Invasive English holly (*Ilex aquifolium* L.) in Clear-Cut and Forest Units in a Western Washington Managed Forest

Elliott D. Church

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Committee:

Sarah Reichard (Chair)

David Stokes

Warren Gold

Martha Groom

Program Authorized to Offer Degree:

School of Environmental and Forest Sciences

University of Washington

Abstract

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Elliott D. Church

Chair of the Supervisory Committee:

Dr. Sarah Reichard
School of Environmental and Forest Sciences

English holly (*Ilex aquifolium* L.) is a dioecious, shade-tolerant evergreen shrub or small tree native to Europe, Western Asia and North Africa that is becoming a prominent invasive in west-side Pacific Northwest (PNW) forests, with potentially negative impacts on native biodiversity and forest succession. Little is known about the susceptibility of managed forests and the potential influence timber harvest practice has on English holly invasion (e.g., through edge creation). In a managed forest in western Washington State, I surveyed for English holly in belt transects running from recent clear-cuts (harvested 2000-2005) into adjacent mature forest units (established 1925-1935). For all holly in transects measuring ≥ 1 cm basal diameter, I recorded location relative to the forest edge, size (basal diameter and height), and presence or absence of berries. For smaller English holly, only total number was recorded. Additional English holly samples were collected for a comparison of pre- and post-harvest growth rate (annual Basal Area

Increment; BAI). A total of 286 holly were present in two out of four transects sampled, with 158 appearing in one forested unit and 128 appearing across two clear-cut units. The oldest holly sampled established in 1976, and total number of holly steadily increased in the study area up to the time of sampling. Proportion of fruit producing trees was more than 7 times greater in clear-cuts compared with forest. Growth rates of holly (calculated as basal diam. ÷ age) were higher on average, and more variable, in recently harvested areas compared to forest and decreased with distance into the forested unit. After-harvest growth rate (estimated with BAI) was larger on average compared to before harvest in both environments. The largest after-to before-harvest growth ratios were exhibited by holly located in clear-cuts. These results suggest that managed forests are susceptible to holly invasion, and that timber harvest without holly control could result in an increase in holly growth and fruit production, possibly accelerating holly spread.

1. Introduction

Invasive non-native plant species pose a major threat to forest ecosystems, causing serious negative impacts to forests and the functions and services these important ecosystems provide (see Kohli *et al.* 2009 for a review). Woody perennial invasive plants have been identified as a group that poses unique and significant challenges; in the Eastern United States, several non-native woody perennial plant species are spreading in forests, suppressing or displacing native species and altering forest structure and composition (Webster *et al.* 2006). These types of invasions have the potential to alter forest successional pathways (Webster and Wangen 2009). Shade-tolerant woody perennial invaders pose a unique threat to undisturbed forest communities, which have been generally considered more resistant to invasion by non-native plants (e.g., Martin & Marks 2006, Martin *et al.* 2009). Shade-tolerant, invasive trees pose a special case: due to relatively slower growth and a long lag time, their presence in forest habitats may go unnoticed or seem benign until the population enters a rapid expansion phase (Frappier *et al*. 2003; Wangen and Webster 2006; Webster and Wangen 2009). Shade-tolerant woody invasive plant species make up a significant part of the diverse suite of invasive species threats to sustainable management of commercial forestlands in North America (Moser *et al.* 2009).

English holly (*Ilex aquifolium*) is a shade-tolerant evergreen shrub or small tree, native to Europe, North Africa, and Western Asia, which is becoming a prominent non-native invasive in the Pacific Northwest (PNW) (Stokes *et al.* 2014). English holly is dioecious, and spreads via bird-dispersed berries in its native range (Peterken and Lloyd 1967) and in the PNW (Zika 2010). It also spreads vegetatively forming dense monotypic stands in its native range (Peterken and Lloyd 1967; Peterken and Newbould 1966; also in the PNW, Stokes *et al.* 2014). Long cultivated in the PNW (Jones and Reichard 2009), English holly's invasive capabilities have been demonstrated (Stokes *et al.* 2014), and it appears to be rapidly spreading in low elevation western Washington forests (Stokes *et al.* 2014, Olmsted 2006). Due to its growth form as an evergreen, thicket-forming shrub or small tree that can grow up to 23 m in height (Peterken and Lloyd 1967), English holly introduces a novel structural element to western Washington lowland forests that appears to have a negative impact on vegetation under its canopy (Stokes *et al.* 2014; Church and Stokes, in prep).

The threat that English holly poses to timberland is of particular interest given the prevalence and economic importance of these lands in the PNW. Western Washington contains 9.5 million acres of unreserved timberland (WA-DNR 2007), and posted a harvest of 2.759 billion board feet in 2014 (Smith 2015). Historically intensive timber harvest in Washington state is reflected in the current age structure of forestland, in which 99% of forests on non-federal lands are less than 100 years old (WA-DNR 2007). In addition to colonizing the understory of PNW low elevation timber-managed forest, English holly established in these environments could withstand and perhaps benefit from the disturbance and edge-creation which characterizes commercial forest harvesting. There are examples of other woody, shade- and sun-tolerant invasive plant species displaying potential to respond to disturbance with increased growth and fecundity in newly exposed environments and the adjacent forest edge (e.g., Moore *et al.* 2013, Webster and Wangen 2009).

If English holly is capable of invading and becoming widespread in managed forests, it could have negative impacts to forest management –e.g., through resource competition with timber trees, control costs, and displacement of native species in the understory. I examined holly presence in a managed forest in Western Washington, hypothesizing that due to increased light availability associated with timber harvesting, forest management would facilitate holly invasion.

Specifically, I predicted that: a) English holly is present in both recent clear-cuts and forested stands, b) there is a difference in growth and berry production between forested and recently-harvest habitats, and c) distance from forest edge is a significant predictor of English holly abundance, growth, and berry production. I expect light availability will influence holly invasion because it is an important factor for plants in forest ecosystems (Chen 1997) and it has direct influence on English holly berry production (Peterken and Lloyd 1967), seedling vigor and mortality (Peterken 1966, Arrieta and Suarez 2005), and on factors that influence English holly establishment and growth (e.g., drought; Peterken and Lloyd 1967).

2. Methods

Study area

I studied English holly in Charles L. Pack Experimental Forest (hereafter, Pack Forest), a 4300 acre experimental research and demonstration forest operated by the University of Washington, located at the confluence of the Nisqually and Mashel rivers in Eatonville, Washington (approx. 1.25 miles from the town of Eatonville, Figure 1). In 2004, the Center for Sustainable Forestry was established at Pack Forest with the goal of advancing the science and practice of sustainable forest management (CSFPF 2014).

Pack Forest timber management is similar to typical Western Washington commercial forests, with a few differences that make it unique compared with the typical commercial forest landscape. Pack Forest comprises a patchwork of "Forest Management Units" (FMUs) which are more variable in shape and size and smaller on average than harvest units in adjacent commercial timberlands (Swanson 2006). In addition, a smaller percentage of stands are in early stages of succession than typical of surrounding commercial timberland (Swanson 2006), a result of Pack Forest's diverse management goals that include preservation of semi-old growth stands and unique vegetation communities. Included in these unique communities, designated as "ecological areas" and exempt from harvesting, is a patch of Douglas-fir (*Pseudotsuga menziesii*) & western redcedar (*Thuja plicata*) forest called Hugo Peak which established in the year 1800 after a fire and contains large old individuals from the previous stand. Hugo Peak stands exhibit many of the characteristics attributable to old-growth low elevation west-side forests, providing an opportunity to shed light on the vulnerability of this rare and important habitat.

Vegetation at Pack Forest is representative of the *Tsuga heterophylla* zone (Franklin and Dyrness 1988) in which it resides. Douglas-fir dominates Pack Forest, and other common shade-intolerant tree species include red alder (*Alnus rubra*), bigleaf maple (*Acer macrophyllum*), and black cottonwood (*Populus balsamifera* ssp. *trichocarpa*). Western redcedar (*Thuja plicata*) and western hemlock (*T. heterophylla*) are the most common later-seral, shade tolerant canopy species (Swanson 2006). Several species common to lowland western Washington forest are intermittently present, including grand fir (*Abies grandis*), Pacific madrone (*Arbutus menziesii*), and western white pine (*Pinus monticola*); with Sitka spruce (*Picea sitchensis*) and Oregon ash (*Fraxinus latifolia*) appearing in some riparian areas (ibid). There are several invasive species present at Pack Forest, most commonly Himalayan blackberry (*Rubus armeniacus*), Scot's broom (*Cytisus scoparius*), and reed canary grass (*Phalaris arundinacea*) in disturbed areas.

Timber harvesting in Pack Forest at the time of this study is largely occurring on a second rotation of FMUs established mainly between the years 1925-1935. This process has given rise to a pattern of recent clear-cuts that border forested stands which display "mature" characteristics (*sensu* Franklin *et al.* 2002). The border between clear-cut and forested FMUs are characterized by a microclimatic gradient running from the clear-cut into the forested FMU (see Chen *et al.* 1995). These gradients present several opportunities to address my hypotheses, mainly: 1) a before- and after-harvest comparison on local English holly growth and spread, and 2) information regarding English holly's response to clear-cut, forest edge, and forest "interior" conditions.

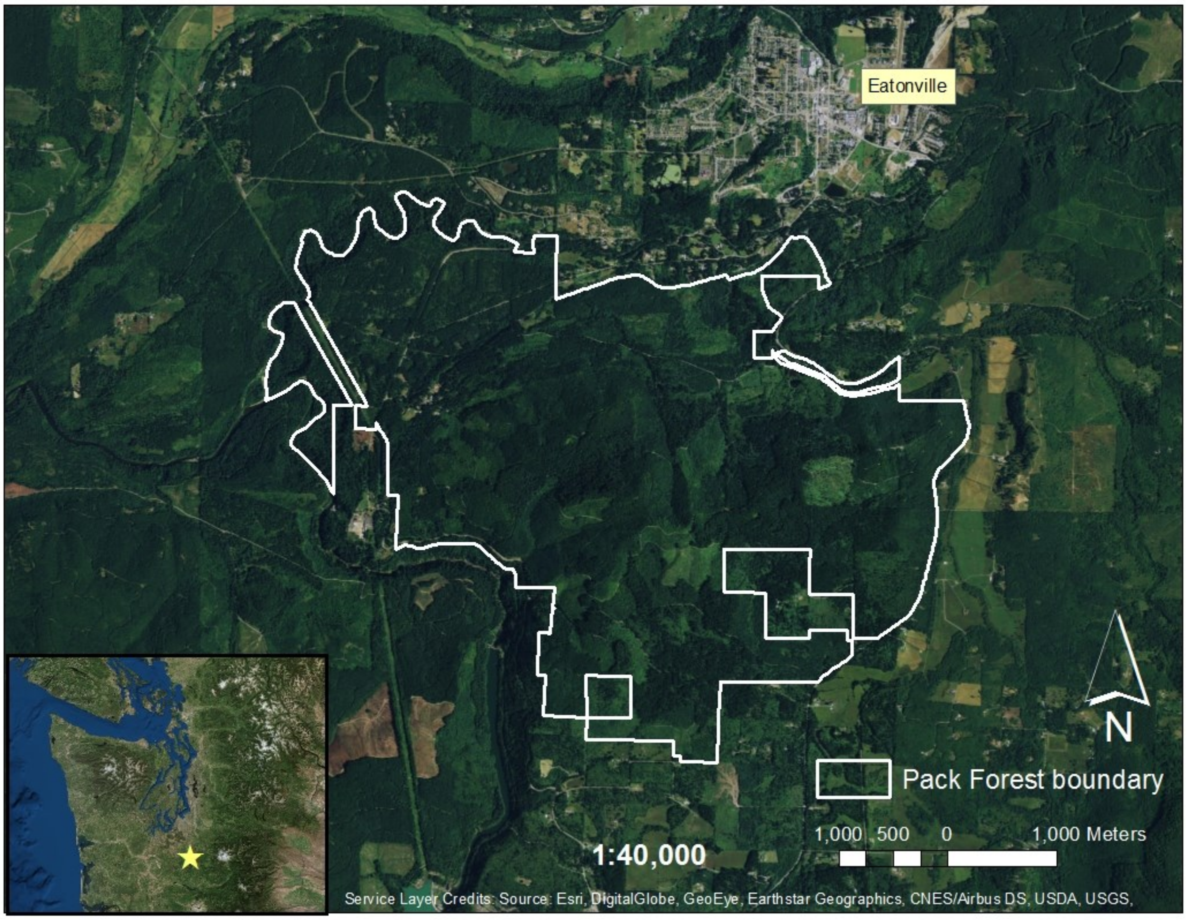


Figure 1. Boundary of Charles L. Pack Experimental Forest, Eatonville, and location within Washington state (insert). Imagery source: ESRI.

