through 1998, and since 2002. Ponderosa pine chronologies contained extended periods with less than expected growth from 1960 through 1977 and from 1995 through 1999.

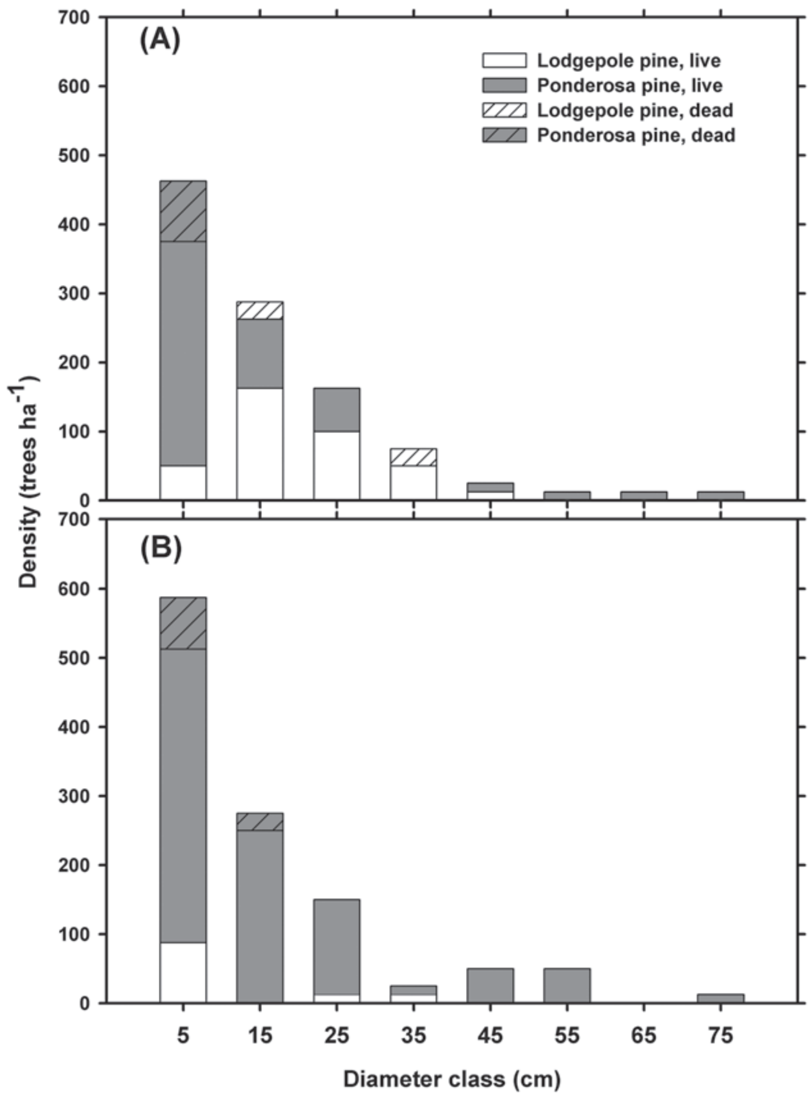

Figure 3—Diameter distribution of lodgepole pine and ponderosa pine at (A) a lodgepole-dominated stand and (B) a ponderosa-dominated stand sampled for biofuel potential at Lookout Mountain, Pringle Falls Experimental Forest, central Oregon.

