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Termiticidal Activities in the Heartwood, Bark/Sapwood and Leaves of *Juniperus* Species from the United States

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Abstract—Twelve taxa of *Juniperus* from the United States were investigated for termiticidal activities of the heartwood, bark/sapwood and leaves. All taxa exhibited termiticidal activities for the fresh heartwood sawdusts. All except *Juniperus scopulorum* showed high termiticidal activities for the bark/sapwood sawdusts. The activity in the sawdusts could be removed by washing with hexane followed by methanol for about half of the taxa. Both hexane and methanol (sequential) extracts of the heartwoods showed termiticidal activities. Hexane and methanol (sequential) extracts of intact leaves displayed termiticidal activities for most of the taxa.

Introduction

Juniper wood is the domestic source of cedarwood oil for the United States but the junipers are also known to contain natural wood preservatives [1]. The control of wood rot and termites is a perennial problem in much of the world. Many of the methods for wood preservation have used arsenic and/or chlorinated hydrocarbons which may present some environmental hazards. Carter [2] has found that the subterranean termite *Reticulitermes flavipes* Kollar 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.

Oda *et al.* [3] conducted insecticidal screening of individual components of the sesquiterpenoids from the wood of *J. recurva* Buch., which has been used as an insecticide by the natives in Nepal against household insects. They found the highest insecticidal activity in thujopsene (widdrene) (LD_{50} mg/mosquito = 4.5) and 8-cedren-13-ol (LD_{50} mg/mosquito = 6.6), with less activity by cedrol and alpha-cedrene.

The two sources of domestically produced cedarwood oil for the United States are central Texas (*J. ashei*, 'Texas cedarwood oil') and the eastern United States (*J. virginiana* L., 'Virginia cedarwood oil'). In many parts of the United States the weedy junipers have invaded abandoned fields and overgrazed rangelands. They often occur in almost continuous stands for hundreds of kilometres. The most important weedy junipers of the United States are *J. 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 are an estimated 21.5 million acres of juniper-invaded grasslands [4]. Ranchers are paid (USDA–ASCS) for juniper removal to improve the range conditions. The opening of the shade canopy appears to be very important for foliage production [5]. Thus, a natural renewable source of termiticides may be available from plants that are currently not utilized.

The purpose of this study was to determine the termiticidal activities of heartwood, bark/sapwood and leaves of the dominant Juniper species in the United States as part of the evaluation of the commercial potential of plants that are now considered to be a noxious tree species in rangelands.

Results and Discussion

The first test conducted to determine the bioassay for termiticidal activity used fresh

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sawdust. Both the heartwood and bark/sapwood sawdusts had extremely high activity except for the bark/sapwood of *J. scopulorum* (62 and 57% survival, Table 1). There were essentially no survivors when the heartwood of any juniper was used.

In order to determine if the active components could be removed from the sawdust, heartwood sawdust was extracted 10 times (by shaking) with hexane and then 10 times with acetone. This did not remove all of the termiticidal activity from any species (Table 2). Several of the heartwood sawdusts were still 100% lethal (Table 2) but about half of the species lost most of their bio-active components (Table 2).

Additional samples of heartwood were Soxhlet extracted with hexane and the hexane soluble material was bioassayed (Table 3). The hexane extracts proved to be highly active. The termites did not survive for more than one week on most extracts (Table 3). However, at these low concentrations one can begin to see differences among the species. At 1 ml/pad there is some survival on the extracts of *J. monosperma* and *J. osteosperma* and considerable survival on *J. californica* extracts (Table 3).

TABLE 1. RATES OF SURVIVAL FOR TERMITES FED ON JUNIPER HEARTWOOD AND SAPWOOD/BARK SAWDUSTS.

