Biomass **12** (1987) 129–139

Yields and Seasonal Variation of Phytochemicals from *Juniperus* Species of the United States

Robert P. Adams

Bio-Renewables Institute and Biology Department, PO Box 423, Baylor University, Waco, Texas 76798, USA

(Received 22 November 1986; accepted 29 January 1987)

ABSTRACT

The ten most widespread and dominant species of Juniperus *(juniper) in the United States were analyzed for their yields of hexane and methanol soluble phytochemicals from the leaves, bark/sapwood, and heartwood. The yields of volatile heartwood oils, a commercial commodity, varied from 4·92% to a low of 0·21%. The yields of hexane soluble components in the heartwood ranged from 0·44 to 7·6% dry wt, while the methanol soluble fraction varied from 2·8 to 6·8% dry wt. The yields from the bark/ sapwood were comparable: the hexane fraction ranged from 0·46 to 4·4% dry wt; the methanol fraction varied from 2·4 to 6·2% dry wt. The majority of the extractable chemicals in the above-ground biomass were found in the leaves with the hexane extraction accounting for 5·4 to 16·7% dry wt and the methanol extraction yielding 23·8 to 35·2% dry wt. Seasonal examination of the leaves of* Juniperus monosperma *and* J. osteosperma *revealed that the hexane extractable components increase toward the end of the growing season, reaching a maximum during the winter and declining in the spring. The methanol extractables exhibited minor, but significant, changes throughout the year.*

Key words: Phytochemicals, *Juniperus*, juniper, biomass, seasonal.

INTRODUCTION

There have been a number of reports on the yields of phytochemicals from plants during the past several years. Since this area has been recently reviewed,[1] it need not be further reviewed. The phytochemicals that have been investigated over the past several years for use as possible sources of liquid fuels and industrial chemicals can also be viewed as

Biomass 0144-4565/87/$03.50 — © Elsevier Applied Science Publishers Ltd, England, 1987. Printed in Great Britain

resource allocations for the plant. These resource allocations serve many functions in plants ranging from insect defenses and disease resistance to drought tolerance.[2] Changes in resource allocation not only impact the species defenses but may be important considerations in the production of industrial chemicals from biomass. For example, seasonal data on smooth sumac (*Rhus glabra* L.) indicated that the acetone extract yields could be maximized by harvesting at the full flower stage, whereas the hexane extract yields could be maximized at the seed-set stage.[3] In both milkweed (*Asclepias speciosa* Torr.) and gumweed [*Grindelia squarrosa* (Pursh) Dunal], the hexane extract yields reached a maximum at seed-set (October) and the methanol extract yields exhibited two maxima: very early growth (June) and full flower (August).[2] For the woody, perennial, rabbitbrush (*Chrysothamnus nauseosus* ssp. *consimilus* (Green) Hall & Clements), the hexane extract yields reached a maximum in the summer (June to August) and the methanol extract yields showed non-significant changes.[2]

Most of the previous reports on the yields of phytochemical resources have been from herbaceous plants or shrubs.[1,2] Due to the fact that junipers are already being harvested for their wood to produce cedarwood oil in the United States and other countries, it was felt a whole-plant utilization approach should be explored for the junipers. Not only could the cedarwood oil be removed but bio-active components could also be obtained from various plant parts.[4,5]

The junipers are well known to contain natural wood preservatives.[4,5] Carter[5] has found that *Reticulitermes flavipes* Kollar (southern termite) could not survive on sawdust from *J. virginiana,* nor could they survive on filter paper treated with a pentane extract of the *J. virginiana* sawdust. The preferred status of juniper wood (cedar) for use as fence posts comes from a long history of its resistance to rotting.

In many parts of the United States the weedy junipers often occur in almost continuous stands for hundreds of kilometers. The most important weedy junipers of the United States are *Juniperus ashei* Buch., *J. californica* Carr., *J. erythrocarpa* Cory, *J. deppeana* Steud., *J. monosperma* (Engelm.) Sarg., *J. occidentalis* Hook., *J. osteosperma* (Torr.) Little, *J. pinchotii* Sudw. and *J. virginiana* L. These species have invaded millions of acres of grasslands and old fields. In Texas alone, there is an estimated 21·5 M acres of juniper-invaded grasslands.[6] Ranchers are paid (US Department of Agriculture) for juniper removal to improve range conditions. The opening of the shade canopy appears to be very important for forage production.[7] Cropping machinery has been utilized by the PAKS Corporation at the Texas cedarwood oil production plant at Junction, Texas, to harvest *Juniperus ashei.* The trees are cut, chipped and trucked to the plant at Junction where the wood oils are removed by steam distillation. The cedarwood oil is then shipped to International Flavors and Fragrances Corp. (New York) for processing into flavors and fragrances.[8] All of the fuel for steam distillation comes from the burning of a portion of the spent chips.

