SEED ORIGIN STUDIES

NOBLE-CALIFORNIA RED FIR SPECIES COMPLEX

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Use of off-site seed or seedlings in reforestation programs can have serious consequences ranging from reduced growth rates to outright failure. However, in order to properly match seed source and reforestation site, the forester must know the origin of the seed. This is no problem when he supervises collection of the seed himself, but he rarely can do so. Therefore, the forester occasionally needs some way of verifying the source of cones or seed collected by others prior to purchase.

Previously, source verification has not been possible except in very general terms, e.g., distinguishing of coastal and interior seed sources. Now we report a study which may provide a means for identifying noble, California red, and Shasta red fir source to within 50 miles or less from the seed itself.

In 1967 we started this cooperative Region 6--Pacific Northwest Forest and Range Experiment Station study to determine if some seed or seedling features could be used to identify broad seed source localities for noble and Shasta red fir. Although the study is only about half completed, cotyledon number and seed weight have proven very promising as source indices. The preliminary data reported here illustrate the potential of the method for verifying source of commercially collected noble and Shasta red fir seed.

Methods

The study was designed around collection of seed from many locations spread throughout the range of noble, Shasta red, and California red fir with 8 to 20 trees individually sampled and studied at each location. In 1967 cone

collections were obtained from about 250 trees in 33 locations ranging from southern Washington to the central Sierra Nevada in California. In 1968 a wider range of collections was obtained from about 450 trees in over 50 locations, including samples from northern Washington and the southern Sierra Nevada. Seed weight and cotyledon data are not yet available for most of the 1968 material.

Seed was extracted, dewinged, and dried to about 8 percent moisture content. Some of the extracted seed from each tree was X-rayed. On the X-rays, sound seeds were identified and separated from the remainder. Counted samples of sound seed were weighed and used to determine number of sound seed per pound. Cotyledon counts were obtained by germination or by cutting the seed and counting embryonic cotyledons.

Results

Cotyledon Number

Average number of cotyledons increases from north to south (table 1 and fig. 1). Lowest average numbers observed (4.88) were in collections from the Stevens Pass area at the north end of the noble fir range. Cotyledon counts average 5.51 to 5.76 on the Mount Hood and northern Willamette National Forests. South of the McKenzie River counts increase sharply to around 6.0 at 44° north latitude and 6.6 at 43°40'. Populations to the south in Oregon consistently have seedlings averaging between 6.7 and 7.4 cotyledons. Shasta red fir trees from Mount Shasta average only 7.37 cotyledons, but California red fir populations to the south reach as high as 8.82 cotyledons. There are indications that cotyledon number again declines (to around 7.0) in the southern Sierras.

These data confirm preliminary observations that there is a latitudinal gradient or cline in cotyledon number (fig. 1). The regression of cotyledon number on latitude for the 1967 collections is highly significant ($r^2 = 0.89$), explaining all but 11 percent of the variation.

The absolute range in cotyledon numbers changed with the mean count (table 2). North of the McKenzie River seedlings with 8 cotyledons were rare or absent and only one with 9 cotyledons has been tallied. In southwestern Oregon seedlings had 4 to 11 cotyledons, but 4's are rare (none were encountered in most populations), and 5's make up 0 to 4 percent of the total. Seedlings with 8 cotyledons are common and those with 9 generally make up 1 to 8 percent of the total. The range in cotyledon numbers was greatest in California -- 5 to 13. There, excepting the Mount Shasta and Inyo County collections, seedlings with 5 or 6 cotyledons were rare or absent and 8 or 9 cotyledons were typical.

Seed Weight

Seed weight, using number of sound seed per pound as an index, varies in a similar pattern. Seed is lightest in the north, averaging 9,000 to 14,000 sound seeds per pound. In southwestern Oregon (south of the Willamette River) there are 5,100 to 6,900 sound seeds per pound. California red fir seed is heaviest with only 4,400 to 5,400 seeds per pound. The maximum number of seeds per pound was 23,000 on two trees near Mount Hood. The minimum was 3,190 on a tree at Lake Tahoe.