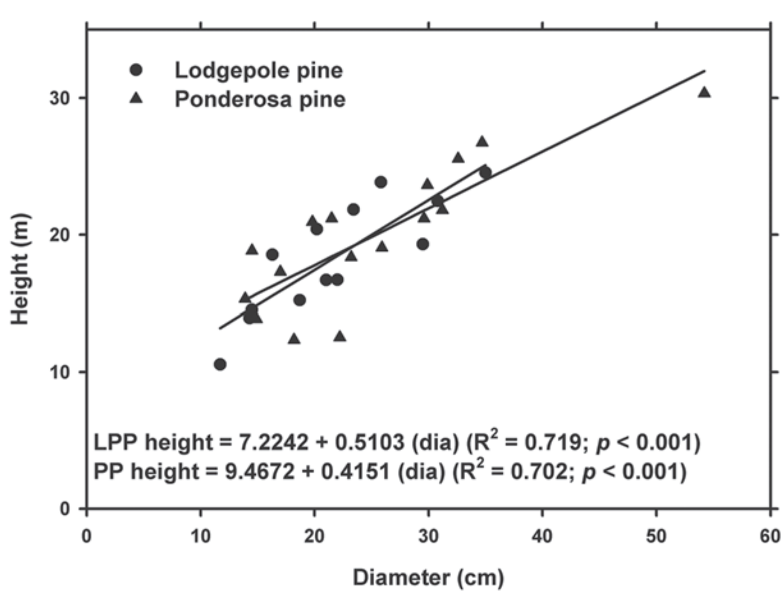

Figure 4—Breast-height diameter-total height distribution in a lodgepole-dominated stand (n = 13) and a ponderosa-dominated stand (n = 16) sampled for biofuel potential at Lookout Mountain, Pringle Falls Experimental Forest, central Oregon.

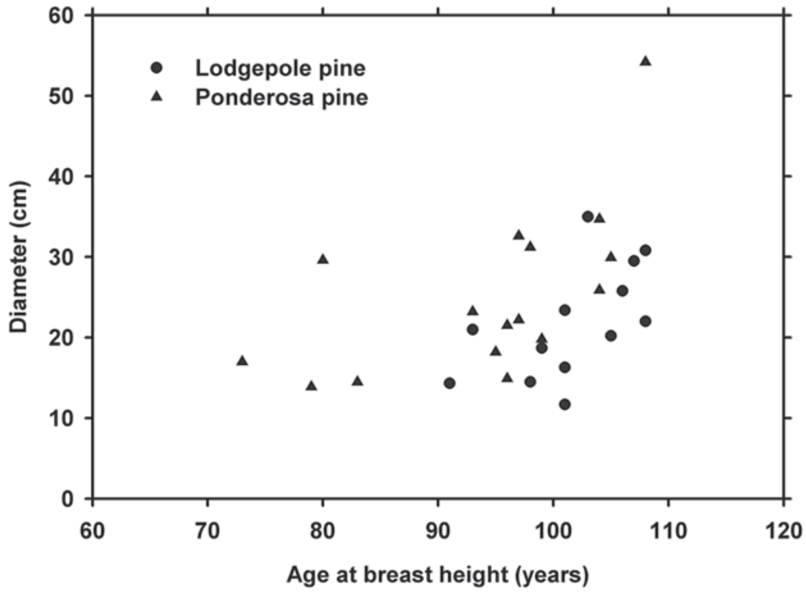

Figure 5—Breast-height age-diameter distribution in a lodgepole-dominated stand (n = 13) and a ponderosa-dominated stand (n = 16) sampled for biofuel potential at Lookout Mountain, Pringle Falls Experimental Forest, central Oregon.

Table 2—Statistics of tree ring chronologies by species in two stands sampled for biofuel potential at Lookout Mountain, Pringle Falls Experimental Forest, central Oregon.

Site	Sample size	First year	Ring width	MS	AC	$\overline{r}_{bt}$	$\overline{r}_{wt}$	EPS
			mm					
Lodgepole pine stand								
Suppressed	24	1900	0.94	0.179	0.858	0.144	0.608	0.94
Unsuppressed	2	1905	1.59	0.132	0.918	NA	0.701	NA
All trees	26	1900	0.99	0.176	0.862	0.162	0.615	0.87
Ponderosa pine stand								
Suppressed	30	1900	1.04	0.221	0.902	0.034	0.523	0.59
Unsuppressed	2	1900	1.99	0.209	0.964	NA	–0.015	NA
All cores	32	1900	1.11	0.220	0.906	0.034	0.489	0.61

Mean ring width; MS, Mean sensitivity; AC, first-order autocorrelation; $\overline{r}_{bt}$, mean correlation between trees; $\overline{r}_{wt}$, mean correlation within trees; EPS, expressed population signal, a measure of the amount of year-to-year growth variations shared among trees of the same chronology.

