Comparing Growth and Form of Coast Redwood Selfs and Outcrosses[1]

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Abstract

We now report 22 years of new data and observations from the third of three small projects evaluating the effects of inbreeding in coast redwood (*Sequoia sempervirens* (D. Don) Endl.). We also briefly summarize previously-reported effects of inbreeding on redwood's cone production, seed set, germination percentage, nursery growth and survival, rooting of juvenile cuttings, and their growth and survival in field plantings. Offspring resulting from self-pollinations were compared to offspring from controlled outcrosses of the same seed-parents. Milder forms of inbreeding, likely among offspring of wind-pollinated redwoods, are likely to exhibit performances intermediate between those of selfs and outcrosses. Effects of self-level inbreeding were found to be small or absent in favorable conditions, increasing to serious or disastrous when the redwoods encounter competition or are grown in unfavorable or stressful environments. Deploying clones of outcrossed offspring will eliminate the possibility of inbreeding-caused poor performance of planted redwoods.

Keywords: form traits, genetics, inbreeding depression, *Sequoia sempervirens*, tree improvement

Introduction

With a few important exceptions, survival, growth and other conifer traits are adversely affected by inbreeding. In the 1970s, we and others wondered whether coast redwood (*Sequoia sempervirens* (D. Don) Endl.), uniquely hexaploid among conifers, might be protected from the effects of inbreeding by heterozygosity fixed or homozygosity slowed due to having three diploid sets of chromosomes. We first observed and measured control-pollinated selfs and outcrosses of a single redwood family of unknown origin, also including open-pollinated sibs, planted in 1965 on a high-quality mid-elevation redwood site near the Mad River in Humboldt County, California.

Shortly after joining the Berkeley faculty, one of us (WJL) noted in 1963 an easy-to-climb unusually fecund redwood of unknown origin (tree 10) growing on the University of California's newly acquired Russell Research Station. Using techniques recently learned at the Institute of Forest Genetics (Placerville, California), control crosses were made using its own pollen and pollens of trees 12 and 19, two nearby planted redwoods, thus producing families 10×10, 10×12, and 10×19. Open-pollinated cones (10×Wind) were also harvested when the cones matured. Seeds from these four families were germinated and grown in a greenhouse in 1964.

In spring 1965, five seedlings of each of the two outcrosses, four of the wind-pollinated family, and 14 of the selfs were planted at 3-m square spacing on available land within a deer-fenced Simpson Timber Company research area above the Mad River in Humboldt County, at 380 m elevation. The inbreeding-trial plot was on a high-quality northeast-facing redwood site. Selfs alternated with the other entries, such that the nearest four neighbors of each plot-interior self were four of the other entries, and the nearest neighbors of each of the other plot-interior entries were four selfs. The site had been well-prepared and there was no early mortality.

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All 28 seedlings survived the first year, and only two 10×19 outcrosses and two selfs had died by August 1979. Only modest competition from weeds and brush had occurred between 1965 and 1979. Average heights recorded after the first growing season were: family 10×19 0.15 m; 10×Wind 0.13 m; 10×12 and 10×10 both 0.09 m. Differences in relative size increased during the next 14+ years. By August 1979, average heights were: 10×19 4.7 m; 10×12 4.1 m; 10×Wind 2.7 m; and 10×10 1.8 m. Bole diameters were measured at 2 dm above ground: 10×19 8.5 cm; 10×12 7.3 cm; 10×Wind 3.5 cm and 10×10 2.2 cm. These observations were consistent with increasing adverse effects of inbreeding on growth with time. Differences in average numbers of main stems were also noted in 1979: 10×19 1.3; 10×Wind 1.5; 10×12 1.7 and 10×10 2.3. Surprisingly, 10 of the 12 surviving selfs were forked (> one main stem) in 1979. The trial was discontinued after 1979, because the selfs were beginning to be strongly suppressed by their neighbors (Libby et al. 1981).

During 1977 to 1979, we conducted controlled crosses employing a total of eight additional parents from Humboldt County. They had been cloned as mature cuttings, which continued to produce female strobili and/or pollen, thus allowing the crosses to be performed soon on very small ramets of the clones. The crosses were made on small field-grown ramets of the parent clones at Russell Research Station in 1977, and in 1978 and 1979 on even smaller ramets of the parent clones growing in a greenhouse or lathhouse.

We followed cone development of bagged female strobili protected from pollen, pollinated with self pollen, or pollinated with outcross pollen from different males. There were no consistent differences in cone abortion between selfs and outcrosses. This was less surprising as we learned that the female strobili protected from pollination also developed into normal cones. They developed normal-appearing seeds at about the same rates as pollinated cones, but apparently contained no viable embryos, since none of the 12,000+ seeds from the unpollinated strobili of 10 different parents germinated. Differences among selfed, wind-pollinated and outcrossed families in seeds per cone were highly variable and overall not statistically significant.

