Needle Oils of Three Pine Species and Species Hybrids

BY
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THE COMPOSITION and characteristics of the needle oils of western pines may provide criteria for distinguishing pine hybrids and may help explain why some needle-feeding insects select certain pine species as hosts.

Many insects feed on the foliage of pines during some part of their life cycle. One such insect is the pine reproduction weevil (*Cylindrocopturus eatoni* Buch.). This weevil is particularly destructive to seedling pines in California plantations and forests (Eaton, 1942), but the pine species differ in susceptibility. Ponderosa pine (*Pinus ponderosa* Laws.) and Jeffrey pine (*P. jeffreyi* Grev. and Balf.) are particularly susceptible to attack. Coulter pine (*P. coulteri* D. Don) is immune. Miller (1950) demonstrated that the Jeffrey-Coulter interspecific hybrid possesses some of the resistance of its Coulter parent.

Because the weevils have to feed on pine foliage before they attack the stem,[1] the hypothesis was developed that selection of susceptible hosts by the weevils is related to the needle-feeding habit. As one step in testing the hypothesis, the volatile oils contained in the needles of three pine species and three hybrids were investigated to determine whether qualitative differences between host species might be one factor influencing such selection. The species and hybrids studied were ponderosa, Jeffrey, and Coulter pines, the F_1 Jeffrey-ponderosa hybrid, the backcross of a natural Jeffrey-Coulter hybrid to Jeffrey, and the wind-pollinated progeny of the same natural hybrid.

Few articles dealing with the yield and composition of pine-needle oils have been published. This literature is reviewed adequately by Bailey (1948), who studied interspecific differences in yield of oil. Schorger (1919) showed that the yield of needle oil is dependent upon the number and size of the resin canals in the needles of each species. Bailey showed that yield also was affected by time of harvesting needles, age of the trees, and the prevailing growth conditions. Schorger contributed most to knowledge of the composition of the oils. He and a few others reported that the needle oil of each pine species has a characteristic chemical composition, each differing in the amounts of terpenes, sesquiterpenes, and terpene alcohols and their

[1]The late J. M. Miller, Entomologist, Berkeley Forest Insect Laboratory, showed that foliage feeding by the adult is obligatory.

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esters present. Of the terpenes, alpha- and beta-pinene occur most frequently. The pleasant odors of the oils are due primarily to borneol and bornyl esters.

Of the three pine species included in this particular study, only ponderosa pine had been studied previously. Schorger found that the physical properties of 10 samples of ponderosa pine leaf oil were: Specific gravity (d^{15}_{4}°), 0.8718 to 0.8849; specific rotation (D_{22}°), —15.73° to 19.59°; index of refraction (D_{15}), 1.4789 to 1.4815.

Procedure

Needle collection. Needles were collected at the Institute of Forest Genetics, Placerville, California, in February and early April, 1951. According to Bailey, the largest accumulation of volatile oils in the needles occurs during these spring months. Only the most recent (1950) twigs with the attached needles were collected. All twigs were taken from the lowest branches so as not to deform the young trees. The trees were from 9 to 22 years of age. Foliage was taken from several trees of each species or hybrid.

The twigs from different trees varied considerably in their rate of growth, but the effect of this factor on the qualitative or quantitative variation in needle oils is unknown. As twigs were removed from the trees, they were placed in heavy canvas bags. Then the bags of foliage were stored at 0°C. pending distillation. Some were stored as long as 4 weeks. Bailey's work indicates that 4-week storage at low temperature does not cause any significant change in either the yield or characteristics of the needle oils.

The bags of foliage were removed from cold storage and the needles were stripped from the twigs. Stripping was greatly facilitated by having frozen needles. As the needles warmed to room temperature it become more difficult to pull them from the twigs without removing large strips of phloem and cortex. To avoid any contamination of the volatile needle oil by the turpentine from the stem, special care was taken to prevent including stem tissues with the needles. An example of such contamination was reported by Schorger.

Oil distillation. The needles were chopped into short segments just before distillation. The objective of this treatment was to expose the resin ducts and thus to facilitate extraction of the volatile oil. A small sample of the chopped needles was reserved for a moisture determination. The remaining needles (4,000 to 6,000 grams per species) were weighed before being placed in the still.

The still was made from a 5-gallon can with a friction lid. The can was fitted with a pressure release, condenser, and screen, which was placed near the bottom to prevent submerging the needles in the boiling water. The distillate passed from the can through a glass "L" sealed in the lid and thence through a straight water-cooled glass condenser into a flask immersed in an ice bath. A small amount of boiling water was poured over the needles; then the still was sealed quickly. Most of the oil (90 to 95 percent) was collected in the first 30 to 40 minutes of distillation. However, the distillations were continued until there were no droplets of oil in a 10 to 15 cc. sample of distillate. This usually required 3 to 4 hours. The oil was separated from the water in a separatory funnel and was placed in a small vial. A few crystals of anhydrous sodium sulfate were added to remove the water from the oil. The vials were sealed and refrigerated at 5° C. for at least 24 hours.

Oil analysis. The percent yield, both on a dry- and on a wet-weight basis, was determined. Then measurements were made of the following physical properties of the oil: Specific rotation, index of refraction, and specific gravity.

Results

The data (Table 1) show no striking interspecific differences in physical properties of the needle oils analyzed for this study. Most properties of the ponderosa pine

TABLE 1. Physical properties and yield of needle oil from pine species and species hybrids.