English holly control had not been previously undertaken at Pack Forest, and there is no herbicide application for vegetation control post-harvest.

Data collection

I sampled for English holly in Pack Forest locations where recently clear-cut FMUs (harvested from 2001-2006) bordered "mature" forested FMUs (established between the years 1925-1935). Forested and clear-cut FMU pairs were located through geographic information systems (GIS) software (ArcMap 10.2.1, ESRI 2014) using Pack Forest GIS data supplied by the Center for Sustainable Forestry at Pack Forest. Only pairings with a southern-facing forest edge were selected, so that there was enough light penetration to allow for a substantive "edge" environment to sample. Chen *et al.* (1995) found short-wave radiation reached interior forest levels at 60 meters for southern facing edges, whereas northern facing edges did not exhibit an "edge zone" of intermediate light levels. A total of six clear-cut to mature FMU pairs were sampled (Table 1)

English holly was sampled within these FMUs using two different sampling schemes. To investigate the questions of English holly abundance, establishment, reproduction, and potential edge effects in these environments, four clear-cut to mature stand pairs were sampled with one 20-meter wide belt transect originating at the forest edge and running to the approximate inner-most point of the forested stand and while sampling an equal amount of clear-cut (see Figure 2). Transects were oriented as perpendicular relative to the forest edge while avoiding FMU borders and roads. Transect length varied with stand geometry and 2.0-6.4% (SD: 1.65) of the total area of the FMU pairs was sampled. Transect location, direction and distance was determined in ArcMap by randomly generating potential origin points along the forest edge and calculating the centroid of the forested stand geometry. The direction and distance was selected so that the transect ran towards the interior of the forested stand (the centroid) while also being contained by the clear-cut unit. The selected transect origin and target endpoint were then loaded onto a handheld GPS unit (Trimble Juno 3B) running ArcPad software (ESRI 2014b), and the origin was located in the field using a proximity alert applet (Kennedy and Sawada 2012). Transects where constructed in 20 meter segments using a meter tape and compass, sighting the bearing pre-determined from ArcMap.

Table 1. Sampling pairings, with descriptive information from Pack Forest stand database. FMU type codes: CC = clear-cut, F = forested. Harvest year is presented for recent clear-cuts, and establishment year for forested FMUs, as planting usually occurred in the year after a harvest year. SDI = stand density index, a measure of tree spacing. Higher number indicates higher density of trees. Site index is the standard forest productivity measure: average height in feet of dominant and codominant trees, here reported at 50 years. Aspect mean is presented for the overall topography of the FMU.

Pairing	FMU name	Mgmt. type	Area (ha)	Harvest / establishment year	Site index	SDI	Elevation (ft.)			Slope (deg.)			Aspect mean (deg.)
							Min	Max	Mean	Min	Max	Mean	
1	PWT	CC	.529	2005	105	0	1620	1924	1776	2	58	22	183
1	Silvi Demo Control	F	.192	1930	105	445	1555	2031	1831	2	85	32	88
2	Lower Little Mashel West	CC	.192	2000	120	0	997	1114	1091	0	171	19	111
2	Waterfall	F	.123	1930	112	177	837	1134	968	3	152	35	22
3	Falling Firs	CC	.253	2003	105	0	1485	1550	1513	5	21	12	217
3	Thins East	F	.227	1935	120	280	1445	1603	1521	0	49	9	171
4	Cougar	CC	.268	2001	105	0	1416	1607	1493	1	46	18	154
4	Thins	F	.385	1935	120	280	1445	1603	1521	0	49	9	171
5	1200 Rd	CC	.178	2004	120	0	932	1039	983	6	31	17	17
5	HR2030	F	.046	1925	120	364	855	917	881	7	28	15	311
6	Jungle	CC	.315	2002	105	0	1298	1646	1546	2	107	34	247
6	2000 Rd Thin	F	.083	1932	85	397	1688	1871	1757	8	69	28	240

Transects were surveyed for English holly in the periods July – August 2014 and April – July 2015. Each individual English holly was assigned an identification number and data were collected on location (distance along transect into forested or clear-cut FMU with 0 = edge/origin), size (basal diameter and height), and presence or absence of berries. Data collection on location, size, and berry presence was limited to English holly ≥ 1 cm basal diameter with smaller stems just recorded for their presence. Clumps of English holly were noted where multiple individuals grew closely together to form a continuous canopy, as Stokes et al. (2014) found that holly formed a highly clustered distribution, with clumps of holly forming predominately via vegetative spread from older seed-established individuals. I did not attempt to differentiate between genets and ramets within clumps; rather, an individual constitutes an English holly that has developed a root system for its main stem or stems, and may or may not have a vegetative connection to another individual. For aging and growth ring analysis, a cross-sectional stem segment was collected from the base of individual English holly or the largest English holly in a clump, up to three individuals. These samples were used to determine the age of holly plants through growth ring analysis (Stokes and Smiley 1968). The samples were allowed to dry for at least a week, and then prepared by sanding with 60 (when cut was particularly rough), 120, 220, then 400-grit sandpaper (as in Stokes *et al.* 2014). Annual growth rings were then counted under a dissecting microscope.

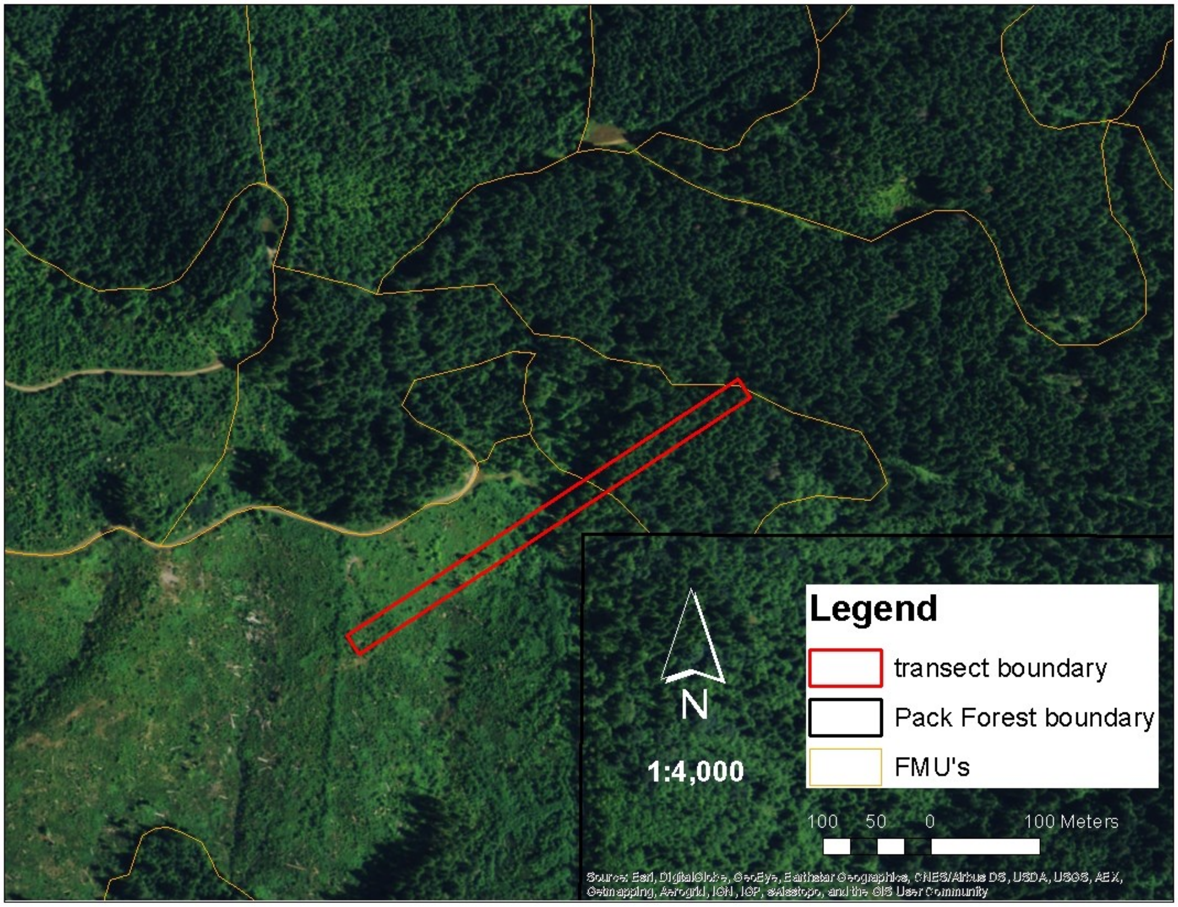


Figure 2. Location and extent of PWT-SDC transect (to scale) in Pack Forest.

To examine holly growth rate before and after timber harvest in clear-cut and forested FMUs, additional English holly samples were collected between October 2015-January 2016 from each FMU type using a paired sampling design with the goal of comparing the annual Basal Area Increment (BAI) in each holly for a five year period before and after harvest. BAI was selected as the growth rate metric because it removes any age-related growth trend resulting from adding the same volume of wood to an increasingly larger stem while preserving growth responses to suppression and release (LeBlanc 1990, Phipps 2005, Speer 2010). English holly large enough to be of sufficient age was located in a recent clear-cut, and was paired with the nearest English holly in the bordering mature stand. Thirteen total pairs were located from four

FMU pairings (which includes samples from transects) All holly collected from forest for this analysis was located within 100 meters from forest edge. An additional 4 unpaired English holly (two in each FMU type) were collected for a non-paired statistical analysis of before and after-harvest BAI between FMUs with a larger sample size. A stem cross section of each English holly was taken as near to the base as possible, but in one case where there were multiple stems originating from the base of the English holly, a cross section was taken further up on the largest stem to get a readable sample. Cross sections were prepared in the same manner as above. For each sample, five years of growth were measured along 4 radii before and after the year *prior* to harvest by using a sliding scale micrometer under a dissecting microscope. Radii were drawn as perpendicular as possible (originating from pith), while also keeping the radii as closely perpendicular to the tangent of the growth rings as possible. The reason for selecting the year prior to harvest was that harvest records for Pack Forest only listed the year of harvest, not month or day; therefore the harvest could have been before or after the formation of the annual growth ring in the summer. In some cases, one radius was excluded in BAI calculation due to the presence of reaction wood, stem traces, or injuries preventing accurate measurements. Total surface area was calculated for the 5 years of annual growth rings before and after year prior to harvest (YPH) using the average of the 4 radii, and then divided by 5 to get the average annual BAI over that period. Annual BAI was then converted to a percentage of the basal area of the overall cross section to reduce variability attributable to size differences between English holly.