One clone (RB37) appeared highly self-compatible, with unusually high germination rates of selfs. But in most other comparisons, the germination rates of the outcrossed seeds exceeded the germination rates of selfed seeds of the common parent, in some cases substantially so. Furthermore, the germination rates of wind-pollinated seeds were lowest, probably because of insufficient windborne pollen in the Russell research planting where the 1977 control-pollinations were done and the wind-pollinated cones collected (Libby et al. 1981).

The first nursery run (December 1977), including selfed, outcrossed and wind-pollinated seedlings, was soon heavily infected with a *Phytophthora* root pathogen, and nursery survival percentages were: 56 percent for outcrosses, 44 percent for wind-pollinated sibs, but only 14 percent for the selfs ($p < 0.001$). In the second (June 1978) and third (December 1978 to June 1979) nursery runs, the root disease was greatly reduced and no wind-pollinated families were included. Survival was about 81 percent in the second and 89 percent in the third, with differences between the selfs and outcrosses small and statistically non-significant.

Ten 25-tree blocks of plants were organized in leach-tube racks from the second nursery run: each rack had 10 seedling selfs, 10 related seedling outcrosses, one ramet each of four ‘standard clones’ (random clones of Humboldt-County origin used in most of our experiments), and one seedling from select family RB17×RB23 from the Simpson breeding program. Three of the selfed parents (S series) were from the same stand on the upper Mad River, and thus the three parents of those three outcross families (S6×S3, S3×S1, & S1×S6) were possibly related. A fourth outcross was SI×ARC154. The ARC154 was at that time Earth’s tallest known tree, located in Redwood National Park, and thus not likely related to S1. Parents of the fifth outcross, RB17 and RB23, are similarly from different mid-elevation Humboldt County stands, and thus not likely related. The average heights of these families and the standard clones following 1 year’s growth in a lathhouse were, in rank-order: RB17×RB23 13.9 cm; S1×ARC154 12.8 cm; S6×S3, S3×S1, and S1×S6 11.6 cm; standard clones 11.0 cm; S1, S3 and S6 selfs 8.5 cm; and ARC154 selfs 4.6 cm ($p < 0.001$). Given a hypothesis of some inbreeding depression, this rank-order is what one might expect.

Dead plants in the racks were replaced by spares of the same identity and approximate size in March 1979. Half of the 25-tree blocks were then planted on a deep alluvial flat next to the Mad River in Humboldt County, at elevation 185 m. The other half were planted on a relatively xeric side-ridge near Philo, Mendocino County, at elevation 489 m.

After 5 summer months at the favorable Humboldt County site, all five RB17×RB23 outcrosses survived, only one of three planted ARC154 selfs survived, and the rest survived similarly: S1, S3 and S6 selfs 89 percent; S1×ARC154 81 percent; standard clones 80 percent; and S6×S3, S3×S1 & S1×S6 79 percent. These differences in early survival were not statistically significant. Early survival was much lower at the more-stressful Mendocino County site. RB17×RB23 survived 80 percent and two of three planted ARC154 selfs also survived. The rest were clustered: S6×S3, S3×S1 & S1×S6 28 percent; S1×ARC154 27 percent; S1, S3 and S6 selfs 26 percent; and standard clones 25 percent. These differences also were not statistically significant. Because of the low survival, this Mendocino trial was discontinued.

Average 1979 height after the March through August first-season growth on the Humboldt site, in rank order, was: RB17×RB23 17.5 cm; S6×S3, S3×S1, and S1×S6 15.8 cm; standard clones 14.6 cm; S1×ARC154 14.2 cm; S1, S3 and S6 selfs 12.3 cm; and ARC154 selfs 12.0 cm. Although average August 1979 height was in a fairly small range, the number of surviving plants allowed the differences to be statistically significant at the $p < 0.01$ level and the rank-order was consistent with some adverse inbreeding effect. In the more-stressed Mendocino trial, average August height was: S6×S3, S3×S1 and S1×S6 22.8 cm; RB17×RB23 15.3 cm; S1×ARC154 13.2 cm; S1, S3 and S6 selfs 11.7 cm; standard clones 10.0 cm; and ARC154 selfs 3.0 cm. With fewer surviving plants but a greater range in growth, these differences were barely statistically significant at $p \sim 0.05$, and the rank-order was consistent with a strong inbreeding effect.

As of the late 1970s, that was about what we knew about inbreeding effects in coast redwoods. We then launched the next small study. The 1977 to 1979-produced families were germinated and then a small sample of each was clonally propagated by rooting cuttings (Kirchgessner and Libby 1985). Planning to put clonal replicates of this third study on more than one site, we first observed and recorded rooting performance of the then-available selfed and outcrossed families, with selfs of one or both of their outcrossed parents included in the study.