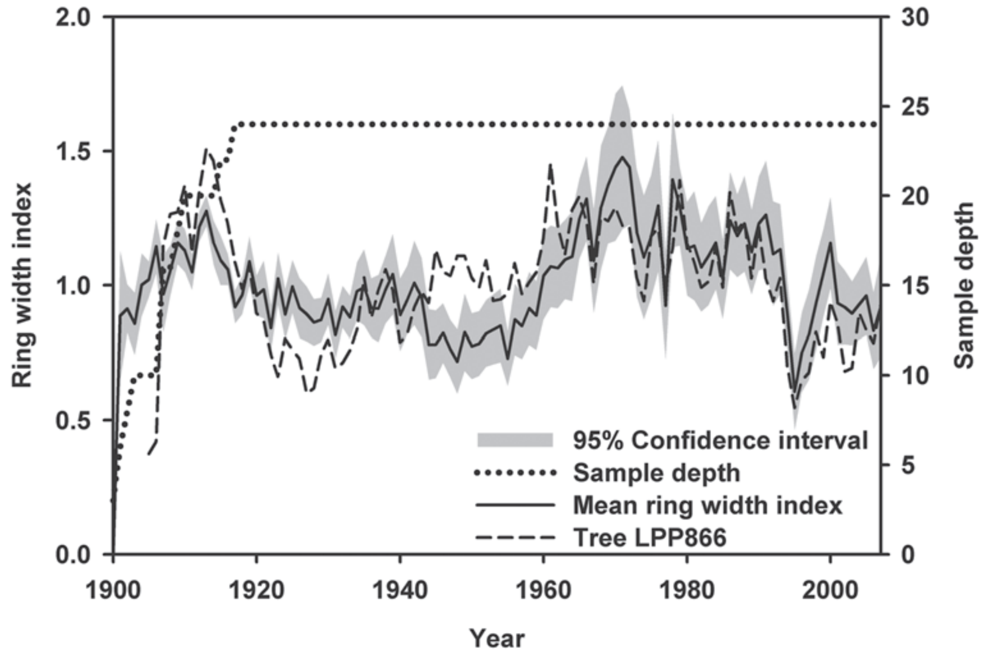

Figure 6—Standardized ring-width chronology with 95% confidence interval (left axis) and sample depth (right axis) from a suppressed lodgepole pine-dominated stand sampled for biofuel potential at Lookout Mountain, Pringle Falls Experimental Forest, central Oregon. Standardized ring-width index for the "unsuppressed" tree is overlaid.

Growth Analysis and Wood Chemistry

Preliminary analysis relating wood chemistry to tree growth focused on the reputed unsuppressed ponderosa pine. Ten discrete sections with unique growth characteristics were identified in the disk extracted at breast height (table 3). The first four sections (A through D) contained the first 17 years of growth and include rings formed under relatively rapid radial growth. Section E began beyond the apparent extractives region of heartwood and contained rings formed by rapid and

Table 3—Statistics of radial growth patterns by section in a reputed unsuppressed ponderosa pine sampled for biofuel potential at Lookout Mountain, Pringle Falls Experimental Forest, central Oregon.

Section	Number rings	Cumulative age	Section width	Cumulative radius	Mean ring width	Section area	Disk area	Ring area	Growth rate
		year	*mm*	*mm*	*μm*	*cm^2*	*cm^2*	*%*	*cm^2yr^{-1}*
A [a]	6	6	23.6	23.6	3937	18	18	1.1	2.9
B	3	9	17.5	41.1	5842	36	53	2.3	11.9
C	4	13	25.4	66.5	6350	86	139	5.6	21.5
D	4	17	21.1	87.6	5271	102	241	6.6	25.5
E	11	28	49.0	136.6	4457	345	587	22.3	31.4
F	20	48	39.1	175.7	1956	383	971	24.8	19.2
G	7	55	12.7	188.4	1814	145	1116	9.4	20.7
H	11	66	12.7	201.1	1155	155	1271	10.0	14.1
I	18	84	10.5	211.6	586	137	1408	8.8	7.6
J	28	112	10.2	221.8	363	138	1546	8.9	4.9