Data analysis

To evaluate whether holly was distributed across the sampled areas in equal proportions, I used a Fisher's test. To evaluate whether distance from forest edge affected a number of response variables (abundance, growth rate, frequency of berry presence), I used regression models appropriate to each set of variables. Each FMU type (forested and clear-cut) was tested separately, with distance = 0 representing forest edge.

To test for a significant association between English holly abundance and distance from the forest edge, a Poisson regression model was used with abundance calculated as the number of stems at a given transect distance. In order to avoid violation of the independence assumption, only the oldest English holly of each clump was included in the analysis, as multiple English holly from the same clump could be influenced by each other due to English holly's vegetative reproduction capabilities (whereas the oldest is presumably seed established). Distance was consolidated into 5-meter segments, and sections of transects where no holly was present was given a "0" abundance value at the corresponding distance.

To look at English holly growth rates in transect data, basal diameter of aged samples was divided by the sample age to get a measure of growth over time. Basal diameter was chosen over height as the best measurement of growth increase over time because the main stem of English holly pre-existing harvest in clear cuts could have been damaged by harvest operations, and heights in the forest environment were impacted by dieback (symptomatic of *Phytophthora* infection) which seemed to have been occurring for some time . Also, Stokes *et al.* (2014) found that basal diameter was most closely correlated with age. Simple linear regression was used to examine associations between the distance from the forest edge and growth rate (basal diameter ÷ age), using only the oldest holly trees in a clump to avoid violation of independence assumption (as above). Issues of non-normal data distributions were addressed via log transformation of basal diameter ÷ age values or by comparing tests on data with and without the three greatest outliers removed (McDonald 2014). To test for differences between FMUs in mean growth rate, Wilcoxon test was used due to non-normality. Levene's test was used to compare growth rate variability between FMUs, with the response being each group observation's deviation from the group median.

Logistic regression was used to test the hypothesis that frequency of berry presence was influenced by distance from forest edge. Berry presence was represented as a binary response variable. Tests were run on individual stands and the entire length of the transect with 0 representing the forest edge, and negative distance values representing the forested FMU. Wald's test was used to test significance of distance in the model.

To analyze BAI data, two analysis of variance models were used to test for significance in differences between before and after harvest growth and between FMU types, as well as a significant interaction between stand type and before and after harvest growth that would indicate different responses within forest vs. clear-cuts. All factors were treated as fixed-effects. For the paired English holly samples, a randomized complete block design was employed, with pairs treated as blocks. A two-way ANOVA was used to test for differences in both FMU types without pairing.

All statistical analysis was carried out in R through the RStudio GUI (R Core Team 2013, RStudio Inc. 2016), following Zar (2010) and McDonald (2014).

3. RESULTS

Quantitative analysis

A total of 4 clear-cut and forested FMU pairings were sampled with transects, in which English holly presence was spatially variable: 216 English holly stems ≥ 1cm basal diameter were concentrated in 3 out of 4 total FMUs within two transects, and no English holly were found in the other two transects (Table 2). Clear-cuts with English holly present were harvested in 2005 (PWT) and 2000 (LLMW). English holly displayed a spatial clumping tendency, aggregating into 18 clumps of 2 or more individuals which formed a contiguous canopy, with just 12 individual English holly ≥ 1cm basal diameter located outside of a larger clump (see Table 5). These solitary individuals were of similar age to the largest clumps of English holly trees.

The abundance of English holly was not associated with the distance along transect from forest edge into interior in the SDC FMU ($z = 0.567$, $df = 1$, $p = 0.571$) (Figure 3). However, the abundance of holly was negatively associated with the distance from the forest edge into the clear-cuts for pooled clear-cut data (although marginally statistically significant: $z = -1.920$, $df = 1$, $p = 0.055$). When looking at clear-cut FMU's individually, this same trend held for the PWT FMU ($z = -1.898$, $df = 1$, $p = 0.057$) (Figure 4); in the LLMW FMU, distance was not statistically associated with abundance ($z = -0.089$, $df = 1$, $p = 0.929$) (Figure 5).

Proportion of English holly with berries was more than 7 times greater in clear-cuts than the forested stand (Table 3). Over 40% of clumps in clear-cuts contained holly with berries,

Table 2. Total English holly individuals sampled in two transects conducted in Pack Forest (Eatonville, WA) between July –August 2014 and April– July 2015. Differences in total # of holly between all FMUs statistically significant (Fisher's exact test; ≥ 1cm basal diameter $p < 0.000$, < 1cm basal diameter $p = 0.002$).

Transect	FMU type	FMU id	Clumps	# of holly ≥ 1 cm basal diam.	# of holly < 1 cm basal diam.	Density (holly/m^2)*
1	forest	SDC	16	110	48	.0359
	clear-cut	PWT	11	51	17	.0156
2	forest	Waterfall	0	0	0	0
	clear-cut	LLMW	3	55	5	.0188
3	forest	Thins E	0	0	0	0
	clear-cut	Falling Firs	0	0	0	0
4	forest	Thins	0	0	0	0
	clear-cut	Cougar	0	0	0	0
Totals	**forest**		16	110	48	.0104
	clear-cut		14	106	22	.0084
	Overall		30	216	70	.0094

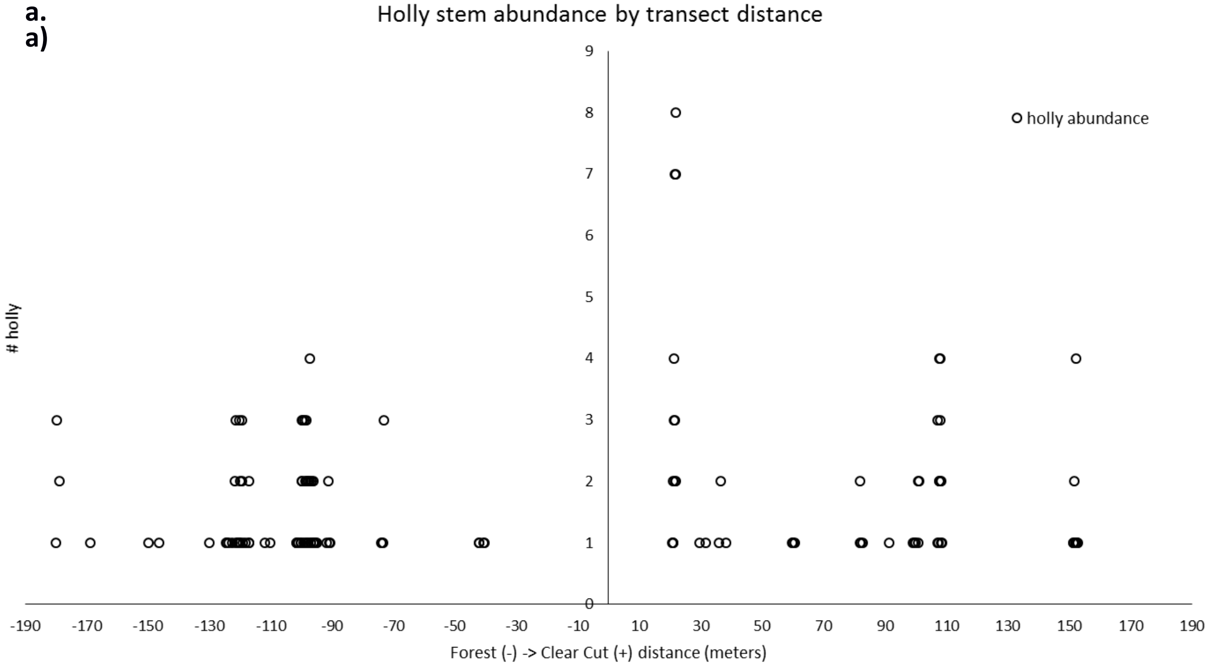
*total sample including English holly <1cm basal diam.

approximately twice that of clumps in the SDC FMU. However, this difference is mostly attributable to the difference between the largest clumps, as smaller clumps have similar proportions of berry producing trees in both environments (Table 3). Distance from forest edge was marginally positively associated with berry presence in the SDC FMU ($\chi^2 = 2.9$, df = 1, p = 0.087), however, this result is only based on three holly stems in the forested unit producing berries. Distance from forest edge was negatively associated with berry presence in the pooled clear-cut data ($\chi^2 = 9.8$, df = 1, p = 0.0017). However, statistical significance appears to come from the one large clump in the LLMW FMU, because berry presence is not statistically associated with distance when clear-cuts are examined individually (PWT: $\chi^2 = 1.8$, df = 1, p = 0.18; LLMW: $\chi^2 = 0.47$, df = 1, p = 0.5).

Table 3. Counts of fruit-bearing holly and proportion of fruit-bearing holly out of total number of holly in two transects sampled in Pack Forest (Eatonville, WA) between July –August 2014 and April– July 2015. Data presented for individual holly and clumps. Different letters indicate statistical differences in forest-clear-cuts & between clear-cuts comparisons (α = 0.05, Fisher's exact test).

	SDC (FORESTED)	PWT+LLMW (CLEARCUTS)	PWT alone	LLMW alone
holly with berries / total holly	3/111 | 2.7% a	20/104 | 19.2% b	6/51 | 11.8% a	14/53 | 26.4% a
Clumps with berries / total clumps	3/16 | 18.6% a	6/14 | 42.9% b	5/11 | 45.5% a	1/3 | 33.3% a
holly in clump with berries	1/2, 1/28, 1/1	1/1, 3/5, 1/1, 1/3, 14/42	1/1, 3/5, 1/1, 1/3	14/42

a.