Pine species or hybrid	Specific gravity $d_4^{23°}$	Specific rotation $[\alpha]_D^{23°}$	Index of refraction $n_D^{21.5°}$	Yield		Date of Collection
				Wet wt.	Dry wt.	
				Percent	*Percent*	
Ponderosa	0.8814	—17°25′	1.4846	0.23	0.46	2/23/51
Jeffrey-ponderosa	0.8803	—13°55′	1.4842	0.29	0.56	4/ 3/51
Jeffrey	0.8707	—25°05′	1.4762	0.13	0.27	2/23/51
Jeffrey × natural Jeffrey-Coulter	0.8600	—23°45′	1.4741	0.15	0.32	2/23/51
Natural Jeffrey-Coulter × wind	0.8666	—29°00′	1.4830	0.19	0.41	2/23/51
Coulter	0.8744	—25°40′	1.4851	----	----	2/23/51

needle oil were close to the ranges given by Schorger. However, the index of refraction was somewhat higher than the value given by Schorger.

About 6 grams of Jeffrey pine oil were subjected to a crude fractional distillation. The object was to determine whether the needle oil of this species contained the large quantities of n-heptane present in Jeffrey pine oleoresin (Schorger). The physical properties of the needle oils from Jeffrey pine and the Jeffrey pine hybrids indicate that no large quantity of n-heptane was present. Furthermore, the results of the fractional distillation (Table 2) showed no discernible n-heptane (boiling point 98.4° C.) in the needle oil of this species. Since n-heptane is highly volatile, however, any small amounts which might have been present in the distillate could have been lost through evaporation during the steam distillation. There was not a sufficient amount of distillate in any of the fractions for determinations of the compounds present.

Discussion

The results of this investigation, which was limited to only a few trees of each species, do not indicate any striking interspecific differences in the physical properties of the needle oils. This does not preclude the possibility that the constituents of the turpentines differ. Further study of these constituents may determine whether needle oils are important in influencing the selection of host species by adult pine reproduction weevils.

The physical properties of the needle oil of the Jeffrey-ponderosa hybrid are intermediate between those of the parental species, as one might expect for F_1 hybrids. On the other hand, most properties of the Jeffrey-Coulter hybrids transgress the parental values. Logically, for a character controlled by many genes, one might expect to find such transgressive variation in the progenies of hybrids. The lack of interspecific differences in needle oils and

TABLE 2. Fractional distillation of the needle oil of Jeffrey pine.

Fraction number	Boiling point range	Yield
	°C.	*Percent*
1	0-150	0
2	150-160	32
3	160-170	35
4	170-200	12
5	200-240	11
6	Green oil	10
Total	------------	100

TABLE 3. Physical properties of oleoresin turpentine[1] and of needle oil from Jeffrey and Coulter pines and Jeffrey-Coulter hybrids.

Pine	Index of refraction		Specific rotation		Specific gravity	
	Turpentine	Needle	Turpentine	Needle	Turpentine	Needle
Jeffrey	1.3957	1.4762	— 1°	—25°	0.697	0.871
PJ × (PJ × PC)	1.4155	1.4741	— 6°	—24°	.732	.860
(PJ × PC) × (Wind)	1.4319	1.4830	— 9°	—29°	.767	.867
Coulter	1.4711	1.4851	—18°	—26°	.839	.874

[1]Turpentine properties determined by Zobel (1951).

the transgressive variation in the hybrids suggest that needle oils would not provide discriminating characters for studies of hybridization between these pine species.

It is possible to compare the needle oils from the Jeffrey-Coulter hybrids with the oleoresin turpentine from the same trees. Zobel (1951, 1951a) tapped the same hybrid trees and many Jeffrey and Coulter pines throughout California. The obvious specific differences and hybrid intermediacy shown by Zobel's data are not found in the needle oil data (Table 3).

From his work, Zobel concluded that Coulter pine could have been the pollen parent of the natural Jeffrey-Coulter hybrid. This conclusion would be substantiated by the index of refraction of the needle oils, but not by the values of specific gravity. The parentage of the hybrid could not be decided from the needle oil data.

Normal-heptane apparently is absent from the needle oil of Jeffrey pine, although it is present in the oleoresin turpentine (Mirov, 1954). A similar situation was found by Schorger in Digger pine (*P. sabiniana* Dougl.). The turpentines both of Jeffrey and of Digger pines have been reported as composed of 95 percent n-heptane. The apparent absence of n-heptane from the needle oils of both of these species adds further evidence in support of the statement by Schorger (1919, p. 733): "The phytochemical processes occurring in the wood and in the needles are . . . entirely different."

Summary

The physical properties of needle oils from three pine species and three species hybrids were analyzed to determine whether there were any major differences in oil composition between pines resistant and susceptible to the pine reproduction weevil. No striking differences between species were found. Further study of turpentine constituents is needed to determine whether differences in needle oils influence host selection by adult weevils. The properties of the needle oils of an F_1 pine hybrid were found to be intermediate between those of the parents. In the progenies of another hybrid some properties transgressed the parental values. The properties of needle oils do not appear to be as useful in studying pine hybridization as are the oleoresin turpentines. Normal-heptane, a major constituent of Jeffrey pine turpentine, was not found in a crude fractional distillation of Jeffrey pine needle oil.

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