In late December 1980, 5 to 15 juvenile cuttings had been taken from seedlings of the selfed and outcrossed families. After auxin treatment of fresh-cut bases, they were set in leach supercells that had potting mix topped with about 5 cm of a complex organic rooting mix. This eliminated the need to transplant recently-rooted cuttings from rooting mix to growing substrate as the cuttings grew into field-ready plantable rooted cuttings (i.e., stecklings). The labeled tubes were randomly placed in supercell racks, which were then set into shallow water maintained at 23 ^{o}C to supply warm moist air to the tubes. Mist was delivered five or more times per day, and the entire setup was partially enclosed in clear plastic to maintain heat and humidity. A cutting was declared 'rooted' when one or more roots emerged from the bottom of its supercell, and it was then moved in its supercell to a lathhouse to grow to steckling status.

Observations were noted frequently, and data on rooting and cutting mortality were taken weekly. The first roots emerged from the bottoms of the supercells after about 10 weeks, and the data were analyzed in three periods: early (mid-March to mid-April); middle (mid-April to mid-May); and late (mid-May to late July). In the early period, there was no recorded mortality of either self or outcross cuttings. The original 496 self cuttings had rooted 11 percent and the original 517 outcross cuttings had rooted 26 percent ($p < 0.001$). There was minor cutting mortality during the middle period: selfs 3 percent; and outcrosses 1 percent. The 443 surviving self cuttings had rooted 36 percent and the 382 surviving outcross cuttings had rooted 44 percent. Neither the mortality nor rooting percentages in the middle period were significantly different.

During the late period, the warm high humidity conditions had favored an algae growth that increasingly covered the remaining cuttings, a development we judged to be stressful to the cuttings. During this late period, the 271 surviving self cuttings had rooted 18 percent and the 211 surviving outcross cuttings had rooted 13 percent, not a statistically significant difference. But the 271 surviving self cuttings had 18 percent mortality while the 211 surviving outcross cuttings had only 7 percent mortality ($p < 0.001$).

By mid-July, we had sufficient rooted cuttings for our planned field trials, so the rooting trial was discontinued. Overall during mid-March to mid-July, 53 percent of the self cuttings and 64 percent of the outcross cuttings had successfully rooted. The self cuttings had suffered 12 percent mortality and the outcross cuttings only had 3 percent mortality. Differences in both rooting and mortality were statistically significant at $p < 0.001$. In summary, self cuttings rooted more slowly than related outcross cuttings, and self cuttings suffered greater mortality in conditions judged stressful (Kirchgessner and Libby 1985).

We were then ready to begin the third study, a larger comparison of selfs and related outcrosses in field trials, reported below. The questions addressed in this study were: 1) Is coast redwood, being hexaploid, buffered from inbreeding effects by having three copies of its genome? 2) Given that redwood suffers from inbreeding, how severe are such effects, and in what conditions might they be important?

Materials and Methods

Four nearly-identical clonal replicates were planted on:

(a) a flat valley-bottom site on 8 September 1981 as a hedge-orchard at the Russell Research Station (Latitude 37° 54’ 59” N, Longitude 122° 09’ 12” W, elevation 240 m);

(b) Simpson Timber Company site on 15 December 1981 just east of Maple Creek Rd, south of Snow Camp Rd and near Ward Rd (Latitude 40° 50’ N, Longitude 123° 53’ W, elevation 950 m);

(c) a ridgetop Masonite site on 26 January 1982, just south of the Navarro Ridge Rd (Latitude 39° 11’ N, Longitude 123° 38’ W, elevation 260 m); and

(d) 4 June 1982 at a second Russell Research Station site, described more fully below.

At each location, the fundamental research units were 2×2 squares, with selfs on one diagonal and their outcrossed sibs on the other diagonal (fig. 1). For data-taking purposes, these were mapped in five 25-tree blocks, but there was no additional space between the blocks, with 2×2 squares and two self-outcross single-pairs at the right-hand edge of each block. Ideally, a four-tree square contained two full-sibs on one diagonal and a self of each of the full-sibs’ two parents on the other diagonal. However, the available selfs and their outcrossed relatives did not permit that in most cases, so the relevant comparison was often between a paired self and its related outcross. Five ‘standard clones’ were planted in the same order down the center of each block. Spacing was square, with 3 m between trees except in (a) the hedge-orchard, where spacing was 1 m between hedges. Note that the same number of selfs and outcrosses occur as border trees, but that only two standard-clone trees are in the border.

All self and outcross stecklings were sourced from the rooting trial reported in Kirchgessner and Libby (1985). Stecklings of the five standard clones were sourced from the ongoing production of those clones for use as shared entries in various trials. With few exceptions, the same clone was planted in the exact same mapped location in each of the four plantings. Substitutions were made in the field trials when there was no ramet of the designated clone of proper size and condition available at the time of planting. While these substitutions may weaken paired comparisons of a self and its related outcross, it little affected analyses comparing averages of all selfs, outcrosses, and standard clones in or among trials.

