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MONOTERPENE VARIATIONS IN VAPORS FROM WHITE PINES AND HYBRIDS*

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Abstract—Possibilities of altering the monoterpene composition of vapors given off by white pine twigs, bark, and internal tissues by means of hybridization were investigated by gas chromatographic analyses of fifty-four trees. Considerable variation in the chemical composition of vapors was found among the hybrids produced by crossing *Pinus strobus* with *P. griffithii*, *P. koraiensis*, *P. flexilis*, and *P. ayacahuite*. The intraspecific variation of *P. strobus* was nearly as large, however. Vapors from needles and bark closely resembled those given off by internal tissues, but exceptions were noted for several compounds.

INTRODUCTION

VARIATION in the odors of species that are hosts of the white pine weevil, *Pissodes strobi* (Peck), may be partly responsible for differences in susceptibility. Weevils in an olfactometer were observed by Barnes[1] to be attracted by the odors of eastern white pine and Norway spruce (which are severely attacked), less so by the odor of Scotch pine (less frequently attacked), and not at all by the odor of red pine (rarely attacked). Anderson and Fisher[2] demonstrated that essential oils distilled from the twigs of black spruce and red spruce (rarely attacked) were more repellent to weevils in an olfactometer than oils from other spruces and white pine. Stroh and Gerhold[3] noted that adult weevils feeding in the cortex of eastern white pine consistently avoided resin canals. Heimburger[4] found that *Pinus peuce* grafts were more attractive to weevils but were damaged less than *P. strobus* grafts. *P. peuce* grafts selected for resistance to weeviling were less attractive than others of the same species, and their resistance was believed to be based mainly on a heavier resin flow after weevil attack, and also on some unknown factor that influenced the attractiveness of leaders. Wright and Gabriel[5] stated that western white pine (*P. monticola* D. Don), Himalayan white pine (*P. griffithii* McClel.), Macedonian white pine (*P. peuce* Griseb.) and Mexican white pine (*P. ayacahuite* Ehrenb.) were promising sources of weevil resistance, used either alone or in hybrid combination with eastern white pine (*P. strobus* L.).

The composition of the oleoresins of pines may be altered through hybridization,[6–8] and such a technique might be useful in breeding for white pine weevil resistance. This study explores the extent to which the content of monoterpenes (including associated aliphatic

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[1] T. C. BARNES, Doctoral Dissertation, Harvard Univ., Cambridge, Mass., U.S.A. (1928).
[2] J. M. ANDERSON and K. C. FISHER, *Can. J. Botany* **38**, 547 (1960).
[3] R. C. STROH and H. D. GERHOLD, *Silvae Genet.* **14**, 160 (1965).
[4] C. HEIMBURGER, *Proc. 14th Northeast For. Tree Imporv. Conf.* 64 (1967).
[5] J. W. WRIGHT and W. GABRIEL, Northeast Forest Exp. Sta. Paper 115 (1959).
[6] M. B. FORDE, *N. Zealand J. Botany* **2**, 53 (1964).
[7] N. T. MIROV, *Can. J. Botany* **34**, 443 (1956).
[8] R. H. SMITH, Forest Service Research Note PSW-135 (1967).

TABLE 1. PARENTAGE OF THE TREES WHOSE VAPORS WERE ANALYZED

Species	Parents: Female	Parents: Male	Number of progeny
P. strobus × *P. strobus*	831	wind	3
	980	selfed	1
	1951	1936	3
	1951	1955	3
	7382	1636	6
	7382	1955	6
			22
P. griffithii × *P. griffithii*	798	1628	1
	798	selfed	1
			2
P. strobus × *P. griffithii*	832	mix	1
	980	mix	1
	1625	mix	1
	1938	mix	2
	1951	mix	1
	1957	mix	2
	7382	mix	3
			11
P. griffithii × *P. strobus*	798	mix	1
	1632	wind	3
			4
P. strobus × *P. koraiensis* (hybridity questionable)	1951	619	2
	1951	mix	2
	7382	mix	1
			5
P. strobus × *P. flexilis*	740	726	1
	980	1532	1
			2
P. strobus × *P. ayacahuite*	831	1533	2
	979	1533	1
	1625	wind	1
	7383	1533	1
			5
P. ayacahuite × *P. strobus*	1533	wind	3
			3
			54

hydrocarbons such as heptane) of eastern white pine may be modified through hybridization. It is limited to two species and four hybrid combinations in the F_1 generation, and each category is represented by a rather small number of individuals.