[a] Section A closest to pith; section J closest to bark

consistent growth over the next 11 years, with mean ring width of 4.5 mm. This section likely represents normal or unsuppressed growth. Radial growth slowed over the next 27 years (sections F and G) and then rapidly declined. Over the next 57 years (sections H through J), annual radial growth dropped from about 1814 µm $year^{-1}$ to 363 µm $year^{-1}$ and the corresponding disk area only increased by 27 percent. Over a period of 7 years from 1994 to 2000, annual rings averaged only 3 earlywood fibers and 2 latewood fibers in the radial direction. These sectional results are consistent with the full ponderosa pine chronology results presented above. Despite its outward appearance, this reputed "unsuppressed" tree was as suppressed as other trees selected on the basis of physical appearance to represent suppression.

Numerous polysaccharides, including glucan, were identified in the wood of the reputed unsuppressed ponderosa pine (table 4). Only modest differences in glucan concentration were found among the various sections of the reputed unsuppressed ponderosa pine (sections E through I described above). In addition, sampling of short bolts from higher in the same tree, representing areas of recent growth, failed to establish a clear trend of increasing or decreasing glucan concentration. Other samples, corresponding to the most recent 60 years from several trees with the smaller diameters and thus representing potentially the more suppressed trees of those sampled, also contained glucan in similar amounts.

A similar preliminary evaluation of polysaccharide composition of lodgepole indicated that component polysaccharides occur in similar concentrations to those found in ponderosa pine, with perhaps slightly higher concentrations of galactan and xylan.

Enzymatic Cellulose Conversion

Substrate chemical composition after SPORL pretreatment differed from untreated samples at pH 4.2 and pH 1.9 (table 5). For both pretreatment levels of pH, lignin loss was about 10 percent while most of the hemicellulose (xylan and mannan) was removed. Glucan loss, undesirable for our purposes, was undetected at the pH 4.2 level (measurement uncertainty resulted in no loss and repeated measurements showed consistent results), and was moderate (10 percent) at the pH 1.9 level. Enzymatic cellulose conversion of fiberized lodgepole pine using the SPORL pretreatment process was time dependent as expected (fig. 7). Preliminary results suggest that near complete cellulose conversion to glucose can

Table 4—Composite polysaccharide composition (%) by component and glucan/carbohydrate ratio (G/C ratio) of lodgepole pine and ponderosa pine cross-sectional samples analyzed for biofuel potential at Lookout Mountain, Pringle Falls Experimental Forest, central Oregon, and reported values from other sources.

	Component composition									
	Ash	Lignin	Arabinan	Galactan	Glucan	Xylan	Mannan	Carbohydrates	Yield	G/C ratio
	percent									
Lodgepole pine	0.0	27.9	1.6	2.1	42.5	5.5	11.6	63.4	91.1	0.67
Ponderosa pine	0.0	26.9	1.8	3.9	41.7	6.3	10.8	64.6	91.4	0.65
Aspen[1]		23.0	0.0	0.0	45.9	16.7	1.2	63.8	86.8	0.72
Corn stover[2]	13.3	19.7	2.1	0.8	34.0	19.5	0.5	56.9	89.9	0.60
Hybrid poplar[2]	1.0	23.5	0.5	0.7	43.7	16.6	2.8	64.3	88.8	0.68
Switch grass[2]	5.8	19.5	2.9	1.2	33.6	23.1	0.4	61.2	86.5	0.55
Wheat straw[2]	10.2	16.9	2.4	0.8	32.6	19.2	0.3	55.3	82.4	0.59

[1] Wang et al., 2009.

[2] DOE.

Table 5—Chemical composition of lodgepole pine wood, untreated and pretreated using a Sulfite Pretreatment to Overcome the Recalcitrance of Lignocellulose (SPORL) process at two initial levels of pH, evaluated for biofuel potential as suppressed trees sampled at Lookout Mountain, Pringle Falls Experimental Forest, central Oregon.