The purposes of this study were to determine the extractable yields of phytochemicals from leaves, heartwood and bark/sapwood of juniper species and to examine the seasonal variation of the phytochemical yields from the leaves of two juniper species, *J. monosperma and J. osteosperma.*

MATERIALS AND METHODS

Samples of wood and herbarium vouchers were collected from natural juniper populations (Table 1). Voucher specimens are deposited in the

TABLE 1

Species of *Juniperus* Collected, Location of Populations Sampled and Specimen Voucher Numbers of the Samples Used in the Study. *Juniperus californica* 'A' and 'B' Refer to the Two Chemical Races Discovered by Vasek and Scora[9] and Reconfirmed by Adams, von Rudloff, and Hogge[10] Using Leaf Volatile Oils

Species	*Collection site*	*Voucher numbers*
J. ashei	9 km W of Ozona, Crockett Co., TX	Adams 5007–5009
	2 km E of Junction, Kimble Co., TX	Adams 5010–5016
J. californica 'A'	13 km NE of I-40, Granite Mtns, San Bernardino Co., CA	Adams 5067–5071
J. californica 'B'	30 km SE of Yucca, Yuma Co., AZ	Adams 5072–5076
J. erythrocarpa	32 km N of Alpine, Jeff Davis Co., TX	Adams 4987–4996
J. deppeana	32 km NW of Ft Davis, Jeff Davis Co., TX	Adams 4974–4983
J. monosperma	2 km W of Santa Rosa, Guadalupe Co., NM	Adams 5027–5036
J. occidentalis	8 km W of Juntura, Malheur Co., OR	Adams 5077–5086
J. occidentalis var. *australis*	2 km W of Sonora Jct., Mono Co., CA	Adams 5057–5066
J. osteosperma	25 km E of Monticello, San Juan Co., UT	Adams 5047–5056
J. pinchotii	28 km E of Ft Stockton, Pecos Co., TX	Adams 4997–5001
	10 km W of Sheffield, Pecos Co., TX	Adams 5002–5006
J. scopulorum	5 km E of Clines Corner, Torrance Co., NM	Adams 5037–5046
J. virginiana	7 km W of Bastrop, Bastrop Co., TX	Adams 5017–5025
Permanently tagged trees used for the seasonal study:		
J. monosperma	12 km E of Gruver, Hansford Co., TX	Adams 2905
J. osteosperma	Cottonwood Creek and Wasatch Blvd, Sale Lake Co., UT	Adams 3133

herbarium (BAYLU) at Baylor University, Waco, Texas, USA. The samples consisted of: wood (section 20 cm long × 5–10 cm in diameter) and leaves (400 g). All samples were kept cool (February collections) in the field and then frozen in the lab until analyzed. The samples for the seasonal studies consisted of three samples of leaves (400 g each), taken at monthly or bimonthly intervals at 9am local time. All samples were taken from the south-facing portion of the trees at heights from 0·5 to 2·0 m to minimize intra-tree variation.

The wood samples were separated into heartwood and bark/sapwood. Each subsample was then kept separate for analysis. Portions of the heartwood, bark/sapwood and leaves were dried (48 h, 100°C) to determine the percentage moisture. Approximately 12 g of the heartwood was steam distilled (20 h) to remove the volatile oil.[11] The percentage oil yield was calculated as: 100 times oil wt/(corrected dry wt of wood distilled plus oil wt). Portions of the heartwood, bark/sapwood and leaves were dried (48 h, 100°C) to determine the percentage moisture. Extracts were obtained from fresh heartwood, bark/sapwood and leaves by Soxhlet extraction of each set of materials for 6 h.[12] In every case the first solvent used was hexane and the second (sequential) solvent used was methanol. The material was dried (4 h, 70°C) after the hexane extraction to remove residual hexane and then extracted with methanol (see Ref. 13 for detailed notes on the extraction protocol).