The increase in seed weight with decreasing latitude is also statistically significant. Preliminary analysis of the 1967 data indicates a highly significant curvilinear regression line ($r^2 = 0.69$). However, much as with cotyledon

number, there is a rather abrupt seed weight increase between the McKenzie and Willamette Rivers.

Use in Seed Source Verification

The data presented here are preliminary; complete cotyledon tallies for some of the 1967 collections and most of those from 1968 are not yet available. Analysis and interpretations of the data are even more incomplete. However, bearing these limitations in mind, they can still be useful in broad seed zone verifications.

The procedure is simple once you know the average cotyledon numbers of noble or red fir in a given district or tree seed zone. It requires only (1) a representative sample of the seed for which verification is desired from which cotyledon counts are obtained and (2) comparison of data with those for known populations in the designated locale.

Cotyledon counts of the representative seed sample can be obtained in two ways -- by germinating the seed or, much more quickly, by counting cotyledons on the embryo. The latter technique is not as difficult as it sounds. Embryonic cotyledons are well developed in mature seed. They can be counted by:

(1) cutting the seed in half as close to the base of the embryonic cotyledons as possible with a razor blade or sharp knife - a hand lens (10X will usually do fine) is used to count the cotyledons; or

(2) slicing the seed lengthwise and removing the embryo from the endosperm -- the count is made using a hand lens and a probe to peel the cotyledons back as they are counted.

The first method is easier but the other is more accurate, especially when dealing with embryos typically having more than 7 or 8 cotyledons (southwest Oregon and California).

When cotyledons have been tallied on 100 or more embryos or seedlings and an average count has been calculated, a comparison is possible. Does the average compare favorably with average cotyledon counts for noble or red fir populations which have been studied in the tree seed zone or district it is supposed to have come from? With our present state of knowledge an average cotyledon count within $\pm$ 0.3 cotyledons of known sources would seem to be at least an acceptable comparison. For example, an average cotyledon count between 5.4 and 5.8 on a lot identified as being from Mount Hood (Government Camp) seems reasonable (table 1). On the other hand, an average count of 5.0 or 6.0 cotyledons is unlikely and warrants further investigation.

The percentage distribution of seedlings by number of cotyledons should also be considered. For example, from the McKenzie River ($44^{\circ}10'$ north latitude) north occurrence of more than 1 percent seedlings with 8 cotyledons or 0.1 percent seedlings with 9 cotyledons seems highly unlikely (table 2). From the Willamette River ($43^{\circ}40'$) south occurrence of more than 0.5 percent seedlings with 4 cotyledons or 10 percent seedlings with 5 cotyledons is questionable.

Table 1. Number of cotyledons and seeds per pound for selected populations of noble, Shasta red and California red fir (preliminary data, based on at least 250 cotyledon tallies at each location)

Vicinity	Latitude	Average Cotyledon Number	Number Seeds Per Pound
Stevens Pass, Washington	47°45'	4.88	13,760
Red Mountain, Washington	45°55'	5.56	9,210
Lookout Mountain, Washington	45°49'	5.47	9,180
Government Camp, Oregon	45°18'	5.58	10,880
Mount Wilson, Oregon	45°05'	5.67	9,800
Iron Mountain, Oregon	44°24'	5.76	10,460
Roaring River Ridge, Oregon	43°55'	6.00	8,040
Wolf Mountain, Oregon	43°37'	6.64	6,350
Logger Butte, Oregon	43°34'	6.68	6,910
Reynolds Ridge, Oregon	43°24'	6.90	5,880
Hershberger Mountain, Oregon	43°02'	7.01	5,860
Cavern Creek, Oregon	42°54'	7.44	5,210
Crater Lake, Oregon	42°53'	7.10	6,060
Huckleberry Mountain, Oregon	42°52'	7.15	5,640
Blue Rock, Oregon	42°32'	7.01	6,200
Mount Baldy, Oregon	42°15'	6.93	7,000
Dutchman Peak, Oregon	42°02'	6.82	6,580
Mount Shasta, California	41°22'	7.37	5,430
Mount Lassen, California	40°38'	8.38	5,490
Swain Mountain, California	40°25'	8.82	4,420
Lake Tahoe, California	38°38'	8.77	4,560
Inyo County, California	36°46'	6.94	4,470

