Viable Seeds from the Duff and Soil of Sugar Pine Forests

BY
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SINCE 1937, methods developed by the author[1] have been used successfully to isolate, identify, and germinate seeds of *Ribes spp.* from samples of duff and soil collected in sugar pine forests. As here described, similar methods were used to show variation in kind and quantity of seeds of many species occurring in soil and duff samples taken from the Stanislaus Experimental Forest, near Pinecrest, Tuolumne County, California.

Timber species found on the sampled areas were sugar pine (*Pinus lambertiana* Douglas), pondersoa pine (*Pinus ponderosa* Lawson), Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), white fir (*Abies concolor* (Gord. & Glend.) Hoopes), red fir (*Abies magnifica* A. Murr.), and incense cedar (*Libocedrus decurrens* Torrey.)

Rapid intrusion of brush into cutover timber land in the Sierra Nevada has long been a vexatious problem in timber management, and has been especially troublesome where regeneration of sugar pine is concerned. Climate, and the varying shade tolerance of the several timber species as described by Baker (1944, 1949), are important factors in this complex problem of forest land management which has been analyzed by Dunning with respect to tree classifications (1928) and site quality classification (1942), by Fowells for site improvement (1944), for the system of unit area control by Hallin (1951, 1954), and from the standpoint of the status of pine forests in California by Hughes and Dunning (1949).

On the Stanislaus Experimental Forest the principal species of brush which had markedly increased on adjacent previously logged areas, and which were expected to increase after the prescribed logging, were snowbrush (*Ceanothus cordulatus* Kellogg), deerbrush (*Ceanothus integerrimus* H. & A.), sweetbirch (*Ceanothus parvifolius* Trel.), green manzanita (*Arctostaphylos patula* Greene), bush chinquapin (*Castanopsis sempervirens* Dudley), Sierra Nevada gooseberry (*Ribes roezlii* Regel), Sierra Nevada currant (*Ribes nevadense* Kellogg), ground waxberry (*Symphoricarpos mollis* Nuttall), ground rose (*Rosa spithamea* Watson), bitter cherry (*Prunus*

[1]Unpublished reports 1937-39 in files of Blister Rust Control, Methods Development Unit, Forest Service, Berkeley, California.

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emarginata (Dougl.) Walp.), and western thimbleberry (*Rubus parviflorus* Nuttall).

Collection of Samples

Sixty-four samples of duff and soil were collected on September 23-26, 1947, from the forest floor under virgin stands of mixed conifers in the NW 1/4, sec. 26, and NE 1/4, sec. 27, T4N, R18E, Mt. Diablo Meridian. The samples were distributed over the areas concerned in 8 series. Each series of samples consisted of a set of 8 samples usually spaced at one-chain intervals along a line in a cardinal direction.

Field methods for the collection of samples may be described briefly as follows. The forest floor at time of collection was quite dry. A flat square boxwood frame, 11.2 inches inside measurement (2 square links of area—1/50,000 acre), was placed on the ground at the spot from which a sample was desired. Duff was cut through to mineral soil with a heavy knife around the inside edge of the frame. Duff and soil was removed down to rather solid undisturbed mineral soil, and placed in a 4-mesh sieve resting on a square of canvas. Material which would not pass the 4-mesh sieve was inspected for seeds and then discarded. The material was then worked on a 20-mesh sieve to reduce it to manageable size. This process requires dry soil. All material remaining on the 20-mesh sieve was placed in a cotton bag for greenhouse processing. It is estimated that the 64 samples taken to the greenhouse weighed about 700 pounds.

Some dry seeds in forest duff and soil will pass a 20-mesh sieve. From previous work it was known that practically all ribes and ceanothus seeds would be retained on a 20-mesh screen. All conifer seeds and most brush seeds, except for a very few such as azalea seeds, would likewise be retained. Inasmuch as the seeds of brushy plants were of primary concern in this study, collection and recovery procedures were simplified by discarding all material that would pass a 20-mesh sieve.