RESULTS

Fourteen peaks were detected in the chromatograms (see Fig. 1). These are designated heptane, α-pinene, β-pinene, limonene, and unknowns F, G, H, J, L, and N. J is probably undecane and/or camphene, and L is probably myrcene and/or 3-carene, on the basis of relative retention times compared to those in the literature.[8, 9] F may be octane, and G may be nonane. Peaks A, B, and C were caused by small amounts of contaminants from the container in which branch segments were warmed. Unknown compound D (relative retention time 0·26) often appeared in larger amounts when branches were stored in the freezer more than 14 days, and therefore it was regarded as a degradation product. In general there

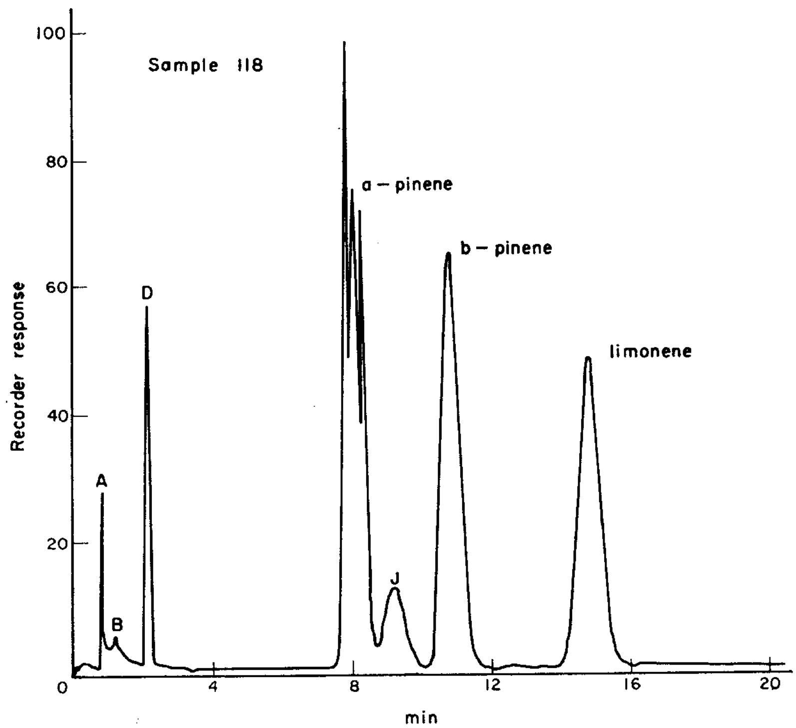

FIG. 1. REPRESENTATIVE CHROMATOGRAM ILLUSTRATING PEAK SYMMETRY AND SEPARATION OF MONOTERPENES FROM WHITE PINES.

was close agreement (± 10 per cent for major components) between duplicate samples, when allowance was made for quantitative distortions caused in some cases by compound D.

In both external and internal samples the largest total amount of monoterpenes was fifty-five times the smallest amount, as measured by the total area of all peaks. This range apparently indicates considerable variation in the quantity of vapor that can be emitted by different trees, both hybrids and non-hybrids. The sampling procedures and storage effects caused considerable inconsistency among duplicate samples, however, as only one-fourth of them agreed within 10 per cent. Therefore further comparisons of total monoterpene quantities are not warranted, but qualitative analyses of their compositions remain valid.

[9] J. W. HANOVER, *Heredity* 21, 73 (1966).

TABLE 2. COMPOSITION OF VAPORS THAT COME FROM EXTERNAL TISSUES (NEEDLES AND BARK) AND INTERNAL TISSUES (CORTEX, XYLEM, PITH) OF 1-yr-old BRANCHES OF WHITE PINES AND WHITE PINE HYBRIDS. THE HYBRIDITY OF STR × KOR IS QUESTIONABLE