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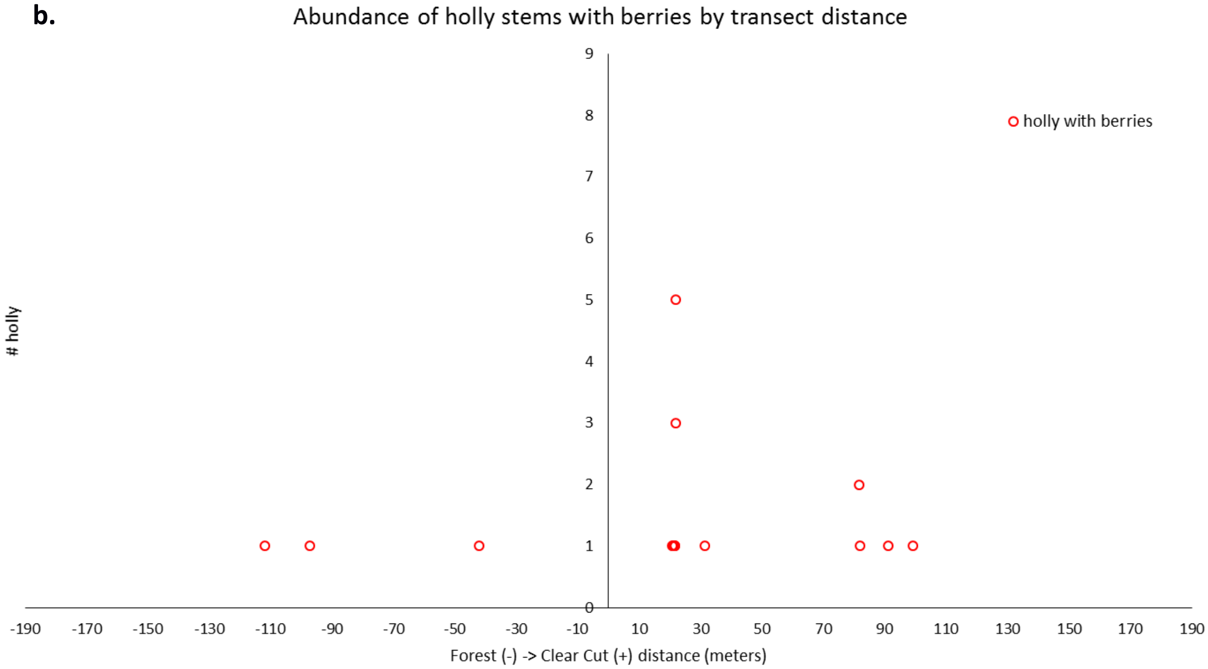
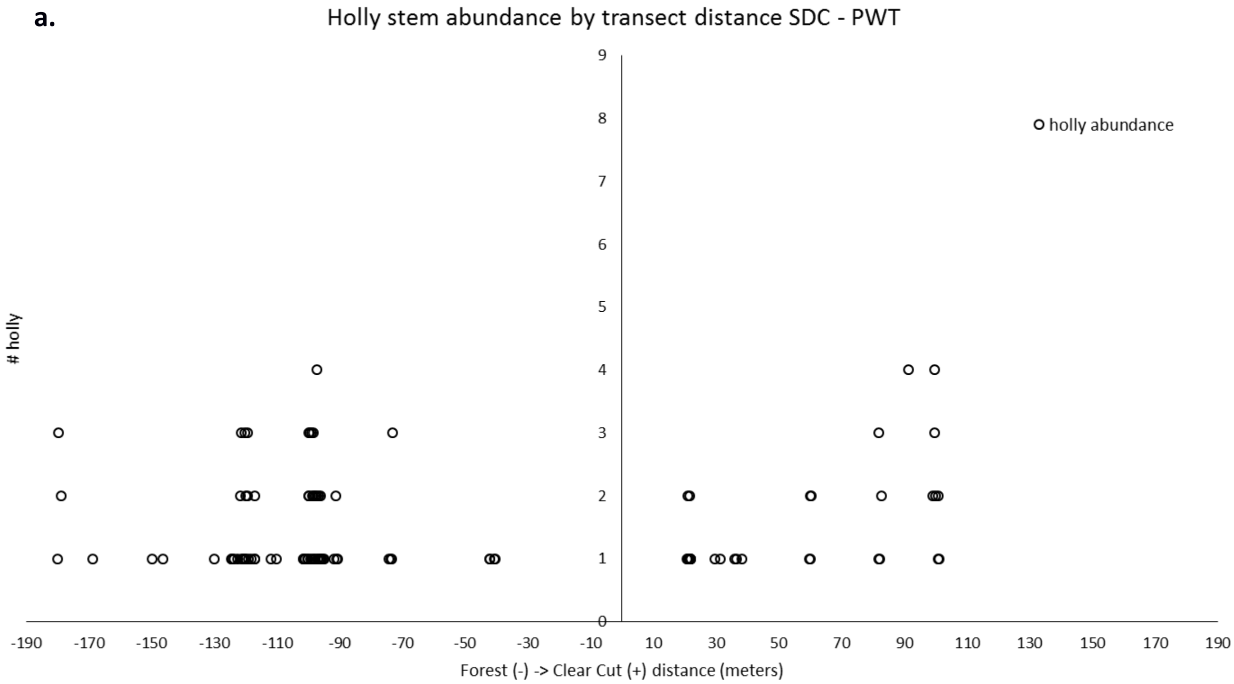


Figure 3. a) Overall abundance and b) abundance of berry producing holly along two transects from forest into clear-cuts. Berry presence statistically associated with distance in clearcuts (distance coefficient: -0.023381, $\chi^2 = 9.8$, df = 1, p = 0.0017, n = 104; PWT & LLMW FMU's pooled), but not in forest (Wald's test on distance term in logistic regression model, $\chi^2 = 2.9$, df = 1, p = 0.087; n = 111).

a.



b.

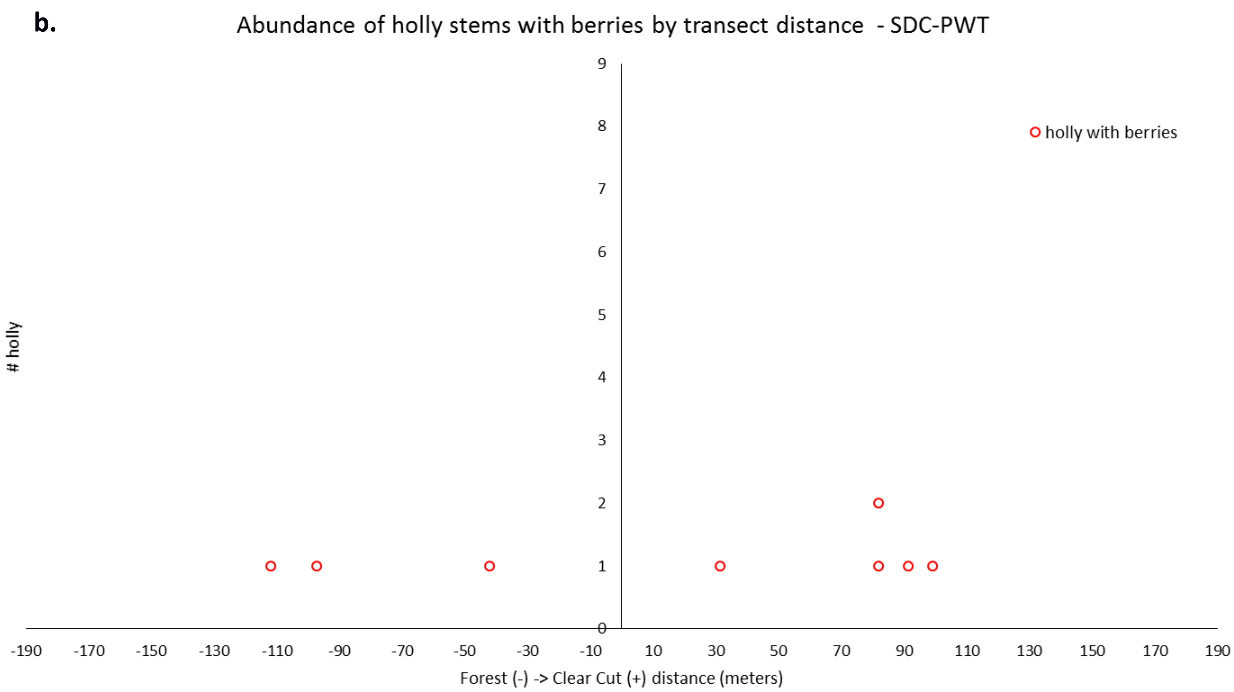
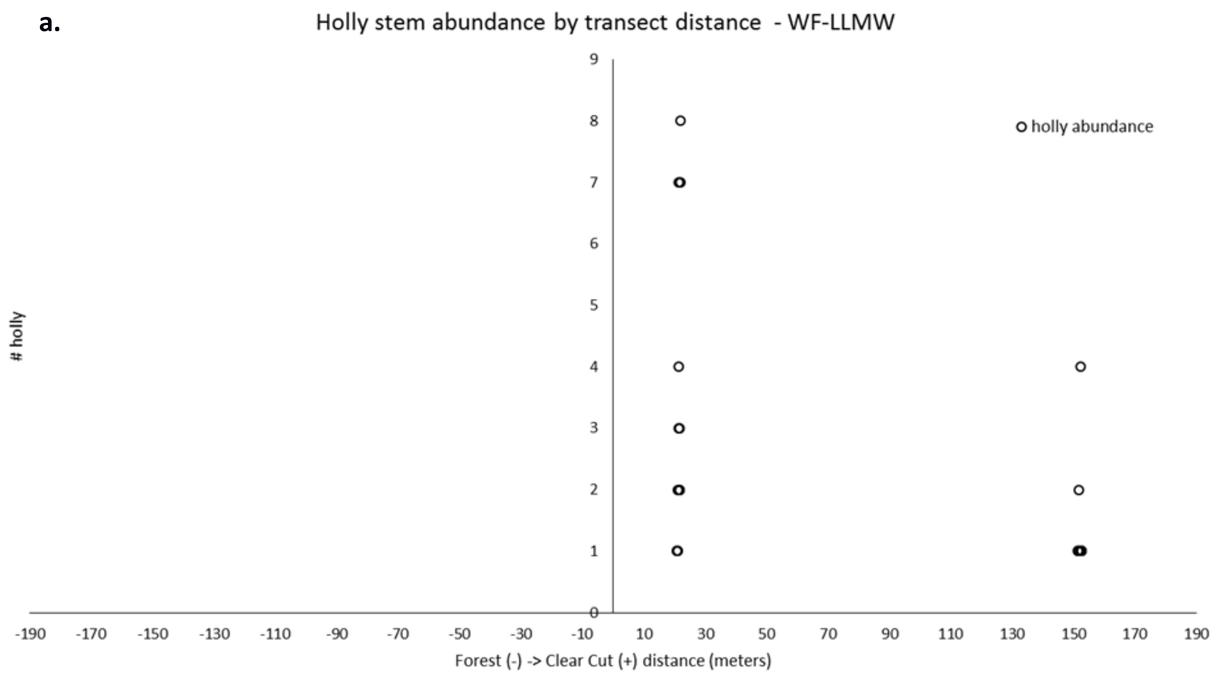


Figure 4. a) Overall abundance and b) abundance of berry producing holly along SDC-PWT transect. Berry presence in PWT not significantly associated with distance (Wald's test on distance term in logistic regression model, $\chi^2 = 1.8$, df = 1, p = 0.18; n = 51). SDC as described in Figure 3.

a.



b.

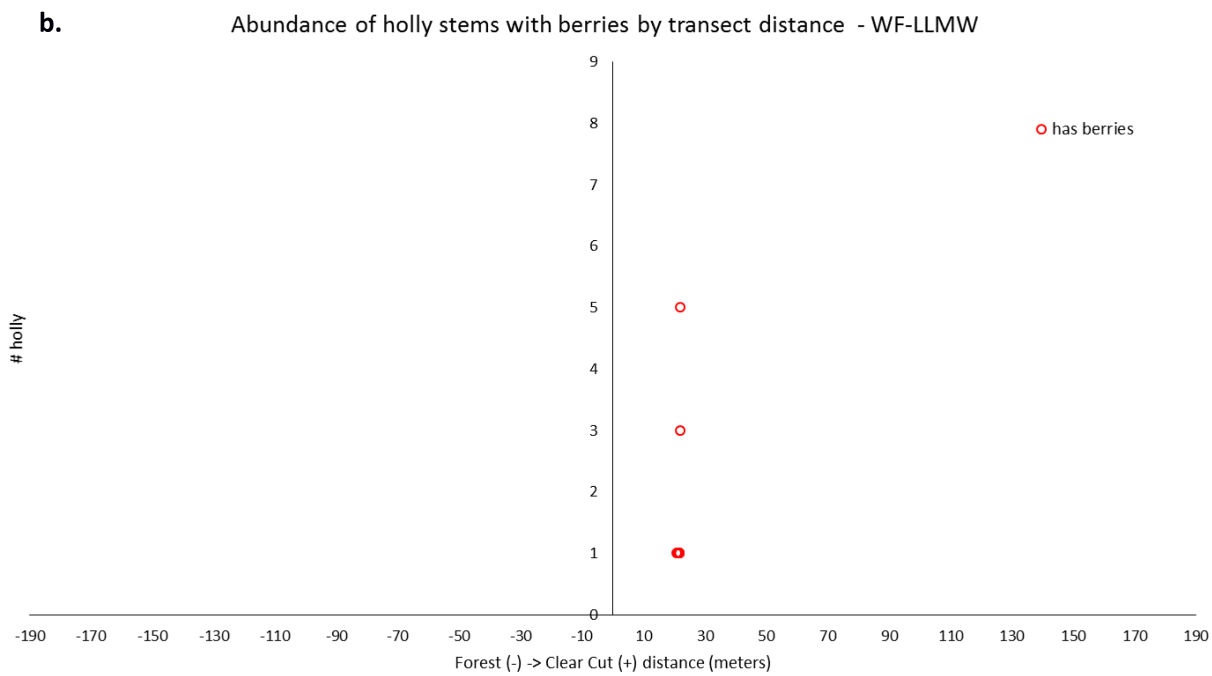


Figure 5. a) Overall abundance and b) abundance of berry producing holly along Waterfall – LLMW transect. Berry presence not statistically associated with distance (Wald's test on distance term in logistic regression model, $\chi2 = 0.47$, df = 1, $p = 0.5$, $n = 53$).