Preparation of Seed Concentrates

Methods used in the greenhouse for reduction of the duff samples[2] were similar to those described by Stone and Holt (1950), and had proven satisfactory on preliminary samples from the Stanislaus area. These methods were as follows: Each air-dry field sample was rubbed vigorously on a 12-mesh sieve. Material retained on the 12-mesh screen was placed in a No. 10 can and soaked over night in tap water. Material passing the 12-mesh screen was rubbed on a 20-mesh sieve. Material passing the 20-mesh screen was discarded; that retained was placed in another can of water and soaked over night.

The soaked subsamples were separately worked on a 20-mesh sieve under a stream of water from a "rose" nozzle until little additional material would pass the sieve. Residues retained by the 20-mesh sieve were separately spread on newspaper and dried. The sample retained wet on 12-mesh was resieved dry on 12-mesh screen. Dry material of this subsample now passing 12-mesh screen was added to the other 12- to 20-mesh subsample of the same field collection. This composite 12- to 20-mesh subsample was resieved dry on the 20-mesh screen.

At this stage of sample reduction each field collection consisted of two air-dry subsamples, one retained on 12-mesh screen and the other on 20-mesh screen. Most of the dirt and partly decomposed duff had been removed from the samples by soaking, washing, rubbing and sieving.

Each subsample was now processed separately in a small "Clipper Grain, Seed, and

[2]Frank A. Patty, Blister Rust Control, Forest Service, Region 5, assisted the writer in reducing the field samples to seed concentrates, in inspecting the seed concentrates, and in recording the data on seeds found by visual inspection.

Bean Cleaner," with screens measuring 9.75 × 15 inches. This seed cleaning mill was run by an electric motor of 1750 RPM belted so that the drive shaft of the mill had a speed of about 430 RPM. The first run of each subsample through the mill was made with a minimum of air from the fan. The "heavy overs" and the "middlings" were passed through the machine twice. The "light overs" and the heavy overs were then discarded. The "downs" and the middlings were combined to make the seed concentrate. This concentrate was now milled with maximum air blast. The downs—largely rocks—were passed through the mill 3 times, and then discarded. The light overs, heavy overs, and middlings were combined to make the final seed concentrate from the mill.

The reduced sample was stirred into a saturated solution of calcium chloride. Materials which sank in this heavy solution—mostly rocks—were discarded. Material which floated was removed to a small 20-mesh screen, washed immediately in much running water, and placed on a paper towel to dry. The specific gravity of a saturated solution of calcium chloride in water is approximately 1.4. Most seeds and most other organic matter will float in this heavy solution, and most rocks will sink. Tests in previous work[1] had shown that dry seeds of ribes and other brush species could be exposed to this solution without harmful effects on seed viability for the short periods necessary for the separation procedure. When thoroughly dry, the seed concentrate was rubbed carefully by hand on a 20-mesh sieve, then placed in a porcelain evaporating dish and cautiously winnowed with the breath. Material remaining in the porcelain dish was inspected for seeds, and then cultured for germinable seeds.

All discarded material was inspected casually, and some material with considerable care. A few seeds were lost in the various discarded portions, but the great majority of seeds retained on a 20-mesh sieve ended up in the final seed concentrates. Most of the mineral matter in the final seed samples was porous volcanic rock which had about the same specific gravity as ceanothus and manzanita seeds.

Identification and Culture of Seeds

Ceanothus, manzanita, and other distinctive kinds of seeds were readily found and identified in the seed concentrates. Ribes seeds and some other seeds, because of their dark color, dull surface, and irregular angular shape, were very difficult to identify in the final seed concentrate. Thus visual inspection detected a higher proportion of some species of seeds than of others.

Seeds of many plants, especially woody plants, native to the timbered portions of the Sierra Nevada will not germinate immediately, even if seed coats are readily permeable to, or are made readily permeable to water. Many such seeds are satisfactorily conditioned for prompt complete germination at greenhouse temperatures by stratification in a moist, aerated condition for some weeks or months. General germination methods, and specific methods for many species of native woody plants of California are described by Mirov (1940), by Mirov and Kraebel (1937), and by the Forest Service (1948). Quick (1935) has described methods for forcing germination of several species of ceanothus seeds.