Page 1

S	O	S	O	S
O	S	O	S	O
C	C	C	C	C
S	O	S	O	S
O	S	O	S	O

Page 2

O	S	O	S	O
S	O	S	O	S
C	C	C	C	C
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S	O	S	O	S

Page 3

S	O	S	O	S
O	S	O	S	O
C	C	C	C	C
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Page 4

O	S	O	S	O
S	O	S	O	S
C	C	C	C	C
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S	O	S	O	S

Page 5

S	O	S	O	S
O	S	O	S	O
C	C	C	C	C
S	O	S	O	S
O	S	O	S	O

Figure 1—Planting design showing five map pages of the (actually contiguous) self (S), outcross (O), and standard-clone (C) trees. Dark borders emphasize the locations of the 2×2 and paired arrangement of related selfs and outcrosses.

In the earlier seedling trials reviewed in the Background above, the seedlings germinated at about the same times and grew similarly in the nursery. There was no attempt to closely match the size of paired selfs and outcrosses at the time of field planting, and thus the (usually small) nursery-size differences in the selfs and outcrosses were carried into the field trials. By contrast, nursery steckling sizes were strongly influenced by when the cutting rooted, and because the self cuttings generally had rooted later, this confounded inbreeding effects on growth with post-rooting growth time in the nursery.

After completing the planting of the Simpson field trial and measuring steckling heights immediately following planting, we noted that the average height of the selfs was only 79 percent the average height of the outcrosses. We therefore attempted to match the sizes of paired selfs and outcrosses at the times they were planted in the Masonite and Russell field trials. Although not all pair heights were thus made closely similar, on average pre-matching was successful. Average newly-planted heights of the self, standard-clone and outcross stecklings were within 1 cm of each other in the Masonite and Russell trials.

The Russell Research Station hedge-orchard still exists, but has not been used to establish additional trials. The Simpson and Masonite trials have both been discontinued (See Results, below). The Russell field trial remains useful and had 22+ years' growth on-site when the most recent data were taken.

The Russell Study Site and Conditions

The Russell trial was established at the University of California's 115 ha Russell Research Station on a small alluvial terrace with a slight-to-modest north-facing slope. The site is 14 km east of the San Francisco Bay, 35 km inland from the Pacific coast, and has a Mediterranean climate characterized by cool wet winters and hot dry summers. Mean annual rainfall of 497 mm falls mainly between the months of November and March and the average daily high temperature is 31 ^oC in mid-summer (www.weather.com). Several below-freezing days occur in most winters, and temperatures near or somewhat above 40 ^oC occur in most summers. Although 20 km north-northeast of the nearest native redwood population, the good growth of previously planted redwoods in Russell Research Station's valley bottoms gave us confidence that redwoods could thrive on this site.

This site was well-prepared prior to planting and well-maintained during the establishment years following planting. Drip irrigation was delivered to each plant in rainless periods during the first 2 years following planting, at which time the roots of most of the then-established redwoods had apparently reached the generally reliable ground water flowing downhill in that watershed in and under the alluvial-terrace soil. The newly-planted redwoods thrived in this combination of abundant-to-sufficient water, warm-to-hot temperature, and abundant sunlight during the growing seasons.

Survival and growth during the 2 establishment years, before irrigation was discontinued, were both good. Growth of the selfs and outcrosses during that establishment period were, on average, nearly identical and not statistically significantly different. One self, one standard-clone and four outcross plants died shortly after planting. They were replaced by somewhat larger ramets of the same clones on 5 December 1982. Subsequent growth bias due to those late replacements would have probably favored the selfs. One self, one standard clone and one outcross were not replaced when other instances of early mortality were replanted, reducing sample size to 49 selfs, 49 outcrosses, and 24 standard clones. In subsequent years, encroaching brush was unusual and periodically cut back. Soon after each tree exceeded 2 m height, it was pruned to half-height. This practice continued until all the trees in the trial exceeding 6 m height had been pruned of branches from their lower 3 m.

As anticipated, as the trial developed and crowns began to close, outcrosses were first commonly and then generally taller than their paired selfs. We have analyzed and now present the most-recent data taken, in August 2004, not only on survival and growth, but on several other traits we thought might be of interest. By 2004, it was apparent that the smaller sizes of the selfs was increasingly due to suppression by their neighboring generally-larger outcrosses, rather than due solely or largely to their inbreeding status. Ten years later, in all surviving self-outcross pairs, without exception the outcross was larger, generally substantially so.