Chemical compound	Source of vapor	Parent species, female × male: str × str	gri × gri	str × gri	gri × str	str × kor(?)	str × fle	str × aya	aya × str	Relative retention time
		range in percentage composition (tr = trace)								
Heptane	External	0–1	0	0	0	0–2	0	0–24	0–24	0·35
Heptane	Internal	0–tr	0	0	0	0	0	0	0	
F	External	0–3	0	0–5	0	0–1	0–tr	0–14	0–23	0·52
F	Internal	0–tr	0	0–2	0	0	0	0	0	
G	External	1–7	1	0–4	1–3	2–9	3–8	tr–3	3–10	0·74
G	Internal	0–3	0	0–tr	0	0	0	0–1	0–tr	
H	External	0–tr	0	0–2	0	0–2	0	0–tr	0–tr	0·89
H	Internal	0–1	0	0	0	0	0	0–tr	0–tr	
α-Pinene	External	20–70	32–52	23–41	32–47	25–61	32–41	20–50	24–31	1·00
α-Pinene	Internal	24–69	27–29	25–47	24–50	25–62	30–33	19–52	22–53	
J	External	3–17	1–9	0–13	8–11	1–9	3–10	2–8	2–11	1·14
J	Internal	3–30	8–16	1–13	8–15	4–9	2–7	1–22	3–12	
β-Pinene	External	10–45	27–52	30–68	27–50	17–47	19–44	11–51	15–29	1·34
β-Pinene	Internal	12–49	47–54	36–56	33–43	23–50	19–61	5–58	30–56	
L	External	0–10	0	0–8	0	0–tr	0–11	tr–23	tr–12	1·56
L	Internal	0–20	0	0–1	0	0–4	0–28	1–27	tr–16	
Limonene	External	0–6	tr	0–7	0–14	0–3	tr–7	0–2	tr–2	1·85
Limonene	Internal	0–18	0–tr	0–9	0–2	0–22	tr–4	0–6	1–8	
Number of trees		22	2	11	4	5	2	5	3	

The composition of vapors given off by the needles and bark of individual trees was very similar to that of vapors given off by internal tissues, principally from cortex oleoresin (Table 2). Some exceptions were noted, however. Several of the *Pinus strobus* × *ayacahuite* and *P. ayacahuite* × *strobus* hybrids had substantial amounts of heptane and compound F in the external samples, but none in the internal samples. Most of the fifty-four trees had compound G in the external samples but not in the internal samples. Limonene and compounds L and N in many cases were lower in the external samples than in the internal samples. These facts imply that there may be qualitative and quantitative differences between the oleoresins produced by different tissues of an individual tree (cf. Ref. 10). Different rates of vaporization can explain only some of the discrepancies. If any of these compounds influence the behavior of the white pine weevil, it will be important to distinguish between the two vapor sources in future studies. The external vapors could influence host finding and selection, while internal vapors could affect feeding and oviposition.

TABLE 3. COMPOSITION OF VAPORS FROM INTERNAL BRANCH TISSUES OF *P. strobus* FULL-SIB FAMILIES

Female parents			Male parents: 1936	Male parents: 1955
			range in percentage composition (tr = trace)	
1951	α-Pinene		24–44	49–67
	J		5–31	3–6
	β-Pinene		18–26	12–27
	L		0–18	0
	Limonene		0–15	4–10
		No. progeny	3	3
7382	α-Pinene		50–67	39–69
	J		5–23	5–7
	β-Pinene		12–23	18–38
	L		0	0–tr
	Limonene		tr–0	0–3
		No. progeny	6	6

The amount of variability among the hybrids was very large for most of the compounds. The ranges in composition would be even more varied if the geographic range of each species were represented more fully among the parents, and if larger numbers of progenies were analyzed. Considerable intraspecific variation in monoterpene composition has been reported in *P. radiata*;[11] *P. sylvestris*;[12] *P. maritima* and *P. pinaster*;[13] *P. muricata*;[14] *P. ponderosa*;[15] *P. coulteri*, *P. washoensis* and *P. contorta*;[8, 16] and *P. monticola*.[9] These reports indicate that provenances and also individual trees within a population may show large differences. We have unpublished data showing that both of these sources of variation also are important in *P. Strobus*. Table 3 gives some idea of the variability within full-sib families,

[10] M. VON SCHANTZ, *Planta Med.* **13**, 369 (1965).
[11] M. H. BANNISTER, A. L. WILLIAMS, I. R. C. MCDONALD and M. B. FORDE, *N. Zealand J. Sci.* **5**, 486 (1962).
[12] H. CYRKAL and J. JANAK, *Collection Czech. Chem. Commun.* **24**, 1967 (1959).
[13] W. SANDERMANN, *Holzforschung* **16**, 65 (1962).
[14] M. B. FORDE and M. M. BLIGHT, *New Zealand J. Botany* **2**, 44 (1964).
[15] R. H. SMITH, U. S. Forest Service Research Paper PSW-15. p. 17 (1964).
[16] R. H. SMITH, *Forest Sci.* **13**, 246 (1964).

though the numbers of trees are small. Hanover[9, 17] has reported that genetic control of the quantities of monoterpenes in *P. monticola* cortex is moderate to strong, and that environmental effects are very small.