Throughout the course of my time at Pack Forest I kept note of FMUs that contained English holly outside of my study areas. I did not systematically search all FMUs; rather, I kept a log of where I saw English holly as I travelled between study sites and explored areas of interest in Pack, including the "old – growth" Hugo Peak FMUs. Pack Forest interns also reported FMUs where they noticed English holly present. Table 4 summarizes the number of stands in which at least one English holly was spotted, including the stands where I carried out my sampling.

Table 4. Stands at Pack Forest in which English holly presence was noticed by myself and Pack Forest interns while carrying out travel between study sites, general exploration, and intern duties. These numbers are not the result of a comprehensive search of Pack Forest FMUs, and therefore not an accurate estimation of proportion of invaded area.

FMU age class (year established)	Number of FMUs containing at least one holly	Total number of FMUs in age class
2002-2009	5	14
1988-2001	13	55
1972-1987	4	52
1925-1935	22	42
1800 ("old growth")	2	4

Table 5. Summary of English holly abundance, location, berry presence and morphological characteristics in two transects sampled between July 2014 and July 2015. Results organized by clumps and individuals. FMU type signified by “f” for forested, “cc” for recent clear-cut. Establishment year is age of oldest individual in clump.

							basal diameter (cm)				height (m)			
transect	clump or individual	FMU code (type)	year established	# stems	transect location*	berries	mean ± 1SD	median	min-max	range	mean ± 1SD	median	min-max	range
1	1	SDC (f)	1992	2	42.1-42.2	yes	3.5 ± 2.4	-	1.8-5.2	3.4	2.4 ± 0.85	-	1.8-3.0	1.2
	2	SDC	1995	2	40.5-40.8	no	2.05 ± 0.92	-	1.4-2.7	1.3	1.45 ± 0.64	-	1.0-1.9	0.9
	3	SDC	1981	6	73.2-73.8	no	2.27 ± 1.77	2.2	1.1-5.5	4.4	1.77 ± 1.58	1.35	0.2-4.7††	4.5
	4	SDC	1992	5	90.8-91.8	no	2.04 ± 1.01	1.6	1.2-3.6	2.4	1.4 ± 0.74	1.1	0.8-2.6	1.8
	5	SDC	1980	28	95.4-100.3	yes	2.0 ± 0.68	1.9	1.0-3.8	2.8	1.79 ± 0.58	1.8	0.8-3.0	2.2
	6	SDC	1982	21	99.3-101.7	no	1.98 ± 0.88	1.95	1.0-4.0	3.1	1.78 ± 0.65	1.9	0.8-3.0	2.2
	7	SDC	1983	1	110.2	no	2.4	-	-	-	1.9	-	-	-
	8	SDC	1993	1	112.0	yes	3.1	-	-	-	2.2	-	-	-
	9	SDC	1982	31	117.1-130.0	no	1.99 ± 0.76	1.9	1.1-4.3	3.2	1.56 ± 0.47	1.4	1.0-3.0	2
	10	SDC	1990	1	124.6	no	4.4	-	-	-	3.3	-	-	-
	11	SDC	1985	1	117.0	no	1.9	-	-	-	1.7	-	-	-
	12	SDC	1990	1	146.4	no	1.1	-	-	-	1.2	-	-	-
	13	SDC	2000	1	168.7	no	1.1	-	-	-	1.2	-	-	-
	14	SDC	1989	1	178.9	no	2.7	-	-	-	2.1	-	-	-
	15	SDC	1994	5	178.9-180.0	no	1.72 ± 0.39	1.6	1.2-2.2	1	2.04 ± 0.40	1.9	1.7-2.7	1
	16	SDC	2001	3	97.8-98.2	no	1.73 ± 0.68	1.5	1.2-2.5	1.3	1.1 ± 0.26	1	0.9-1.4	0.5

	17	PWT (cc)	1994	1	29.6	no	4.2	-	-	-	1.9	-	-	- 1
	18	PWT	1992	1	31.5	yes	5.3	-	-	-	18	-	-	-
	19	PWT	1987	3	35.9-36.4	no	3.43 ± 1.59	4.3	1.6-4.4	2.8	2.0 ± 0.4	2	1.6-2.4	0.8
	20	PWT	2006	1	38.2	no	1.9	-	-	-	1	-	-	-
	21	PWT	1987	4	59.9-60.5	no	2.23 ± 1.20	1.85	1.3-3.9	2.6	1.05 ± 0.3	1.1	0.7-1.3	0.6
	22	PWT	1976	5	81.8-82.8	yes	3.84 ± 2.49	2.8	1.4-7.8	6.4	1.4 ± 0.71	1.2	0.6-2.4	1.8
	23	PWT	1987	1	91.3	yes	3.7	-	-	-	1.7	-	-	-
	24	PWT	1991	6	100-101.1	yes	2.55 ± 1.41	1.75	1.6-4.9	3.3	1.7 ± 0.64	1.55	0.9-2.5	1.6
	25	PWT	1988	3	99.2-99.8	yes	4.4 ± 4.77	1.9	1.4-9.9	8.5	1.17 ± 0.74	0.9	0.6-2.0	1.4
	26	PWT	1992	25	107.2-108.6	no	2.05 ± 0.83	1.7	1.0-3.9	2.9	1.78 ± 0.50	1.6	1.0-3.2	2.2
	27	PWT	1989	1	107.2	no	6.1	-	-	-	2.5	-	-	-
2	28	LLMW (cc)	1995	42	20.7-22.0	yes	3.69 ± 2.04**	3.1	1.1-10.8	9.7	3.08 ± 0.91†	3.35	1.0-5.0	4
	29	LLMW	1998	8	152.3-152.7	no	3.4 ± 2.31	2.7	1.3-7.9	6.6	2.71 ± 1.40	1.95	1.4-4.7	3.3
	30	LLMW	1992	5	151.3-151.9	no	3.12 ± 0.97	3.4	1.7-4.1	2.4	2.43 ± 0.87‡	2.43	1.4-3.5	2.1
SUMMARY		SDC	1980-2001	110			2.05 ± 0.89				1.71 ± 0.67			
		PWT	1976-2006	51			2.73 ± 1.77				1.66 ± 0.57			
		LLMW	1992-1998	55			3.59 ± 1.99				2.97 ± 1.0			
		forested		110			**2.05 ± 0.89**	**1.9**	**1.0-5.5**	**4.5**	**1.71 ± 0.67**	**1.7**	**0.2-4.7**	**4.5**
		clear-cut		106			**3.18 ± 1.93**	**2.7**	**1.0-10.8**	**9.8**	**2.32 ± 1.04**	**2**	**0.6-5.0**	**4.4**

*Distance from origin; location of base of main stem. **missing basal diameter for one sample in this clump.
†missing height for two samples in this clump. ‡Missing height for one sample in this clump. ††Smallest height represents only live tissue on an individual where there was significant crown dieback).

Establishment over time

The oldest English holly sampled established in 1976 in the PWT FMU, and the next aged sample establishment occurred four years later in SDC (Figure 6). Total clumps increased approximately linearly over time to the present (linear regression: $F_{1,28} = 367$, Adj. $R^2 = 0.927$, $p < 0.0000$), as well as total number of all aged samples ($F_{1,60} = 4403$, Adj. $R^2 = 0.986$, $p < 0.0000$). The majority of new clumps and all aged holly samples established prior to harvests. Expansion of the English holly population in the study area appears to be continuing, based on the high proportion of English holly in the smaller basal diameter classes (Figure 7). As I did not collect any data that would allow for an estimate of mortality rates, the influence of mortality on the size class structure and the establishment rate over time is unknown.

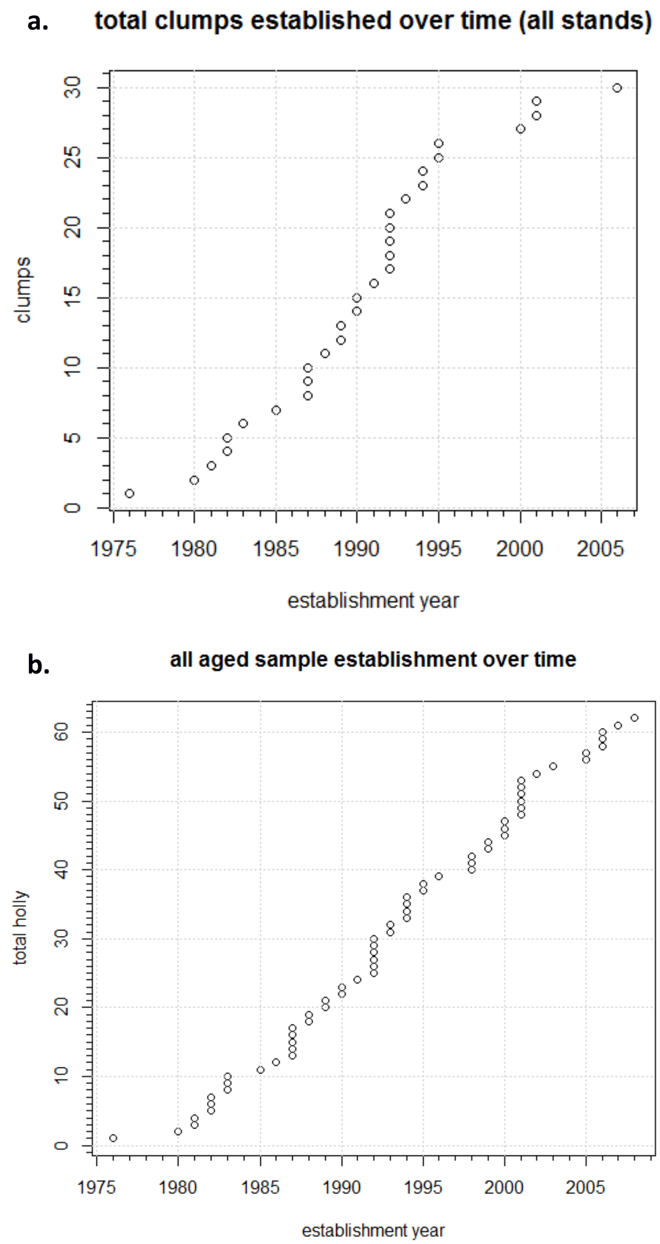


Figure 6. Establishment year for a) clumps (n = 30) and for b) all aged holly samples (n = 62). Clear-cuts were harvested in 2000 (LLMW) and 2005 (PWT).