Russell Trial Data and Analysis

The Russell trial was assessed in August 2004, 22+ years after planting. Bole diameter at 1.37 m height (DBH) and bole height were measured on live trees. Incidence of forking and ramicorn branching (unusually large steep-angled branch) were tallied for each tree (undesirable for timber production objectives). New branches initiating on the lower bole after pruning (i.e., epicormic sprouts) were counted and measured for length. The frequency scale was: 0 = no epicormic sprouts (desirable); 1 = 1-3 sprouts; 2 = 4-10; 3 = > 10 sprouts. The development scale for longest epicormic sprouts present was: 1 = < 0.2 m length; 2 = 0.2-1 m; 3 = > 1 m (most undesirable). Vertically-oriented basal sprouts were also measured, with data similarly categorized on frequency and development scales: 0 = no basal sprouts; 1 = 1-3; 2 = 4-10; 3 = > 10 sprouts; and for the one tallest sprout among those present: 1 = < 0.2 m height; 2 = 0.2-1 m; 3 = > 1 m height. Bole straightness and symmetry – with emphasis on quality of the lowermost (maximum 6 m long) log - was assessed on a five point scale: 1 = unusable; 2 = bad; 3 = some defects; 4 = good; 5 = excellent; with penalties for sweep, sinuosity, crook, elliptical cross-section, and lower-bole (especially lowermost log) deformities. Four aspects of branching were assessed: branch diameter, length, angle, and uniformity of branch distribution and size. Ocular estimates of the typical branch diameter relative to bole diameter at the point of knot formation were assigned relative scores on a 1 to 5 scale : 1 = large; 5 = small (desirable). Typical branch length compared to tree height was assessed on a 1 to 5 scale: 1 = long branches; 5 = short branches (with narrow crown considered desirable). Branch angle was assessed in terms of the typical departure from a horizontal 90 degree angle: 1 = relatively steep droop; 2 = mild droop; 3 = near 90-degrees (preferred); 4 = mild upward angle; 5 = steep upward angle. Branch uniformity in terms of distribution and size was assessed on a 1 to 5 scale: 1 = highly variable in size or distribution; 5 = uniform size and distribution (desirable). Live tree crowns were examined for presence of cones.

Data were summarized and tabulated for each trait assessed: giving number of then-surviving plants observed or measured, means, standard deviation, minimum, and maximum values. Binary survival data for selfs, outcrosses, and standard clones were compared in chi-square tests. Paired t-tests (two-tailed) were constructed to compare live-tree diameter and height in surviving pairs between the adjacent selfs and outcrosses with a common parent. Tree diameter and height data were also compared between all surviving selfs and outcrosses using two-tailed t-tests (not paired).

Characteristics such as forking and epicormic sprouting were not present on all trees, and were compared among selfs and outcrosses using the non-parametric analysis of variance procedure NPAR1WAY of SAS statistical analysis software (SAS Institute Inc. 1989) for Kruskal-Wallis rank tests of different median values. The non-parametric Kruskal-Wallis rank tests were also used to compare lower-bole form and branching scores among all live selfs, outcrosses, and standard clones. Wilcoxon signed-rank tests of two related samples were constructed to compare average live-tree (standing dead trees were not assessed) bole form and branching scores between adjacent selfs and outcrosses with common parents.

Results

The Simpson and Masonite Trials

At the Simpson site, stumps of its recently-logged redwoods indicated that it was a suitable site for redwoods, and the site was well-prepared. But something apparently went wrong following the December 1981 planting of our trial stecklings. It was most likely aggressive regrowth of competing grasses and shrubs, or perhaps some other unobserved stressful events occurred. By March 1983, 64 percent mortality had already occurred. The expected pattern under stress was recorded: the outcrosses suffered 55 percent mortality, the standard clones 61 percent, and 75 percent of the selfs had died. In the following 2 years, an additional 23 percent of the trees surviving in March 1983 had died. The trial was then discontinued.

The Masonite trial, planted in January 1982, at first fared better. It was located on a similarly well-prepared redwood site. By June 1983, only 15 percent of the stecklings had died. Among these, the outcrosses had 10 percent mortality, the standard clones 16 percent and the selfs 20 percent. Prior to our next site visit, an ownership change had occurred, with replacement of the forestry staff. The new staff, unaware of the trial, located a skid road and landing on the trial. Only 33 of the original 124 planted stecklings remained alive around the edges of the skid road and landing, and some of those were damaged. The trial was then discontinued.

The Russell Trial

Following some initial post-planting mortality, soon replanted with larger ramets of the same clones, all stecklings survived the first 2 years, during which drip irrigation was supplied to each plant during rain-free periods. After irrigation was discontinued, the 49 outcross and 24 standard-clone stecklings survived similarly during 1984 to 2004, with mortality of 21 percent among standard clones and 27 percent mortality among outcrosses. The 49 self stecklings suffered much greater mortality of 61 percent. A chi-square test indicated that survival was significantly lower among the selfs than among the outcrosses ($p < 0.001$).