RESULTS AND DISCUSSION

The yields of cedarwood oils varied from 0·21 to 4·92% on a dry heartwood basis (Table 2). *Juniperus ashei* from Ozona, TX, represents a divergent taxon of *J. ashei* (more primitive, slower growing; see Refs 14, 15 for detailed discussions). The wood oil yield from this divergent population is considerably larger (4·92%) than for the typical *J. ashei* from Junction, TX (4·04%). Unfortunately, the divergent form of *J. ashei* (western type) is found in sparse stands on the western edge of the species range in Texas and in a few small populations in northern Mexico. It is therefore not of sufficient density to justify commercial utilization. However, if plantations of juniper were established, these genotypes may be an important source of germplasm. The percentage dry weight of the heartwoods varied from a low of 66·95% (*J. deppeana*) to a high of 83·78% (*J. pinchotii*) with most of the species around 80% dry weight (Table 2). Therefore, the corresponding ranking of the percentage oil yield on a fresh weight basis was not significantly changed (Table 2).

TABLE 2

Yields of Volatile Heartwood Oils from Juniper Species Using a 20-h Steam Distillation. Oil Dry Wt (%) = Percentage Oil Yield on an Oven Dry Wt Basis. Dry Wt (%) = Oven Dry Wt Determined by Drying 48 h at 100°C. Oil Fresh Wt (%) = Percentage Oil Yield on a Fresh Wt Basis

Species	*Oil dry weight (%)*	*Dry weight (%)*	*Oil fresh weight (%)*
J. ashei, Junction, TX	4·04	76·79	3·10
J. ashei, Ozona, TX	4·92	77·31	3·80
J. californica 'A'	0·63	82·02	0·52
J. californica 'B'	0·46	81·69	0·38
J. deppeana	2·69	66·95	1·80
J. erythrocarpa	4·87	78·64	3·83
J. monosperma	1·24	79·76	0·99
J. occidentalis var. *australis*	1·78	80·34	1·43
J. occidentalis var. *occidentalis*	2·33	79·73	1·86
J. osteosperma	1·19	81·40	0·97
J. pinchotii	0·21	83·78	0·18
J. scopulorum	3·40	79·51	2·70
J. virginiana	3·18	80·40	2·56

The yields of hexane soluble components by Soxhlet extraction of heartwood (Table 3) varied from 0·03% (dry wt) for *J. pinchotii* to 7·56% (*J. erythrocarpa*) and 6·95% (*J. ashei*, Junction, TX). It should be noted that the volatile oil may have been lost from these extracts when the hexane was evaporated. Additional research will be needed to determine the composition of these extracts. The second (methanol) extract removed about the same quantity of polar components as resulted from the first (hexane) extract.

The non-polar extractable yields from the bark/sapwood were very low as expected (Table 4) with the exception of the yield from *J. scopulorum* (4·36%). This was not completely unexpected for *J. scopulorum*, because this species exuded considerable resin along the cambium layer when the wood was cut from the tree. This was also observed in *J. occidentalis* var. *australis* which had a moderate yield (2·30%). The methanol soluble yields were generally much larger than the non-polar yields but still low (Table 4). Unless components are found in the bark/sapwood that have very potent bio-activity, liquid extractions would not seem practical. The selection of species with a high heartwood to bark/

TABLE 3
Yields of Hexane and Methanol Soluble Material from Sequential 6-h Soxhlet Extractions of Heartwood of Juniper species Using Hexane Followed by Methanol. All Yields Reported on an Oven Dry Weight Basis

Species	*Hexane ext. %*	*Methanol ext. %*	*Dry wt (%)*
J. ashei	6·95	5·13	82·3
J. californica 'A'	0·57	4·20	89·1
J. californica 'B'	0·44	3·41	85·2
J. deppeana	3·94	5·42	74·1
J. erythrocarpa	7·56	6·76	83·3
J. monosperma	3·00	5·88	83·0
J. occidentalis var. *australis*	2·19	2·97	83·2
J. occidentalis var. *occidentalis*	1·89	2·76	82·4
J. osteosperma	2·42	6·18	84·1
J. pinchotii	0·03	4·18	90·9
J. scopulorum	3·36	4·91	84·2
J. virginiana	2·99	4·01	83·9

sapwood ratio will be important if heartwood components are utilized commercially.

