

there was abundant evidence of rodent habitation in the area and nesting litter mixed with the cached seed, none were observed actually entering the tree stand. It appeared the rodents had been very discriminate. The stored seed was large and good quality. Bitterbrush seed production in 1963 was so much heavier than in previous years the Inyo National Forest felt it warranted an intensive effort at seed collection.

This observation certainly points out the importance of rodents in bitterbrush ecology. Research by Hormay¹ has pointed out the importance of rodents in caching bitterbrush seeds in the ground. Widespread observations and measurements indicate that most of the plants in bitterbrush stands in California become established from seeds cached by rodents. He found the seeds cached 0.25 to 1.5 inches below the soil surface and the seedlings from these varied in number from 2 to 100, although in one case 139 were found. Hormay did not actually observe caching by rodents, but he suspected that chipmunks and golden mantled ground squirrels did most of it, the same rodents that we believe cached the seeds in the hollow aspen tree.

¹ Hormay, August L. 1943. *Bitterbrush of California. Pacific Southwest Forest and Range Experiment Station Research Note No. 34. 13 pp.*

Chromatographic Technique in the Differentiation of Sagebrush Taxa

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A classification of the sagebrushes (genus *Artemisia* section *Tridentatae*), which will represent naturally occurring

biological units, has been advanced by the use of thin-layer chromatography. Systems based solely on morphological characteristics allow considerable taxonomic latitude. However, when they are used in conjunction with a study of the fluorescent lactones found occurring in leaf extracts, clarification may be forthcoming.

Preparation of Extract: Ethanol extracts are prepared from fresh, vegetative leaves of mature or seedling plants. Leaves of flowering shoots are less reliable. The leaves should not be gathered from the plant during winter dormancy. Material is soaked in 95 percent ethanol, the recommended proportion being two grams of material to ten milliliters of ethanol (1 gm: 5 ml). They are allowed to soak for 24 hours before undergoing chromatographic analysis. Good results may be obtained from extracts that are six months old, and those refrigerated may be good for over one year.

Application of Extract: The medium is a silica gel applied on glass plates (OPERATING MANUAL — CHROMATOFILM ASSEMBLY FOR THIN-LAYER CHROMATOGRAPHY. P-118-C, Research Specialties, 200 South Garrard Blvd., Richmond, California) to allow for a separation range of at least ten centimeters. Samples are spotted two centimeters from the bottom of the plate. Five drops should be sufficient for samples that are 24 hours or older. Care should be taken to keep the spots small.

Solvent System: The solvent

system consists of water saturated normal butanol, acetone and ammonium hydroxide (80:15:5). These proportions must be carefully followed since slight quantitative changes will blur the chromatographic patterns.

Developing and Analyzing Chromatograms: Prepared plates are allowed to run in the chromatographic chamber for approximately 80 minutes or over a distance of ten centimeters. Care must be taken to insure that the chromatographic chamber is level in order to obtain an even solvent head. The developed plates are then removed from the chamber and placed wet under a long wave ultra-violet lamp. The color, brightness and R_f values of all fluorescent spots are recorded. The brightness or magnitude of the spots is based on the comparison to the S_1 spot ($R_f = .30$) of *Artemisia tridentata* subspecies *vaseyana*, which has a magnitude of 4+. *Artemisia tridentata* subspecies *tridentata* has the same characteristic S_1 spot but with a magnitude of 1.

The major advantages of this chromatographic technique are as follows:

- (1) it offers a practical method for field identification;
- (2) it may be used in finding variations in a population;
- (3) there is no apparent chromatographic variation due to environmental conditions.

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