Table 2. Percentage of seedlings by cotyledon number at selected collection sites (preliminary data, based on at least 250 cotyledon tallies at each location)

Location	Number of Cotyledons										
	3	4	5	6	7	8	9	10	11	12	13
	-- Percent of Seedlings --										
Stevens Pass	0.5	24.9	60.9	13.3	0.4	--	--	--	--	--	--
Red Mountain	--	1.3	46.5	47.5	4.5	--	0.3	--	--	--	--
Lookout Mt.	--	3.5	50.1	41.8	3.9	0.6	--	--	--	--	--
Government Camp	0.1	1.1	44.6	49.6	4.6	--	--	--	--	--	--
Mount Wilson	--	2.8	36.5	53.1	7.6	--	--	--	--	--	--
Iron Mountain	--	1.1	33.5	54.0	9.9	1.5	--	--	--	--	--
Roaring River Ridge	--	1.0	21.7	52.2	22.4	2.1	0.5	0.1	--	--	--
Wolf Mountain	--	--	4.3	40.2	43.4	11.3	0.8	--	--	--	--
Logger Butte	--	--	1.6	40.4	40.0	8.8	1.2	--	--	--	--
Reynolds Ridge	--	0.5	1.0	29.6	51.9	16.0	1.0	--	--	--	--
Hershberger Mt.	--	--	1.4	25.7	48.8	21.4	2.5	0.2	--	--	--
Cavern Creek	--	--	0.5	12.7	40.5	37.1	7.7	1.4	0.1	--	--
Crater Lake	--	--	0.8	19.0	50.5	26.7	3.0	--	--	--	--
Huckleberry Mt.	--	--	--	16.3	52.6	26.2	4.9	--	--	--	--
Blue Rock	--	--	2.7	29.0	45.4	19.6	3.3	--	--	--	--
Mt. Baldy	--	--	1.1	34.5	47.8	15.4	1.2	--	--	--	--
Dutchman Peak	--	--	1.5	26.9	50.5	19.9	1.2	--	--	--	--
Mount Shasta	--	--	0.9	14.2	39.9	33.8	10.5	0.6	0.1	--	--
Mount Lassen	--	--	0.2	1.0	13.8	37.4	34.5	10.9	1.8	0.4	--
Swain Mountain	--	--	--	--	4.6	33.3	47.2	13.8	1.1	--	--
Lake Tahoe	--	--	--	0.4	7.2	35.9	34.7	19.0	2.5	0.2	0.1
Inyo County	--	--	2.9	25.7	47.1	22.9	1.4	--	--	--	--

Other seed and seedling characters are going to be useful for source verification. The usefulness of seed weight is obvious from even the preliminary data (table 1). For example, seed from Inyo County, California, has about the same cotyledon count as many southwestern Oregon sources (table 1); the heavier seed distinguish it, however. Other characters such as seed color are going to be of interest too. For example, trees with cream-colored seed have thus far been encountered only in some parts of southwestern Oregon and on Mount Shasta.

These are interim data; there are still many thousands of cotyledons to be tallied and seed to be weighed before the basic data will be complete. Statistical analyses necessary to provide confidence limits for cotyledon number within individual tree seed zones remain to be calculated. Perhaps, however, this progress report will give you at least some idea of the excellent possibilities for identifying noble and Shasta red fir source from the seed itself.

It should be noted in closing that cotyledon number may prove useful in predicting where purposeful seed movements are warranted or desirable as well as identifying unwanted movements or erroneous source labeling of seed.