The yields of phytochemicals from unground and ground leaves are compared in Table 5. In general, the yields of hexane soluble components are doubled to tripled when the leaves are ground (*J. monosperma* and *J. osteosperma* are exceptions). The non-polar yields from several species are over 10% dry weight, with both varieties of *J. occidentalis* yielding over 15% (Table 5). The composition of these fractions will need to be examined in future research.

The yields of polar extractables from leaves ranged from 25 to 40% (Table 5) which is not uncommon.[1,12] Unfortunately these polar extracts are probably chiefly composed of simple sugars[13] and may therefore be of low economic value. On the other hand, considerable biological activity has been found in these (polar) leaf extracts (Adams, unpublished); therefore additional research on the composition would be desirable.

It is interesting to note that the yields of methanol soluble components generally were smaller from ground leaves than unground leaves (Table 5). Apparently hexane had already removed a portion of slightly polar material in the ground leaves because the total extractables (hexane plus

TABLE 4
Yields of Hexane and Methanol Soluble Material from Sequential 6-h Soxhlet Extractions of Bark/Sapwood of Juniper Species Using Hexane Followed by Methanol. All Yields Reported on an Oven Dry Weight Basis

Species	*Hexane ext. (%)*	*Methanol ext. (%)*	*Dry wt (%)*
J. ashei, Junction, TX	1·04	4·14	70·2
J. californica 'A'	0·90	3·38	72·9
J. californica 'B'	0·72	2·43	79·9
J. deppeana	0·82	6·21	78·0
J. erythrocarpa	0·80	5·48	83·7
J. monosperma	1·82	4·14	72·0
J. occidentalis var. *australis*	2·30	4·49	66·3
J. occidentalis var. *occidentalis*	0·46	3·25	58·9
J. osteosperma	1·31	5·07	68·9
J. pinchotii	1·13	4·35	76·7
J. scopulorum	4·36	5·15	59·7
J. virginiana	1·05	2·74	64·5

methanol extractables) are approximately the same for both ground and unground leaves. The largest total yields are from *J. occidentalis* var. *occidentalis* (44·8%) and var. *australis* (44·4%).

Because the yields were largest from the foliage, it was felt that some preliminary data should be collected on seasonal variation in the yields from the leaves. In an examination of the hexane and methanol yields from the above-ground biomass of an annual, a herbaceous perennial, and a woody perennial, Adams & Price[2] found considerable seasonal variation. The woody perennial (*Chrysothamnus nauseosus* ssp. *consimilis*) exhibited an increase in the yield of hexane extractables during the growing season with a minor decline in the fall.[2] The methanol extractables displayed no significant differences from May to October (the extent of the study).[2] It should be noted that *Chrysothamnus* has flat, thin leaves as is common in most angiosperms, whereas *Juniperus* has modified needles which are quite turgid, more typical of the gymnosperms. The initial seasonal study of *Juniperus* utilized samples of *J. monosperma* taken from May through November (Fig. 1). The hexane extract was relatively stable from May through July and then began to increase in September and November (Fig. 1). The methanol soluble components showed only minor changes during this period, with a signi-

TABLE 5

Yields of Hexane and Methanol Soluble Material from Sequential 6-h Soxhlet Extraction of Unground and Ground Leaves of Juniper Species Using Hexane Followed by Methanol. All Yields Reported on an Oven Dry Weight Basis

Species	*Hexane Extractables (%)*		*Methanol Extractables (%)*	
	Unground	*Ground*	*Unground*	*Ground*
J. ashei, Junction, TX	2·56	6·49	29·66	35·22
J. ashei, Ozona, TX	N/A	7·39	N/A	26·98
J. californica 'A'	4·91	15·80	30·70	26·33
J. californica 'B'	1·20	15·80	28·30	26·20
J. deppeana	3·15	7·68	24·73	32·71
J. erythrocarpa	4·00	11·27	29·00	24·43
J. monosperma	4·78	5·49	33·46	27·13
J. occidentalis var. *australis*	5·60	16·74	32·60	27·66
J. occidentalis var. *occidentalis*	5·80	15·74	29·60	29·03
J. osteosperma	5·30	5·42	29·00	31·18
J. pinchotii	3·20	10·78	28·50	23·79
J. scopulorum	6·00	10·63	32·60	27·15
J. virginiana	1·94	10·13	39·93	29·15