While I did not age the smaller diameter holly, approximate ages can be inferred from the data collected in St. Edward State Park in Kenmore, WA (Stokes *et al.* 2014) assuming the age to basal diameter relationship does not radically differ between sites. In the St. Edward's known-

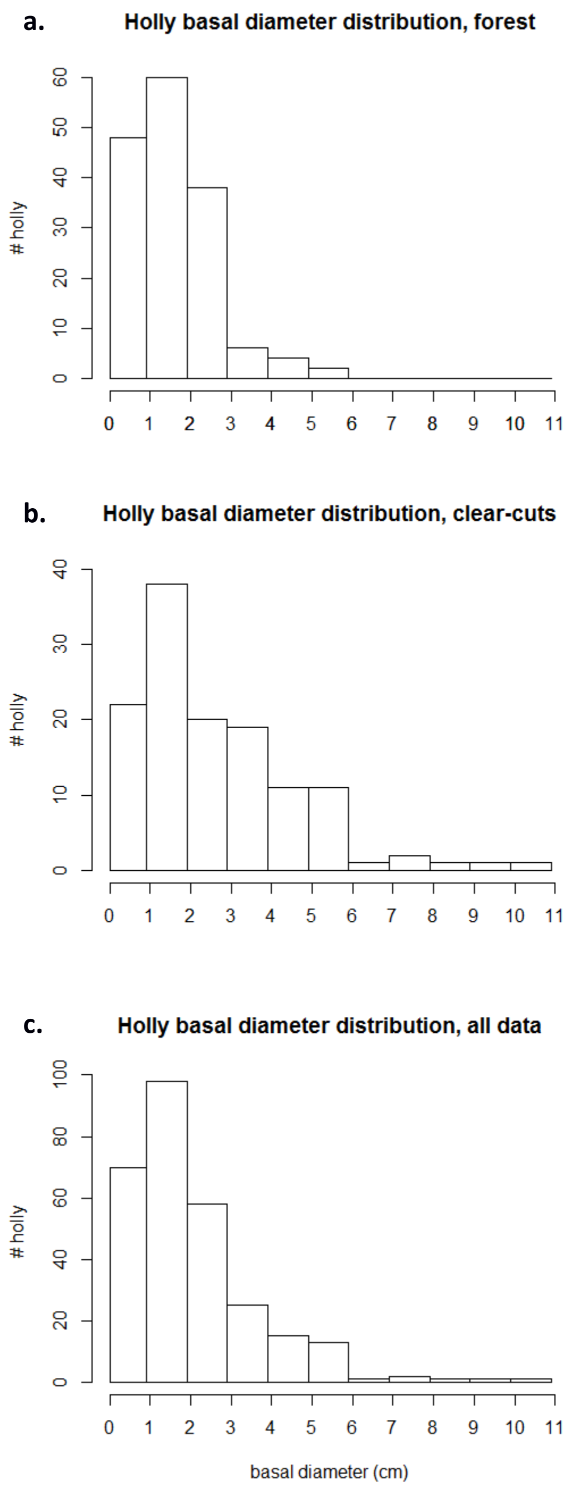


Figure 7. Basal diameter distribution for a) Forested FMU (n = 158), b) Clear-cuts (n = 127), and c) Entire sample (N = 285) including sprouts (< 1cm basal diam.).

age sample, the mean age ± 2 standard deviations of English holly < 1cm basal diameter is 3.8 ± 4.4 years (n = 319), and the mean age ± 2 standard deviations for holly between 1-2cm basal diameter, 9.8 ± 8.1 years (n = 100). As the English holly in the St Edward State Park data set were located in a forested environment, and given the higher growth rate found for English holly in clear cuts in my study area (see below), it is possible that the average age for stems of this size is lower in clear-cuts in my study area. The age range of aged samples in clear-cut FMUs with basal diameters between 1 and 2 centimeters is 6-14 years (n = 9), with stems as large as 3.7 and 3.9 basal diameter being 8 and 9 years old respectively (Table 6). Based on these age approximations for the holly in my study area, it is probably the case that most English holly < 1 cm basal diameter and a substantial number of the un-aged English holly in the 1-2cm basal diameter range established post-harvest in both clear-cuts and the forested stand.

Almost all recent establishment (including sprouts) has occurred within existing clumps. One known-age sample established outside of an existing clump in PWT in 2006 (Tree #114, Table 6), and three sprouts were recorded in SDC that were not associated with any clump.

Table 6. Known-age samples which established post-harvest. PWT was harvested in 2005, and LLMW was harvested in 2000.

Transect	Tree ID	stand	basal diam.	Distance from edge (m)	clump	age 2014	Estab. year	Basal Diam. Avg. (SD)
1	12	SDC	1.3	-91	4	9	2005	1.69 (0.36)
	2	SDC	1.4	-40.5	2	8	2006	
	115	PWT	1.6	36.4	19	6	2008	
	114	PWT	1.9	38.2	20	8	2006	
	116	PWT	2.3	60.5	21	9	2005	
	130	PWT	1.9	99.7	25	8	2006	
	131	PWT	1.4	99.8	25	7	2007	
2	244	LLMW	3.8	152.3	29	13	2001	5.85 (2.90)
	247	LLMW	7.9	152.6	29	13	2001	

Growth rate

Growth rates from transect data (basal diameter ÷ age) limited to the oldest known-age English holly in each clump negatively associated with distance from the forest edge in the forested FMU ($p = 0.0397$, $F_{1,14} = 5.142$, Figure 8a). Statistical significance in this linear trend stems from one observation near the forest edge which if removed results in a non-significant association with distance. Growth rates in clear-cuts were larger, more variable and remained constant relative to distance. Figure 8b displays basal diameter ÷ age for all aged samples (up to 3 per clump), which holds the same trend.

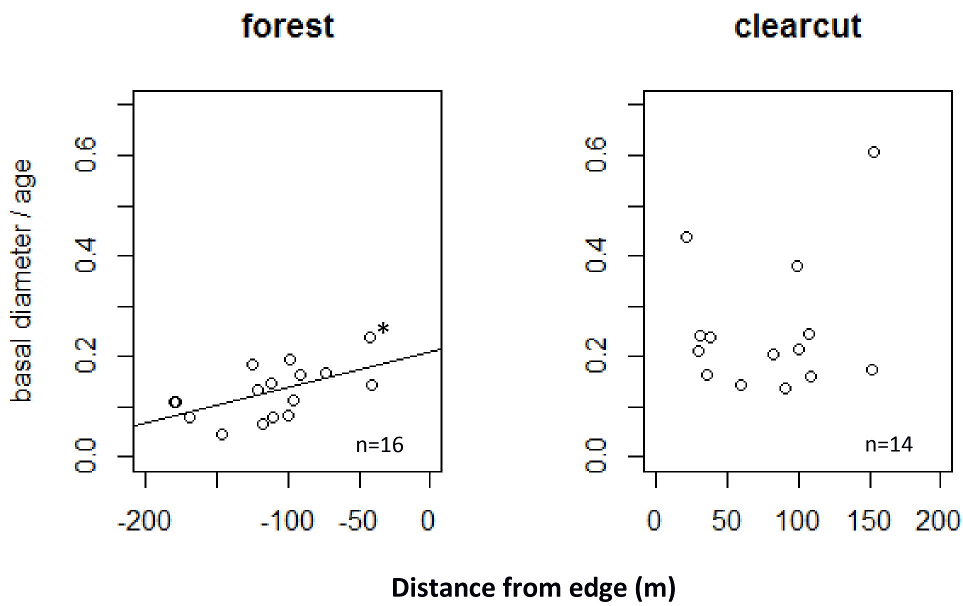


Figure 8a. Holly basal diameter over age at varying distance from forest edge in two transects from clear-cuts into forest. Ages restricted to single oldest holly per clump to avoid violating regression assumption of independence. Association between distance and basal diameter ÷ age in forest significant ($F_{1,14}$ = 5.142, p = 0.0397 on log-transformed values), clear-cut not significant ($F_{1,11}$ = 2.673, p = 0.1303 with top 3 outliers removed to address non-normality issues). When point of highest growth near edge in forest (marked by *) is removed, the association is no longer significant at α = 0.05. Difference between forest and clear-cuts significant (Wilcoxon test, W = 195,p = 0.00028). Differences in variability not significant (Levene's test using median, p = 0.08).

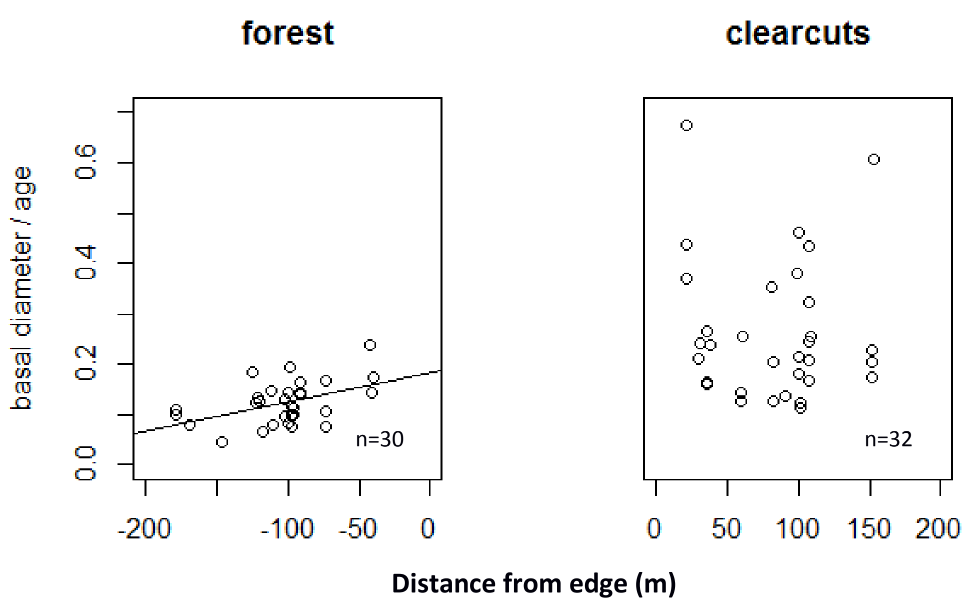


Figure 8b. Holly basal diameter over age at varying distance from forest edge in two transects from clear-cuts into forest. Data presented are for all aged holly samples, up to 3 per clump, which violates regression independence assumption. Forest regression equation: y = -0.0002424x + 0.2832201, $F_{1,28}$ = 7.654, p = 0.0099. clear-cut regression not statistically significant ($F_{1,30}$ = 0.153, p = 0.698).
Difference between forest and clear-cuts significant (Wilcoxon test, W = 105, p < 0.000). Growth rates in clear-cuts more variable than in forest (Levene's test using median, t = -3.17, df = 60, p = 0.001).

Annual BAI for a 10-year period before and after the YPH was higher after harvest than before harvest for both the forest and the clear-cut FMUs (two-way ANOVA, $F_{1, 56} = 103.5$, $p < 0.000$), with no difference in response to harvest between forests and clear cut (Figure 9). For two (out of n=15) English holly samples in the PWT clear-cut, BAI was greater in the 5 year period leading up to YPH than after YPH, a result not seen in the forested FMU samples.

The ratio of after-harvest annual BAI to before harvest annual BAI was generally consistent across the four sampling locations, and showed a substantial increase in growth post-harvest. The difference between mean after- to before- harvest ratios is marginally statistically significant ($V = 67$, $p = 0.073$) and clear-cut ratios were more variable ($p < 0.000$), with the largest ratios being present in the clear-cut. Several holly located in the clear-cut exhibited an abrupt increase in ring width immediately after the harvest year, indicating a suppression-release dynamic is present in some holly (Figure 11).

Some English holly sampled displayed signs of prior injury, possibly as a result of harvest operations. See Appendix for photos of selected English holly cross-sections used in aging.