When the monoterpene compositions of vapors from the hybrids are compared with those from *P. strobus*, only a few differences may be found in the extreme values. Hybridization with *P. ayacahuite* can increase the contents of heptane, compound F, and compound L in the external vapors. Hybridization with *P. griffithii* can increase β-pinene in external and internal vapors, and can eliminate compound J from the external vapors. The other possibilities for changes seem rather small compared to the variability within *P. strobus*.

This limited survey indicates that the monoterpene composition of vapors from *P. strobus* can be changed nearly as much by intraspecific selection and breeding as by interspecific hybridization. For this reason, and because of potential problems due to reduced fertility and uncertainty as to the climatic adaptation of hybrids, it appears that a more complete evaluation of intraspecific possibilities should receive higher priority. In further studies of hybrids the intraspecific selection of parents should also receive careful attention.

EXPERIMENTAL

The trees that were sampled had all been planted at the same location near Washington Crossing, New Jersey, in 1955. The seeds and stock had been produced by personnel of the Northeastern Forest Experiment Station, U.S. Forest Service. The data on parentage are summarized in Table 1. On 8 and 9 July lateral branches from the whorl formed in the previous year were cut to 20-cm lengths, and their ends were wrapped with polyethylene held on by rubber bands. They were enclosed in separate polyethylene bags, and stored in a refrigerated container during transport to the laboratory freezer.

Vapors given off by the branches were analyzed by gas chromatography during 11 to 30 July. Upon removal of a branch segment from the freezer, the needles that extended above the upper end were removed. The branch segment was inserted in a piece of glass tubing 25 mm o.d. by 115 mm, and was sealed in by a slit polyurethane foam plug at each end, through which the branch ends protruded. The upper end of the branch was cut 5 mm above the plug. This assembly was then inserted in a test tube 32 mm o.d. by 200 mm, with the bulging foam plugs forming a tight seal against the inside. A rubber stopper with a sampling port was used to close the test tube. The entire unit was warmed in a 40° water bath illuminated by a daylight lamp. After 15 min, a 1 ml vapor sample was withdrawn by a 5 ml air-tight syringe from the upper compartment in the test tube, which was 25 mm in length and contained the freshly cut upper end of the branch segment (internal sample).

A second 1 ml vapor sample was taken 15 min later from the 90 mm long compartment in the tubing, which contained the branch segment with attached needles, and remained sealed off from vapors given off by the cut ends (external sample). Each sample was injected into the gas chromatograph immediately after it was taken. All of the trees were analyzed in this way once, and then the procedure was repeated with a second set of samples.

The vapor samples were analyzed with an F & M Model 500 gas chromatograph and a Model 1609 flame ionization detector. The column was 4 mm i.d. × 9 ft stainless steel packed with 18% Apiezon L on 100–120 mesh Gas Chrom P. Apiezon L is one of several liquid-phases that are useful for separating terpene hydrocarbons, being non-selective with regard to boiling point and having suitable retention times and volumes for certain compounds.[18] Column temp. was 120°, injection port was 30°, detector was 160°, and helium flow rate was 87 ml/min. Heptane, α-pinene, β-pinene, and limonene peaks were identified by comparing relative retention times with those of known compounds. Identifications were supported by data obtained in a similar way using a different polar column packing, namely Ethylene Glycol Adipate. Calculations of percentage composition were based on areas under the peaks estimated by the product of peak height times width at one-half peak height.

Acknowledgement—The authors thank the Northeastern Forest Experiment Station for permission to use the trees and their records, and for assistance in identification of the hybrids.

[17] J. W. HANOVER, *Phytochem.* **5**, 713 (1966).
[18] M. H. KLOUWEN and R. TER HEIDE, *J. Chromatog.* **7**, 297 (1962).