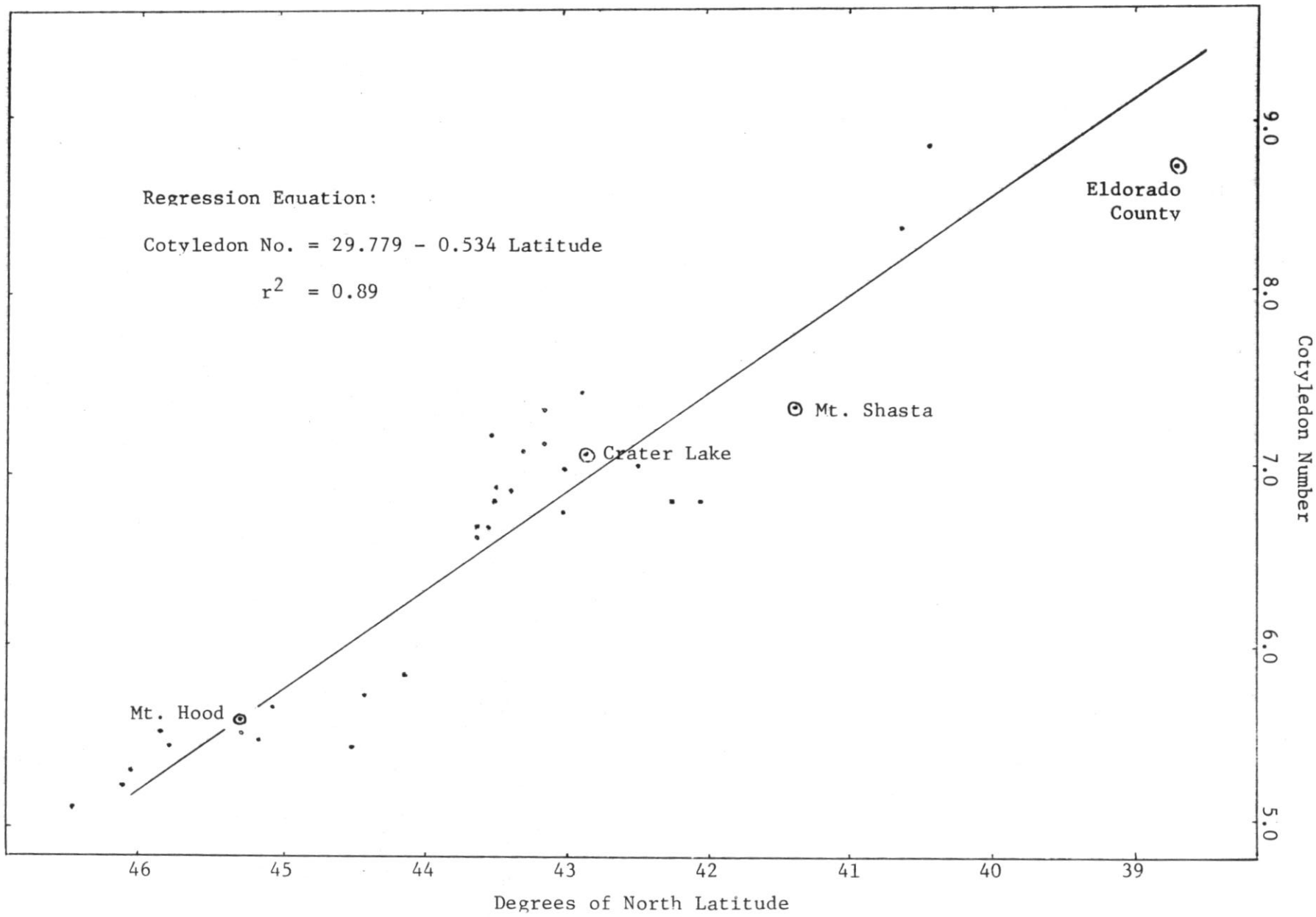

Figure 1.--Relation of cotyledon number to latitude, preliminary data from the 1967 collection.