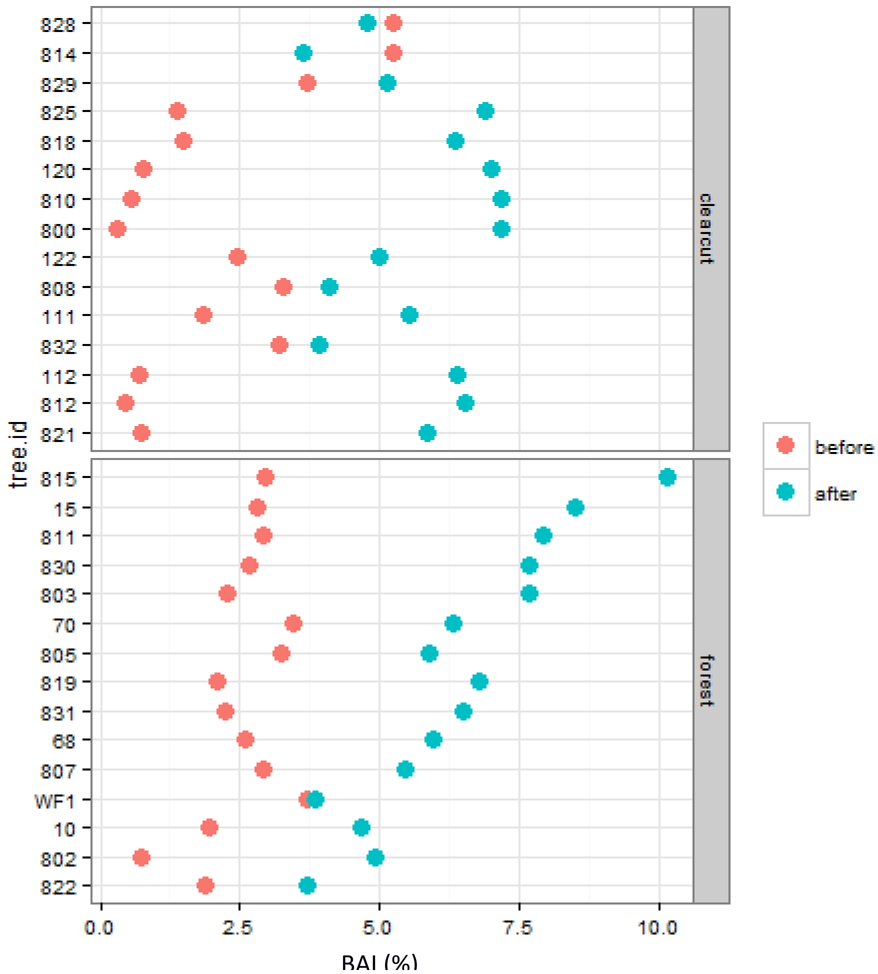


Figure 9. Differences in holly BAI for a 5 year period before and after YPH in clear-cuts and forested stands (n = 15). BAI before and after –harvest results from each holly sample are displayed. Before and after-harvest means significantly different ($F_{1, 56}$ = 103.5, p < 0.000, two way ANOVA).

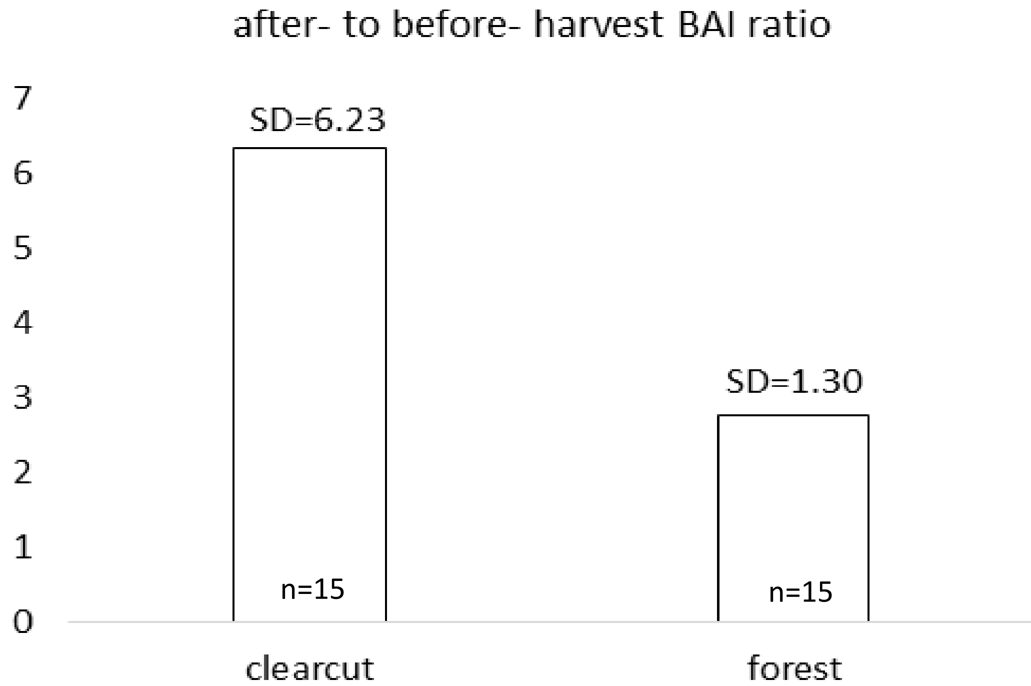


Figure 10. Ratio of post-harvest growth to pre-harvest growth across four clear-cut & forest pairings. Average and standard deviation of ratios displayed for pwt-sdc transect; results of one English holly per FMU presented for the other 3 pairings. Larger average ratio in clear-cut not statistically significant (one-tailed Wilcoxon signed-rank test, $n = 13$ pairs, $V = 67$, $p = 0.073$), but ratios in clear-cuts more variable than in forest (Levene's test using median, $t = -4.18$, $df = 28$, $p < 0.000$).

Qualitative observations

In addition to abundant young timber species (mostly Douglas-fir), clear-cuts were densely vegetated by shrub species with pockets of exposed soil and contained substantial amounts of large woody debris left from logging operations. Large sections of the PWT clear-cut where some English holly was located was covered by dense salal (*Gaultheria shallon*) over which bracken fern (*Pteridium aquilinium*) formed a more or less continuous canopy. Microsites in clear-cuts where English holly rooted included bare, rocky, exposed soil, receiving direct sunlight; next to and underneath decaying logs, alongside and underneath young Douglas-fir, and among native shrubs like salal. In the forested FMU, understory vegetation was less dense, with substantial patches of mostly unvegetated forest floor within the first 100 meters of the transect.

English holly in clear cuts generally exhibited much denser foliage and more instances of multi-stemmed individuals than in the forested stands. For trees which were producing berries, the number of berries produced per tree was much higher for trees in clear-cuts than trees in the forested stand (qualitative observation).

Figure 11. Close-up of rapid increase in diameter growth post clear-cut exhibited by the oldest holly sampled (located in PWT; arrow points to harvest year ring). Photo of entire cross section in Appendix (Photo 1).

Across all FMUs, many English holly displayed some level of current or past leaf and twig dieback, and symptoms were consistent with *Phytophthora* infection (Pscheidt and Ocamb c2016; D. Zuckerman, e-mail message, Nov. 20 2014). Nearly all of the above-ground portion of three trees in the forested SDC FMU had died, but there was live tissue at the very base of the stem in each case from which new leaves were sprouting. These trees were approximately 1 m in height.

I observed two cases of holly showing signs of herbivory, one in the forested SDC FMU, the other on a clump of English holly trees in the LLMW clear-cut. The signs of herbivory stretched quite far up the tallest stems in the LLMW clump, which were approximately 6 meters in height, suggesting a large mammal. There were signs of elk (droppings) and bear (trunk damage on several young Douglas-firs) in the area.

4. Discussion

English holly invasion & implications

English holly has invaded Pack Forest, with a density of 0.0094 stems/m^2 within the area sampled by transects—similar to the density of the invading holly population in St. Edward State Park (0.0141 stems/m^2 ; D. Stokes, unpublished data). However, given the spatially variable distribution of holly within the sampled area, a large sample may be necessary to provide a reliably accurate estimate of the true density of holly in Pack Forest. It is relevant to note that in the invasive species section of Swanson's (2006) otherwise very thorough ecological history of Pack Forest, English holly is not mentioned and the author notes that the invasive species problem at Pack Forest is, like elsewhere in the region, mainly a problem typical of disturbed areas.

English holly growth rates and berry production was higher in the clear-cut units compared with the forest, and the recent clear-cut effect on growth rate appeared to extend into the forest edge. While there were some statistically significant results regarding the association with distance from the forest edge and the abundance of English holly, and the proportion of English holly stems producing berries, not enough data and replicates at the FMU label were collected to be able to draw conclusions from these patterns.

English holly in England in unshaded habitats exhibits denser foliage, higher berry production (Peterken and Lloyd 1967) and overall higher biomass than holly in shaded habitats (Peterken and Newbould 1966). Peterken and Newbould (1966) studied dry matter production in English holly between shaded and unshaded sites in the New Forest and found that standing crop of holly (metric tons of biomass/ha) on sites shaded by oak and beech was 30% of unshaded sites on average, and productivity of holly on shaded sites (measured as metric tons of holly biomass/ha/year) was 20-30% of that on unshaded sites on average. In light of my results, it appears that the larger amount of growth seen in English holly in unshaded environments in its native range translates to invading English holly in the PNW climate, with its characteristic summer drought (Waring and Franklin 1979). The results of Peterken and Newbould (1966) suggest that the increase in growth post-harvest seen on my study sites is largely driven by canopy removal and presumably increase in available light.

If light availability is driving the trend in English holly growth rate in forest relative to distance from (southern facing) edge, one would expect to find that holly growth rates mirror the pattern of change in light levels with distance from the forest edge found by Chen *et al.* (1995): Growth rate should be near to clear-cut levels at the forest edge and then rapidly drop before gradually reaching forest interior levels, in an approximately exponential curve pattern. While association of holly growth rate with distance from forest edge was best fit by a linear model in my data, an exponential curve fit was also statistically significant ($t = 2.268$, $p = .038$). No holly was present within 40 meters of the forest edge in my data, preventing a complete comparison to the exponential pattern. Given the fragmentation of forest habitat in many locales, additional research into edge effects related to English holly invasion dynamics is needed.

Based on the extent of English holly's presence in the forest and the establishment and expansion of the local English holly population in the sampled area, it is clear that without control, English holly can be invasive in an actively managed lowland west-side PNW forest landscape, spreading across and persisting through forest successional stages—provided that there is a source of English holly seed. The town of Eatonville, site of many large berry producing English holly trees (pers. obs.), is located within 2 miles of the boundaries of Pack Forest and is a likely source for invading English holly via avian dispersal of seed.

If the trends in invasion, growth rate and berry production seen at Pack Forest translate broadly across west-side managed forests, they are cause for concern that conducting a timber harvest without any English holly control effort could result in increased growth and berry production and therefore accelerate English holly population growth and spread. The greater growth rate near the forest edge, and the increase in growth post-harvest in forested FMUs adjacent to recent harvests, suggest that the impacts of harvesting on English holly extend beyond the immediate harvest area to some degree. The growth rate by distance results for the SDC FMU suggest an edge effect that appears to propagate a substantial distance into the forest given the general negative linear trend from forest edge. The distance of edge effect in the SDC FMU may be larger than the average case, due to the fact that the distance which light penetrates SDC is likely further than was expected based on the data of Chen *et al.* (1995) due to the slope and aspect of SDC. From personal observation the slope of SDC, facing to the north and beginning at the edge of the forest, allowed for afternoon sunlight to reach further along the forest floor than with a flat or south-facing slope.

Mortality rates of English holly under the differing conditions sampled here is an important component in need of additional study. English holly seedlings are susceptible to drought (Peterken 1966), and there may be a higher rate of mortality for seedlings in recent harvest environments. I did encounter dieback in several English holly, and less severe signs of the presence of an apparent pathogen causing symptoms consistent with *Phytophthora* infection in many English holly across the sampled area. This pathogen appeared to have been affecting English holly for some time: in the forested stand, many English holly showed signs of repeated dieback and re-iteration of the main stem, suggesting that they had been battling infection for much of their lifespan. Two English holly within my transect in the forested stand had recently experienced dieback of most of their aboveground biomass, but were sprouting new branches at the base of the stem where there was still live tissue. Additional English holly outside of the transect also displayed dieback of nearly the entire above-ground portion. In the clear-cuts, extensive above-ground die-back was not observed, but some leaves on several individuals were beginning to brown around the edges and dead steams/branches still attached near the base of some English holly were also observed. *Phytophthora ilicis* is the most common *Phytophthora* disease submitted to the Washington State University Puyallup Plant & Insect Diagnostic Laboratory (J. Glass, e-mail message, Jul. 6 2015); however, the possibility remains that the die-back observed is a result of different cause.