Seed concentrates after visual inspection were cultured individually in steam-sterilized river sand wet with a dilute mineral nutrient solution. Although the coats of most kinds of ceanothus seeds are impermeable to water, they are easily made permeable by a treatment in boiling water for 5-30 seconds. Therefore ceanothus seeds were picked out of the subsample concentrates and immersed in boiling water for 6 to 7 seconds, abruptly cooled in cold water, then cultured in sand. Few manzanita seeds are water-permeable, but no satisfactory pre-stratification treatment similar to the hot-water treatment for ceanothus seeds is known. Thus manzanita

TABLE 1. Seeds observed in sample concentrates, seedlings produced in cultures from 64 duff-and-soil collections, and percent of seedlings produced to seeds observed, Stanislaus Exptl. Forest, Pinecrest, Calif.

Kind of plant	Screen size of sample	Total observed seeds	Total seedlings produced	Mean seedlings per sample[1]	Percent seedlings to seeds
Ceanothus species	(4 -to-12-mesh)	249	188	3.1	75.5
do.	(12-to-20-mesh)	3027	1890[2]	37.3[3]	78.9
Ribes species	All	67	191	3.2	285.0
Manzanita	All	89	14	0.2	15.7
Other dicot species[4]	(4 -to-12-mesh)	126	20	.03	15.9
do.	(12-to-20-mesh)	590	551	9.2	93.4
Grasses[5]	All	----[6]	184	3.1	------[6]
Totals		4148	3038[2]	56.4[3]	86.4

[1]Each collection sample represents two square links of area (1/50,000 acre).
[2]Ceanothus seeds of some collections were subsampled; 635 known seeds were not cultured; percentages were adjusted.
[3]This figure includes an estimate of germination of ceanothus seeds which were not actually tested.
[4]Includes lily and iris seeds and seedlings.
[5]Includes sedge and rush seedlings.
[6]Grass seeds, if seen and identified, were included with assorted dicot seeds.

seeds were cultured for stratification and germination as found. Two field samples, one with 38 manzanita seeds and another with 29, accounted for most of the manzanita seeds recovered.

The final samples resulting from the concentrating procedures were cultured in sand as if to grow immediately, but prior to greenhouse germination test were stratified at 2.2° C. (36° F.) for about 14 weeks. After a 5 weeks' germination test in the greenhouse the cultures were re-stratified, this time at 0° C. (32° F.) for about 20 weeks, and re-germinated in the greenhouse. Seeds of some species of native plants require no stratfiication prior to germination, but for simplicity of procedure and for economy of time all cultures were stratified prior to greenhouse germination tests. This may not have been optimum treatment for some kinds of seeds. With the material at hand, however, it was believed that a greater number of dormant seeds would be killed by long periods in moist cultures at greenhouse temperatures, prior to a necessary stratification treatment, than readily germinable seeds would be killed by an unnecessary stratification treatment.

Discussion of Results

Table 1 summarizes number and kind of seeds found by visual inspection of seed concentrates from the 64 field collections, and compares observed seeds with seedlings produced in sand cultures. All collections were made under stand-condition classes (Hallin, 1951) commonly found on the Stanislaus Experimental Forest. In general this is a virgin, over-mature, mixed conifer forest containing large amounts of sugar pine in the overmature age class.

Seeds of deerbrush in general are considerably larger than seeds of snowbrush and sweetbirch. Almost all deerbrush seeds are retained on a 12-mesh sieve, while almost all snowbrush and sweetbirch seeds will pass a 12-mesh sieve. This difference

TABLE 2. Average number of seedlings produced in selected groups of samples representing five condition classes of timber overstory.