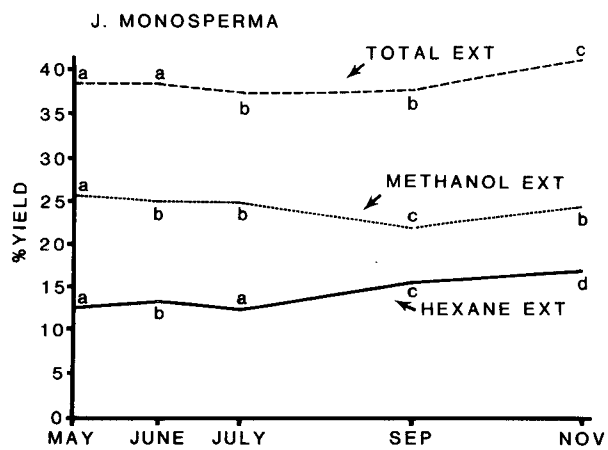

Fig. 1. Seasonal changes in the hexane, methanol and total extractables yields from *Juniperus monosperma* (Hansford Co., Texas, USA). Any data points on a line that have the same letter are *not* significantly different by Student–Newman–Keuls multiple range test at $P = 0{\cdot}05$.

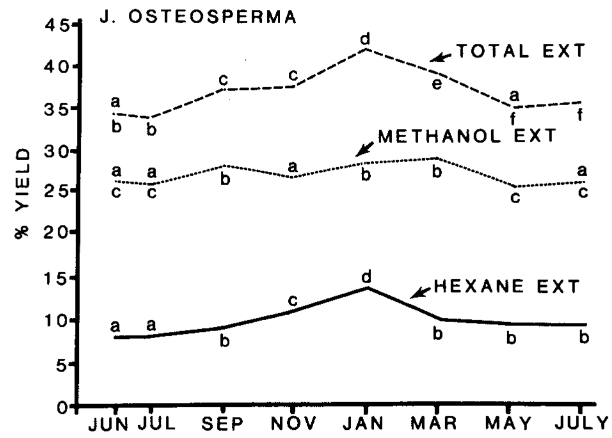

Fig. 2. Seasonal variation in the hexane, methanol and total extractables yields from *Juniperus osteosperma* (Salt Lake Co., Utah, USA). Any data points on a line that have the same letter are *not* significantly different by Student–Newman–Keuls multiple range test at $P=0{\cdot}05$. Note the large increase in the hexane and total extractables during the fall and decrease at the onset of the growing season in March.

ficant drop during the September sample (Fig. 1). The total extractives fell slightly in July and rose in November (Fig. 1).

A longer term seasonal study was then undertaken on *J. osteosperma.* This species is closely related to *J. monosperma* and the yields of extractables were similar during the period from May to November (Fig. 2). The yields of hexane extractables increased during the fall to a maximum in January and then declined in the early growing season to spring and summer levels (Fig. 2). It is interesting to note that this coincides with the browsing pressure when deer are forced to consume juniper in Colorado and Utah during the heavy fall and winter snows. Upon initiation of growth in the spring, these phytochemicals may then be metabolized as energy sources. This same pattern was found in *J. scopulorum* leaf volatile oils[16] which were sampled from a site with a similar environment on the eastern slopes of the Rocky Mountains.

The methanol extractables of *J. osteosperma* varied little during the year (Fig. 2) with a minor increase during the fall and winter. The total yields of extractables showed a moderate, significant increase in the fall and a decline in the spring (Fig. 2).

Overall, seasonal variation in the yields of phytochemicals from the leaves of *Juniperus* would not appear to present a major problem in their utilization. Due to the highly buffered environment in the trunk wood, one would expect the heartwood and sapwood phytochemicals to vary little but research will need to be undertaken if those components appear commercially viable.

Although the juniper wood does not appear to be a promising source of phytochemicals except for the cedarwood oil already being utilized, the foliage is extremely rich in phytochemicals and their composition needs to be fully investigated to determine the economic feasibility for utilization.

ACKNOWLEDGEMENTS

This research was supported by funds from the National Science Foundation (DMB-8460062). Thanks to the PAKS Corporation, Junction, TX, for discussions of their cedarwood oil operation and information on their field operations.

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