It is interesting to compare the results from Pack Forest to Gray's (2005) study in Oregon which examined the importance of vegetation type, management history, climate, and topography on abundance and distribution of several invasive plants species, including English holly, on federal forestlands in western Oregon. In comparing sites representing different management types, English holly was positively associated with mean tree diameter and stand density, and also was among species that significantly increased in abundance after recent thinning; however it was less frequent in young post clear-cut stands. One possible explanation for this trend is a longer lag time since disturbance for holly to re-invade and/or regenerate compared to the shade-intolerant pioneer species (Gray 2005). Given the contrast with my results, in which a comparable amount of English holly is present in recent clear-cuts and forested units, I suggest that the practice of post-harvest herbicide treatments in commercial timberlands (G. Ettl, pers. comm.), which make up 60.5% of the lands in Gray's study, may be underlying this contrast. Additional research into the susceptibility of recent harvests to English holly invasion, and the persistence of pre-existing English holly, should be undertaken on sites where herbicide is applied post-harvest.

Caveats to interpretation of results

It is possibly the case that comparing growth before and after harvest using BAI on a basal cross-section does not capture a significant portion of the post-harvest growth in the clear-cuts. As previously mentioned, English holly in clear-cuts displayed much denser foliage and a higher amount of multi-stemmed individuals overall than their counterparts in the forested FMU, while a similar jump in BAI post-harvest was recorded in both the forest and clear-cuts. I suspect that if additional research were carried out using a more integrated method of measuring biomass production, such as including foliage biomass and accounting for multiple stems, there would be a significantly larger uptick in biomass production in clear-cuts post-harvest compared to forest and compared to pre-harvest growth in both environments. Peterken and Newbould (1966) report a high ratio of foliar biomass to trunk and branch biomass in unshaded holly; also, plants often shift root:shoot allocation toward belowground growth in response to the type of shift to a more exposed, drought-prone environment (Brunner *et al.* 2015) like that seen following timber harvest. A comparison of growth post-harvest should involve root growth and foliar biomass to get a more complete picture. Another potential source of variability in growth rate comparison is sex related growth differences; while males and females were mixed into the sample for my BAI analysis, it would be wise to compare the two exclusively to each other due to potential for sexual dimorphism in growth (Obeso *et al.* 1998, Retuerto *et al.* 2000) as well as obtain a quantitative measure of fruit biomass produced.

In the case that my methods do considerably underestimate English holly biomass increases in recently harvested areas compared to adjacent forest, and the real increase in biomass is larger on average, then the potential for increased impact from English holly on native plant species and the abiotic environment increases. There are several potential impacts related to the root and foliar biomass of English holly. Increased root biomass may create more competition with native plants for soil resources, and possibly make English holly control more difficult. Increase in leaf production may have several effects, including increasing shade effect beneath existing holly canopy (Peterken and Newbould 1966) and increased deposition of holly leaf litter. If this is true, then it is even more important to control English holly in recently harvested environments to avoid the potential impacts of increased English holly biomass production, such as direct competition and inhibition of desired commercial species such as Douglas-fir.

A potential source of variability in before-after harvest growth comparison is the innate biological growth rate in each holly which may be contributing to some of the increase in BAI in the period after harvest. I was not able to determine a way to get an accurate estimate of this trend and whether it was influencing the difference in BAI before and after-harvest; however it is quite apparent that there is a harvest effect given the abrupt and dramatic increase in growth post-harvest seen in several of the holly sampled in the clear-cut units.

Additional thoughts

While not the main focus of this study, the presence of several English holly in two of the "old growth" Hugo Peak FMUs raises concerns regarding the threat English holly poses to old-growth west-side lowland coniferous forest. A range of sizes were encountered, from small sprouts at the base of large Douglas-firs to larger trees approximately 4-6 meters tall, displaying the diffuse canopy and sparse foliage associated with English holly growing in low light environments (Peterken & Lloyd 1967).

Increased sunlight availability is presumably an important driver (to an extent) behind increased berry production in recent clear-cuts, and there is another factor affecting berry production that could be impacted by canopy removal as well. Being dioecious and entomophilous (Peterken & Lloyd 1967), changes to potential insect pollinator populations and behavior as a result of timber harvest could have substantial influence on English holly reproduction dynamics. There are several studies from other ecosystems that suggest pollinator behavior could be altered by woodland fragmentation (e.g., Goverde *et al.* 2002, Kreyer *et al.* 2004). Also, forest fragmentation due to timber harvest may also have an influence on the behavior of dispersers of holly berries, such as birds – e.g., perhaps via perch locations and spatial interconnectivity. Zika (2010) identifies bird dispersers of English holly berries in an urban Seattle environment, which included native and non-native bird species. Further research should be undertaken to determine the suite of bird species dispersing holly seed in more remote natural areas, and whether forest disturbance influences disperser behavior and seed predator behavior.

It was interesting to find that many of the English holly individuals which were not part of a larger clump established at approximately the same time as the largest clumps of English holly in their vicinity. This raises the question of what combination of events and factors are responsible for initiating the horizontal spread of some English holly and not others. Seed rain patterns may play a role, but much of the spread is vegetative. A mechanical force pinning English holly stems and branches to the forest floor, thus initiating root formation, may play a substantial role. Peterken and Lloyd (1967) report that English holly will form suckers from shallow lateral roots, and will layer when lower hanging branches reach the forest floor. Stokes *et al.* (2014) report cases where English holly were bent over by fallen branches, with the branches rooting where they touched the ground. Within two of the largest clumps in SDC, there were multiple sizeable fallen branches from Douglas firs layered over several of the English holly stems, possibly falling on top of the English holly and initiating this process. Others in these clumps were growing parallel to the ground instead of vertical, perhaps pinned down or bent over at an earlier age.

The English holly individuals occupying the dry, exposed micro-sites within PWT – such as areas with exposed, rocky mineral soil in direct sunlight – were larger holly which probably established prior to harvest. Small holly within the clear-cuts (< 1 cm basal diameter) were for the most part growing within the larger clumps, although three were noted outside of the transect in PWT, growing among salal and below several bracken fern. One of these was either deceased or suffering dieback similar to that in the forested SDC FMU. Study of holly occupying these dry, exposed sites should help build knowledge regarding English holly's specific habitat tolerances in the PNW. English holly is often referred to as a shade-tolerant, understory invader in PNW forests, and while English holly is reported to tolerate exposure in its native range, its seedlings are also reported to be sensitive to drought (Peterken 1966, Prentice and Helmisaari 1991). Therefore, the persistence and establishment of English holly in exposed microsites, given the summer drought that is a defining characteristic of the western-Washington climate (Waring and Franklin 1979) suggests that holly should not be considered excluded from these microsites in the PNW. Of course, a population study that tracks mortality in these environments would be needed to establish the level of tolerance in these drier, exposed environments relative to forest understories.

Finally, like the species that are the subject of the papers cited in the introduction, English holly has to a large extent been "off the radar" of many land managers. This is likely due to the dispersed invasion pattern of English holly, which appears to follow a stratified dispersal model (Lockwood *et al.* 2013). Long distance dispersal is achieved via birds, starting new colonies spatially separated from established source colonies. These colonies spread locally, independent from another, via vegetative reproduction and some seed fall (Stokes *et al.* 2014), and potentially go largely unnoticed until clumps expand and satellite populations begin to conglomerate into much larger continuous colonies. Another potential reason for the apparent tendency of English holly to go relatively unnoticed in forest understories is that land managers are used to looking to disturbed areas as Swanson (2006) relates, and the dark green foliage of English holly may not be immediately noticeable against the dark green foliage of native understory species common of PNW lowland forests. Fortunately, the awareness and concern appears to be increasing, and none too soon, as the threat English holly poses to ecological and economic resources continues to grow and control efforts will become increasingly costly the longer the wait for a concerted English holly control effort.

Management recommendations

Results indicate that English holly should be controlled in a managed forest environment. Harvest operations alone do not reliably kill pre-existing holly, and the increase in berry production post-harvest provides increased opportunities for holly spread. Holly can be controlled by uprooting when small enough, but holly quickly becomes too well rooted to mechanically remove without considerable soil disturbance, particular in clear-cuts. After it is too large to uproot, holly must be treated with herbicide, as it will only respond to cutting by vigorous re-sprouting. Stem injection with imazapyr is report to be the most effective and time-efficient method out of several methods tested in an Earthcorps trial which included frilling and cut-stump with glyphosate or triclopyr (Salisbury 2013). Priority should be given to fruiting trees, and an early detection and rapid response approach should be employed to eradicate local holly infestation before it becomes well established.

Further research needed

As I have stated through this discussion, there are several areas which require further research in order to better understand English holly invasion ecology and inform management efforts. Here is a summarized list of research topics:

- Mortality of holly in different habitats, and how/whether disturbance plays a role
- Seed viability and germination success in different habitats
- Impact of disturbance such as timber harvest on disperser and seed predator behavior and pollinators

- Importance of disease in invading holly populations
- Comparison of holly growth / productivity compared to other native species, and assessment of holly competitiveness compared to native species
    - Related question: is holly photosynthesizing during PNW winters? Peterken and Newbould (1966) report that adult holly has a relatively high rate of photosynthesis during winter in the New Forest, England when temperatures are above freezing. If true, English holly ability to take advantage of winter photosynthesis may have implications for the relative susceptibility of evergreen and deciduous forest in the PNW to English holly invasion.

Lessons learned

Based on my experience designing and conducting this study, there are several adjustments that I would make to improve the approach to the same research questions and which may be applicable to future English holly research efforts.

- Sampling: Enumeration of FMUs is probably the best option for getting accurate estimates of densities and abundances in different environments, due to the spatial clustering tendency of holly which randomly placed transects may not accurately characterize. There is a sampling method called Adaptive Cluster Sampling which is optimized for the special case of clustered spatial distributions (Philippi 2005) which may be the best option for sampling areas when enumeration is not practical.
- In addition to obtaining more recent harvest and forested FMU pairing replicates, investigating older clear-cuts may yield additional insights into the influence of forest

edge on English holly abundance and establishment by allowing more time for potential edge effects to influence the spatial distribution of English holly.

- As discussed above, more comprehensive holly biomass measurement methods that have better control for potential sources of variability (such as sexual dimorphism) would allow a more accurate picture of English holly growth response to timber harvest.
- Temporal variability: The amount of fruit production greatly varies year to year for English holly in England, according to Peterken and Lloyd (1967). While more light and heat is needed for holly fruit to be produced in general (Peterken and Lloyd 1967), the potential for temporal variability to influence patterns observed in a single year should be addressed by collection of data across several years.

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APPENDIX: PHOTOS

Photo 1. English holly # 120 from PWT FMU. This is the oldest holly sampled, established 1976. Notice the abrupt increase in ring width after harvest year, which is near to the pencil drawn numbers.

Photo 2. English holly #828 from PWT, exhibiting past injury possibly sustained from logging operations.

Photo 3. English holly #816, sampled as part of BAI study but not analyzed due to the large fissures shown here, which could be damaged sustained during logging operations.

Photo 4. Carrying out transect sampling in the SDC FMU.

Photo 5. Looking out over the PWT clear-cut.

Photo 6. Sampling a PWT English holly clump.