	Liquor		Component weight								
Pretreatment	Initial	Final	Klason lignin	Arabinan	Galactan	Rhamnan	Glucan	Xylan	Mannan	Sum	Total
	- - - - pH - - - -		- - - - g - - - -								
Untreated			27.09	1.56	2.23	0.07	42.55	6.93	10.99	91.42	100.0
SPORL	4.2	2.5	25.26	0.0	0.02	0.0	46.03	1.80	0.86	73.97	76.9
SPORL	1.9	1.5	25.43	0.01	0.05	0.0	38.19	0.32	0.25	64.25	66.7

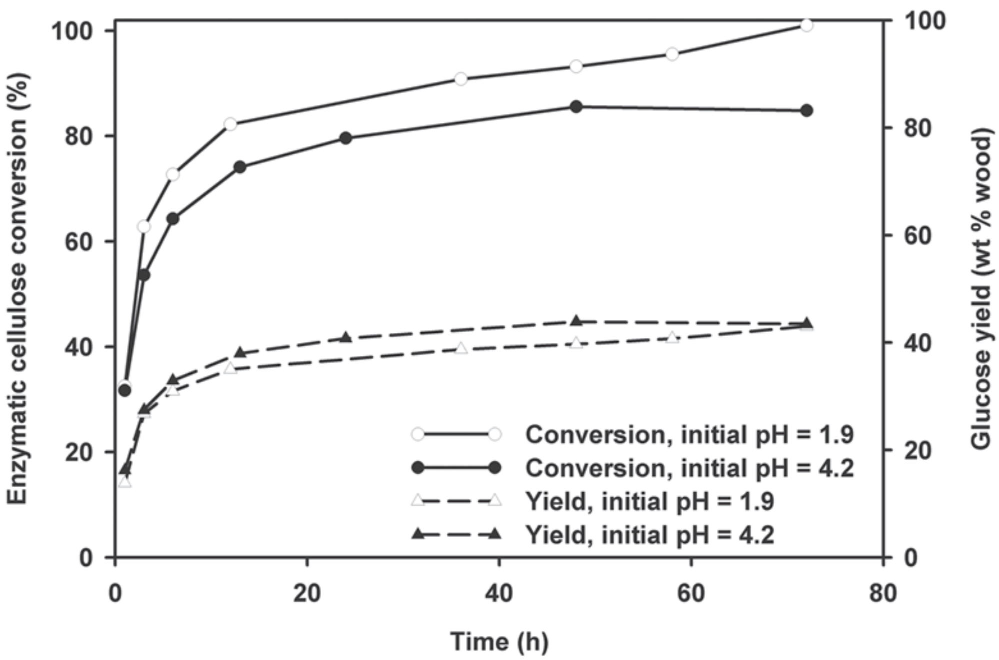

Figure 7—Mean time-dependent enzymatic cellulose conversion (%) (left axis) and glucose yield (wt % wood) (right axis) from substrates of lodgepole pine pretreated using a Sulfite Pretreatment to Overcome the Recalcitrance of Lignocellulose (SPORL) process to evaluate biofuel potential of suppressed trees sampled at Lookout Mountain, Pringle Falls Experimental Forest, central Oregon.

be achieved in 48 hours at an enzyme dosage of 15 FPU/g substrate (equivalent to about 25 FPU/g cellulose). Considering an initial glucan content of about 42 percent in untreated chips, the SPORL pretreatment, with subsequent disk milling and enzymatic hydrolysis, produced high glucose yields from the lodgepole pine chips, suggesting that highly suppressed lodgepole pine could be a viable source of lignocellulosic biomass for conversion to biofuel.

Discussion

In this work, we explored opportunities to use small diameter lodgepole pine and ponderosa pine, scheduled for removal as part of a landscape-scale thinning and fuel reduction project in Pringle Falls Experimental Forest, as biomass to support biofuel production. Using dendrochronological techniques, we confirmed that trees selected in the field representing suppressed crown classes were severely suppressed based on the criterion of reduced diameter growth for at least five years. In addition, we found that both the reputed "unsuppressed" lodgepole pine and the reputed "unsuppressed" ponderosa pine were also suppressed. This limited our comparison of glucose conversion.