Trees in the southern six columns (map page 5 and adjacent column of page 4, fig.1) grew on a drier harsher region within the Russell trial, those conditions being apparent by a change in vegetation and slope. As of 2014, all 12 of those selfs had died, while four of those six standard clones and eight of those 12 outcrosses still survived, appeared healthy, and were growing reasonably well.

The summary data in table 1 are for all surviving self, outcross and standard-clone stecklings. By 2004 the outcrosses were on average 19 percent and 14 percent larger than the standard clones in DBH and height, respectively. By contrast, the outcrosses were on average 79 percent and 38 percent larger than the selfs in DBH and height. In the more sensitive analyses of self-outcross differences between live selfs paired with live outcrosses sharing a common parent (n = 16 data pairs), paired t-tests detected significantly lower diameters ($p < 0.001$) and heights ($p < 0.001$) of the selfs (figs. 2, 3). Higher coefficients of variation (s.d./mean) indicated less stable performance of selfs as a group.

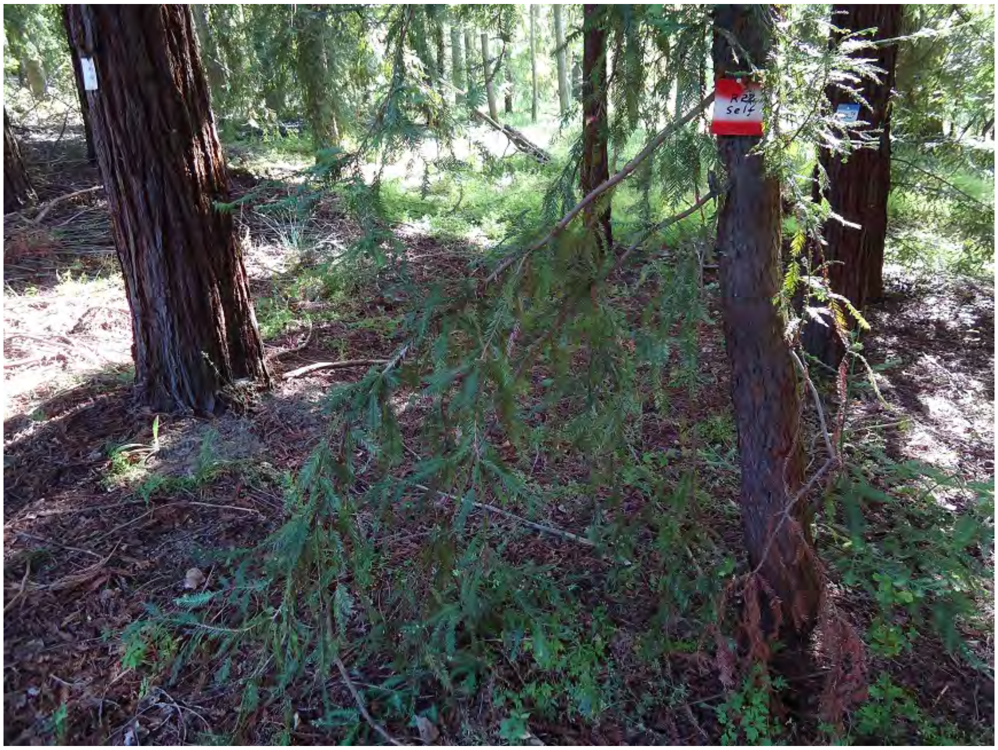

Figure 2—RB22×RB37 outcross (left) vs. RB22 self (right), with age-22 DBH and height of 40.9 cm vs. 13 cm, and 24 m vs. 14.1 m, respectively, at Russell Research Station, Lafayette, California.

Figure 3—RB17×RB37 outcross (left) vs. RB17 self (right), with age-22 DBH and height of 69.0 cm vs. 20.5 cm, and 26.3 m vs. 10.9 m, respectively, at Russell Research Station, Lafayette, California.

Table 1—Summary data for DBH (diameter at breast height; 1.37 m) and total height among selfs, standard clones and outcrosses in the Russell Research Station field trial after 22+ years growth

Trait	Group	n	Mean	s.d.	min.	max.
Diameter (cm)	Selfs	19	23.7	10.2	5.4	42.3
	Outcrosses	36	42.4	9.1	21.2	69.0
	Std. clones	19	35.6	9.1	10.1	47.6
Height (m)	Selfs	19	15.9	5.7	2.0	23.6
	Outcrosses	36	21.9	3.0	13.5	27.8
	Std. clones	19	19.2	3.2	9.7	22.5

Mortality between the time of planting and assessment had reduced the number of adjacent pairs of related selfs and outcrosses available for assessment. However, results from analysis of all live selfs and outcrosses (n = 55) supported results of the paired t-tests (n = 16 pairs). Pairing the same self with two related adjacent outcrosses (and vice versa) gave 26 pairings of selfs and outcrosses including duplicate data for 10 trees used twice (in double pairings). Paired t-test analysis of these data also gave similar results: substantially lower diameter and height growth among selfs than among their related outcrosses over 22 years since outplanting.