Samples	No. of samples	Timber overstory[1]: Major tree species[2]	General age[3]	Reserve[4]	Brush on area[5]	Average number of seedlings produced per sample: Ceanothus species	Ribes species	Other dicot species	Total[6]
CJ-CK	2	SP	Overmature	Plus	Plus	491.5	None	1.0	492 5
BC-BG	5	SP	Overmature	Minus	Minus	7.8	0.6	None	8 4
CR-CT	3	WF,SP	Mature	Plus	Minus	2.0	None	0.3	3 0
CZ-DC	4	SP,PP	Immature	Plus	Minus	33.0	10.5	5.8	49 3
CU-CY	5	SP	Immature	Plus	Minus	51.8	1.8	0.6	54 8

[1]Essentially no timber understory in sample groups listed.
[2]Tree species: PP—Ponderosa pine, SP—Sugar pine, and WF—White fir.
[3]General age of stand: Immature (75 to 150 years of age), Mature (150-300 years of age), and Overmature.
[4]Reserve. Growing stock suitable for carrying as reserve after logging considered adequate (plus), or inadequate (minus) for specific unit areas.
[5]Brush. Kind and amount of brush on specific unit areas expected to require (plus), or not to require (minus), eradication prior to forest regeneration.
[6]A few manzanita seedlings and grass (monocot) seedlings, not recorded in the columns, are included in the totals.

in size was used in the data as an approximate species separation of ceanothus seeds and resulting seedlings.

Because of the relation between numbers of seedlings produced and seeds observed, considerable numbers of ribes seeds, and seeds of assorted herbaceous dicot species in the 12- to 20-mesh subsample apparently were not observed in the seed concentrates. Apparently most ceanothus seeds were found by inspection—and most of them grew. The low yield of manzanita seedlings from the known seeds was consistent with previous experience. Grass (including sedge and rush) seeds and seedlings tended to be concentrated in a few collections. Ceanothus seeds outnumbered all other seeds combined, and were found in more than 75 percent of all collections. Some of the 8 series of samples contained very few ceanothus seeds, however, and a few samples contained no seeds at all. About 70 seeds of a mistletoe, believed to be *Arceuthobium campylopodum* Engelmann, were found in the seed concentrates by visual inspection, but no mistletoe seedlings developed in culture.

A total of 635 ceanothus seeds, picked out of the 12- to 20-mesh subsamples of 3 collections which contained a great many ceanothus seeds, were not cultured. The pertinent percentages of ceanothus seedlings to seeds found by visual inspection have been adjusted in the tables by proportion.

Table 2 summarizes average seedlings per sample resulting from selected samples classified by overstory stand-condition classes. Samples CJ-CK and BC-BG were collected under overmature sugar pine with essentially no coniferous understory. Samples CJ-CK represent a patch of dense ceanothus brush under an open stand of very large old sugar pines. Samples BC-BG were collected under a similar stand of sugar pine with essentially no brush on the forest floor. In Table 2 observe also that samples collected under immature overstory contained many more seeds of all sorts than samples collected under mature overstory, or under overmature stands without brush (samples BC-BG).

Great variability in average number and kind of viable seeds is apparent from all data shown in Tables 1-3. There was even greater variability within some series of samples. For example, a series of samples (CJ-CP) was collected at one-chain inter-

TABLE 3. Average number of seedlings produced in selected groups of samples representing five condition classes of timber understory.

		Timber understory[1, 2]			Average number of seedlings produced per sample			
Samples	No. of samples	Major tree species[3]	Stocking[4]	Brush on area[5]	Ceanothus species	Ribes species	Other dicot species	Total[6]
CB-CC	2	WF	Under	Plus	68.5	2.5	None	71.0
CD-CI	6	IC	Under	Plus	18.9	0.2	13.5	32.6
BV-CA	6	WF	Under	Minus	27.0	None	0.3	27.5
AU-AX	4	WF	Normal	Minus	15.7	10.0	0.5	26.2
BJ-BN	4	IC	Normal	Minus	0.8	None	6.7	7.8
BO-BS	5	WF	Normal	Minus	0.2	0.6	0.8	1.6