Analysis of polysaccharide components was limited to a portion of our ponderosa pine and lodgepole pine samples. While subtle differences were detected among the samples, the apparent differences that were expected to occur in relation to bole height were not readily distinguishable. Overall, we found similar amounts of glucan in the selected wood samples. The amounts we found suggest that both lodgepole pine and ponderosa pine, when suppressed, contain sufficient glucan and other polysaccharides that conversion to biofuel is possible.

Pretreatment is one of the most expensive operations in the conversion of cellulosic biomass to fermentable sugars. Other pretreatment processes have yet to effectively overcome recalcitrance of woody biomass, especially conifer biomass, and thus fail to enhance the conversion of cellulose to glucose through enzymatic saccharification. For example, weak acid pretreatment can achieve only about 40 percent cellulose conversion to glucose (Wyman and others 2009; Zhu and others 2009a). Ammonia pretreatment is not effective for biomass with high lignin content (Sun and Cheng 2002; Wyman and others 2009). Steam catalyzed-steam explosion produced slightly better conversions of cellulose to glucose (up to about 70 percent) with greater energy demands (Tengborg and others 2001; Wyman 2009). Organosolv pretreatment can result in over 90 percent enzymatic cellulose conversion to glucose, but hemicellulose recovery from the solvent is low. The economic production of ethanol from woody biomass using organosolv process requires that high value lignin products be developed and marketed. A proposed $80 million ethanol demonstration plant in Colorado that would use the organosolv process was recently terminated due to the instability of energy prices and uncertainty in the capital markets, before markets for lignin products could be developed. In contrast, pretreatment of lodgepole pine and ponderosa pine chips with the SPORL proved both cost and time effective. The SPORL pretreatment resulted in excellent conversion of cellulose to glucose as demonstrated in this study, has excellent fermentable hemicellulose sugar recovery (Zhu and others 2009a), low energy consumption (Zhu and others 2009b), and commercial scalability. We believe the SPORL pretreatment offers an enormous potential for cellulosic ethanol production using small diameter woody biomass resulting from forest thinning and fuels reduction activities.

While field corn currently is the most common source of biofuel, our preliminary results indicate that the vast area in the western US currently supporting high density stands of lodgepole pine and ponderosa pine could contribute to energy demands by providing sources of woody biomass for conversion to biofuel. Lodgepole pine and ponderosa pine occur across the western US at low elevations as dry forests; few areas in the western US have the potential to support production of field corn at the same extent. Woody biomass in these stands has far greater mass per unit area than field corn, thus transportation and processing costs should be evaluated in more detail. Further, field corn is harvested within a relatively short window of time and requires storage prior to processing. Woody biomass is relatively easy to store, and could be harvested from western forests and transported throughout the year, thus providing greater stability in workforce demands and equipment needs. Finally, the SPORL pretreatment process and other technological advances have removed any remaining technological barrier to bioconversion of woody biomass to biofuel.

Although the two western conifers tested in this study proved capable of contributing to ethanol production through SPORL pretreatment technology, major research and development will be needed before silvicultural treatments with ethanol objectives might be implemented. First, additional effort is essential to ensure that the technology, developed with relatively small digesters and incubators, is scalable in terms of sugar and ethanol yields and chemical and enzyme dosages. Pilot or full-scale production facilities are needed to confirm that energy savings associated with wood size reduction observed in our laboratory study can be expected across a range of commercial facilities. The commercial marketability of dissolved lignosulfonate, as a byproduct of the SPORL process, is largely unknown. While the technology builds on existing equipment, technology, and infrastructure that have long been used in the pulp and paper industry, this infrastructure is not readily available throughout much of the western US where lodgepole pine and ponderosa pine occur. In addition, operational-scale ethanol production would likely require a similar scale logging infrastructure, yet throughout much of the western US the logging infrastructure has disappeared over the past decade. Finally, our work is limited to two tree species, and much of their distribution in the western US is on federally administered lands. Management of forests dominated by lodgepole pine and ponderosa pine on these public lands for ethanol conversion must be balanced with management of other resource objectives.

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