Outcrossed progeny exhibited significantly greater frequency of forking ($p = 0.002$) and occurrence of ramicorn branches ($p < 0.05$) than selfs (table 2). Only one self had a fork and only one had a ramicorn branch. Epicormic sprouts were present on 75 percent of selfs, and on average these sprouts were larger and more prolific than sprouts on outcrosses or standard clones, but differences in epicormic sprout frequency and size were not statistically significant ($p = 0.28$ and $p = 0.12$, respectively). Basal sprouts were less frequent and smaller on selfs, on average, however these differences were not significant ($p = 0.22$ and $p = 0.34$, respectively). Cones were only detected on one outcross tree and two standard-clone trees. Comparisons between the outcrosses and standard clones, although generally not statistically significant, were consistent with the standard clones being at a less-juvenile maturation state than the outcross trees.

Table 2—Summary data for count data on presence of forking and ramicorn branching, and scores for frequency and size of epicormic and basal sprouting among selfs, outcrosses, and standard clones in the Russell Research Station field trial

		n[b]	Count[a]	Mean[b]	s.d.[b]	min.[a]	max.[a]
No. forks	Selfs	19	1	0.05	0.23	1	1
	Outcrosses	36	17	0.69	0.89	1	3
	Std. clones	19	3	0.21	0.54	1	2
No. ramicorn	Selfs	19	1	0.16	0.69	3	3
	Outcrosses	36	11	0.53	0.91	1	3
	Std. clones	19	2	0.16	0.50	1	2
No. epicormics	Selfs	19	15	1.68	1.25	1	3
	Outcrosses	36	25	1.33	1.10	1	3
	Std. clones	19	12	1.16	1.12	1	3
Size of epicormics	Selfs	19	15	1.53	1.12	1	3
	Outcrosses	36	25	1.06	0.98	1	3
	Std. clones	19	12	0.89	0.94	1	3
No. basal sprouts	Selfs	19	3	0.21	0.54	1	2
	Outcrosses	36	11	0.47	0.81	1	3
	Std. clones	19	4	0.42	0.90	1	3
Basal sprout size	Selfs	19	3	0.47	1.12	3	3
	Outcrosses	36	11	0.67	1.12	1	3
	Std. clones	19	4	0.53	1.12	1	3

[a] Summary data for trees with forks, ramicorns, epicormics, or basal sprouts only.
[b] Summary data for all live trees, i.e., with/without forks, ramicorns, epicormics, or basal sprouts.

In terms of form characteristics (table 3), non-parametric Kruskal-Wallis tests indicated that branches on average were more horizontal ($p < 0.0001$) and evenly distributed and of uniform size among the standard clones ($p < 0.05$) than in the selfs or outcrosses whose branching attributes were not significantly different from one another. No other statistically significant differences were detected among these three groups. Among pairs of selfs and outcrosses sharing a common parent (n = 16 pairs), Wilcoxon signed-rank tests indicated that average branch diameter was marginally larger among outcrossed ramets ($p = 0.06$). No significant differences were detected between the selfs and outcrossed progeny in terms of bole straightness and symmetry or branching traits.

Table 3—Summary data for redwood bole straightness and symmetry (Bole SS), and branching characteristics: branch diameter, length, angle, and uniformity of branch distribution and size among selfs, outcrosses, and standard clones in the Russell Research Station field trial

		n	Mean	s.d.	min.	max.
Bole SS	Selfs	19	2.8	0.9	1.0	4.0
	Outcrosses	36	3.0	1.0	1.0	5.0
	Std. clones	19	2.9	0.8	2.0	4.0
Branch diameter	Selfs	19	2.6	1.3	1.0	5.0
	Outcrosses	36	3.2	1.1	1.0	5.0
	Std. clones	19	3.2	1.1	1.0	5.0
Branch length	Selfs	19	2.7	1.3	1.0	5.0
	Outcrosses	36	2.9	1.1	1.0	5.0
	Std. clones	19	3.0	1.6	1.0	5.0
Branch angle	Selfs	19	2.3	0.7	1.0	4.0
	Outcrosses	36	2.5	0.8	1.0	4.0
	Std. clones	19	3.2	0.5	2.0	4.0
Branch uniformity[a]	Selfs	19	3.0	1.3	1.0	5.0
	Outcrosses	36	3.0	1.4	1.0	5.0
	Std. clones	19	4.1	0.9	2.0	5.0

[a] Branch uniformity in terms of distribution and size, assessed on a 1 to 5 scale: 1 = highly variable in size or distribution; 5 = uniform size and distribution (desirable).