[1]Timber overstory in all cases was overmature sugar pine.
[2]General age of understory in all cases was large saplings (10 feet height, to 4 inches DBH).
[3]Tree species: IC—Incense cedar, and WF—White fir.
[4]Stocking. Specific unit areas were considered overstocked, normally stocked, or understocked.
[5]Brush. Kind and amount of brush on specific unit areas expected to require (plus), or not to require (minus), eradication prior to forest regeneration.
[6]A few manzanita seedlings and grass (monocot) seedlings, not recorded in the columns, are included in the totals.

vals along a north-south line. Numbers of ceanothus seedlings resulting from individual collections along this line follow: 618, 365, 156, 106, 19, 30, and 6; a total of 1300, and an average of 186. Numbers of assorted dicot seedlings resulting from a series of samples (DA-DG) collected at one-chain intervals along another north-south line, from timber to timber across a narrow neck of meadow in timber-type, follow: 0, 2, 21, 278, 52, 42, and 1; a total of 396, and an average of 57. Numbers of ceanothus seedlings for this same series of 7 samples follow: 65, 9, 2, 1, 0, 14, and 20; a total of 111, and an average of 16. Numbers of viable ribes seeds varied similarly to ceanothus seeds, and numbers of grass seeds similarly to assorted dicot seeds.

Table 3 summarizes results from selected series of samples classified by understory stand-condition classes. The timber overstory in all cases was overmature sugar pine. Notice that numbers of ceanothus seedlings from brushy areas (samples CB-CC and CD-CI) average appreciably higher than ceanothus seedlings from non-brushy areas, and that understocked understories average well above normally stocked understories.

The occurrence of ribes seeds in the many collections was more sporadic than the occurrence of ceanothus seeds. Many individual samples produced no ribes seedlings, but ribes seeds were widely distributed and surprisingly numerous. One sample produced 38 ribes seedlings, and one series of 8 samples averaged 10 ribes seedlings per sample. One complete series of 8 samples, and a total of 36 individual samples contained no ribes seeds at all.

The column in Table 1 reporting average seedlings per sample is a bit imposing, especially when the average figures are converted to a per-acre basis. Individual field samples represented only two square links of area, that is, only 1/50,000 acre. This extreme smallness of sample and the disproportionate distribution of samples among stand condition classes on the area should be held in mind. It can then be stated that the average viable seed content of duff and soil under virgin, largely overmature, timber on the sampled areas of the Stanislaus Experimental Forest, on a *per acre* basis, was found to be 155,000 seeds of deerbrush, an estimated 1,865,000 seeds of snowbrush and sweetbirch, 160,000 seeds of ribes, 10,000 seeds of manzanita, 475,000 seeds of assorted dicot plants (including seeds of the iris and lily families), and 155,000 seeds of grasses (including seeds of rushes and sedges); an average total of about 2,820,000 viable seeds per acre.

Summary

The increase of brush following logging in the Sierra Nevada of California has long been a troublesome problem. Duff samples were collected from virgin mature forest to determine if kind and amount of post-logging brush could be approximated from the kind and number of viable seeds found in relatively few small duff samples. Sixty-four samples of duff down to mineral soil were collected in September 1947 under virgin mixed-conifer timber on the Stanislaus Experimental Forest, Dodge Ridge, Pinecrest, Tuolumne Co., Calif. Each small sample represented 1/50,000 acre of area. The duff and soil collections were reduced by a variety of methods to seed concentrates of small volume. These seed concentrates were inspected; then cultured in autoclaved sand wet with mineral nutrient solution. Seedlings resulting from germination cultures indicated that the average viable-seed content of duff and soil under virgin, largely over-mature timber on the sampled areas, on a *per acre* basis, was 1,865,000 seeds of snowbrush and sweetbirch (*Ceanothus cordulatus* Kell. and *C. parvifolius* Trel.); 475,000 seeds of assorted dicot plants(including seeds of the iris and lily families); 16,000 seeds of ribes (largely *Ribes roezli* Regel); 155,000 seeds of deerbrush (*Ceanothus integerrimus* H. & A.); 155,000 seeds of grasses (including seeds of rushes and sedges), and 10,000 seeds of manzanita (largely *Arctostaphylos patula* Greene); a total of about 2,820,000 viable seeds per acre.

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