Discussion

Inbreeding can occur at various levels, and inbreeding depression manifests proportionally to the level of inbreeding. For simplicity, both in the creation of inbreds and in the interpretation of inbreeding depression as causing observed differences, we chose an inbreeding level of 50 percent, associated with selfing of non-inbred parents. We employed selfs of seven different parent redwoods in the third set of inbreeding trials. Three parents, S1, S3 and S6, were from an earlier study investigating the distribution of maturation state in large redwoods. The S-series parents had been left as seed-trees in a redwood harvest at mid-elevation near the Mad River in Humboldt County. Parents RB17, RB22 and RB37, were selections from Simpson's breeding-zone B, in mid-elevations of second-growth redwood stands in Humboldt County. The seventh selfed parent, ARC154, was from low elevation next to Redwood Creek in what is now Redwood National Park in Humboldt County. The RB-series selections were made as the best-formed large tree in two hectares, and each came from a different area within the 'B' breeding zone. While not verified by heterozygosity analyses of DNA or allozymes, it seemed likely that none of these seven parents were inbred, and thus their selfs would average 50 percent inbred.

We used some of those same seven parents as pollen-parents in crosses to produce full-sib families. We also used pollen from an additional four parents from the RB selections (RB2, RB5, RB9 and RB23) to produce additional full-sib families related to the seven parents we selfed. Since none of the RB-series selections were from the same stand, it is unlikely they were related to each other, or to ARC154 or the S-series parents. Thus, the full-sib outcross families from crosses involving RB or ARC154 pollens were likely to have zero or near-zero percent inbreeding. However, S1, S3 and S6 were from the same stand and might have been related at the sib or cousin level. Thus full-sib families, S1×S3, S1×S6 and S6×S3, might have had some degree of inbreeding, possibly as high as 25 percent if the parents were full-sibs. We saw no evidence of this. Their performance in terms of average DBH and height (41.3 cm; 22.2 m; n = 5) was similar to the average for all other surviving outcrosses (42.5 cm; 21.8 m; n = 31).

The five standard clones had been chosen much earlier, originated as five random seedlings from a production run of redwood planting stock in Simpson's Korbel nursery. Being from wind-pollinated cones collected in natural stands, which mostly have some family structure, it is possible, even likely, that some or all of these five standard clones were inbred to some currently-unknown degree. Their performance in these trials lends some support to that idea, but the fact they were mostly interior trees also leads to the expectation that their average growth would be less than the outcrosses, which occupied edge positions more than half the time (fig.1).

In nature, or in redwood plantations employing wind-pollinated seedlings, the expectation is that trees with modest-to-strong inbreeding would lose out in competition as the stand develops. That might even be a good thing in plantations in need of thinning, or in overstocked natural regeneration. But one can have too much of a good thing, and most silviculturists likely prefer to manage stands or plantations not suffering from inbreeding depression.

At Russell Research Station, the incidence of forking and ramicorn branching was infrequent and highly variable, but lower among selfs on average (table 2). However, we could not separate spontaneous forking and ramicorn branch incidence from occurrences incited by prior woodrat damage. Woodrats may have favored larger trees, and thus had less impact on selfs that were generally smaller than outcrosses and standard clones. In the large 200 clone Kuser trial, woodrats caused upper bole girdling in a majority of the trees. We followed recovery from that damage. Some formed basket whorls, some forked, and some grew a single sprout from below the girdle, thus keeping a single bole. It is a valid question whether ability to recover from girdling is different between selfs and outcrosses. While it would be nice to know, our small experiment and our available data did not allow us to determine cause. Future studies, if performed, should include additional replicates, preferably on high-quality redwood sites within the native range, to better detect differences in traits occurring infrequently, or in highly variable traits such as epicormic sprouting.

Conclusions

Compared to other conifers, coast redwood has many outstanding features, but hexaploid protection from inbreeding depression is not one of them. Although cone set and seeds per cone were little affected by pollinations that produce inbred offspring, from germination on, inbred redwoods survived at lower rates and grew more slowly than non-inbred redwoods, particularly on harsh sites or as competition or other stresses occurred or intensified.

An earlier reported observation that inbred redwoods had a higher frequency of forking was not supported by this trial. It was observed, however, that pruned inbreds had more and larger epicormic sprouts than did related outcrosses or random standard clones. If this observation is sustained, it could be important if pruned redwoods are grown to produce knot-free outerwood. Inbreds had fewer and smaller basal sprouts than did outcrosses, possibly an effect of the inbreds' smaller size and lower vigor. Other redwood traits of interest, namely bole form and several branch characteristics, seemed little affected by inbreeding.

As redwood silviculture advances, it seems likely that few serious growers will purposely plant inbred redwoods, except possibly for research purposes. Increasingly, clones from outstanding and apparently adapted native trees will be used, as will increasingly tested and characterized outcrossed clones from redwood breeding programs.

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