Species	Material	Per cent survival at 4 weeks* Trial 1	Trial 2
J. ashei	Heartwood	0 (0.5)	0 (0.5)
	Bark/sapwood	5	3
J. californica 'A'	Heartwood	0 (2.5)	0 (2.5)
	Bark/sapwood	1	0 (3.0)
J. californica 'B'	Heartwood	0 (3.0)	0 (3.0)
	Bark/Sapwood	0 (3.5)	0 (3.5)
J. deppeana	Heartwood	0 (1.5)	0 (1.5)
	Bark/sapwood	0 (3.0)	0 (3.0)
J. erythrocarpa	Heartwood	0 (0.5)	0 (0.5)
	Bark/sapwood	0 (3.5)	0 (3.5)
J. monosperma	Heartwood	0 (2.0)	0 (0.5)
	Bark/sapwood	0 (3.5)	17
J. occidentalis	Heartwood	0 (1.5)	0 (1.5)
var. *occidentalis*	Bark/sapwood	0 (3.0)	0 (3.0)
J. occidentalis	Heartwood	0 (0.5)	0 (0.5)
var. *australis*	Bark/sapwood	2	0 (4.0)
J. osteospmera	Heartwood	0 (3.0)	0 (3.0)
	Bark/sapwood	0 (4.0)	0 (3.5)
J. pinchotii	Heartwood	0 (4.0)	10
	Bark/sapwood	0 (3.5)	1
J. scopulorum	Heartwood	0 (0.5)	0 (0.5)
	Bark/sapwood	62	57
J. virginiana	Heartwood	0 (0.5)	0 (0.5)
	Bark/sapwood	0 (3.5)	35

*Values in parentheses are the number of weeks when all the termites had died.

TABLE 2. RATE OF SURVIVAL FOR TERMITES FED ON JUNIPER HEARTWOOD SAWDUSTS SEQUENTIALLY EXTRACTED WITH HEXANE FOLLOWED BY ACETONE

Species	Per cent termites surviving at 4 weeks*
J. ashei	0 (3.5)
J. californica 'A'	0 (3.0)
J. californica 'B'	0 (3.5)
J. deppeana	0 (3.5)
J. erythrocarpa	0 (3.5)
J. monosperma	10
J. occidentalis	11
J. occidentalis var. *australis*	10
J. osteosperma	48
J. pinchotii	38
J. scopulorum	50
J. virginiana	46
Control	93
Sand	0 (2.5)

*Values in parentheses are the number of weeks when all the termites had died in the text.

TABLE 3. RATE OF SURVIVAL FOR TERMITES FED ON PAPER CONTAINING HEXANE OR METHANOL EXTRACTS FROM JUNIPER HEARTWOOD

Species	Per cent termites surviving at 4 weeks* Hexane extract† 1 ml/pad	2 ml/pad	Methanol extract‡ 1 ml/pad
J. ashei	0 (1.0)	0 (1.0)	36
J. californica 'A'	88	0 (1.0)	0 (1.5)
J. californica 'B'	84	84	0 (3.0)
J. deppeana	0 (1.0)	0 (1.0)	4
J. erythrocarpa	0 (1.0)	0 (1.0)	32
J. monosperma	23	20	48
J. occidentalis	0 (1.0)	0 (1.0)	0 (0.5)
var. *australis*	0 (1.0)	0 (1.0)	0 (1.5)
J. osteosperma	20	0 (1.0)	0 (3.5)
J. pinchotii	NT	NT	8
J. scopulorum	0 (1.0)	0 (1.0	0 (2.0)
J. virginiana	0 (1.0)	0 (0.5)	0 (1.5)
Control	96	100	100

*Values in parentheses are the number of weeks when all the termites in the test had died.

†Hexane extracts applied at 1 mg ml^{-1} concentration except for *J. californica* 'A' and 'B', *J. occidentales* and *J. occidentalis* var. *australis* for which 0.5 mg ml^{-1} was used.

‡Methanol extracts were applied at 10 mg ml^{-1} concentration.

NT = Not tested.

A sequential Soxhlet extraction of the heartwood using methanol (following hexane) removed the more polar components. The bioassay of the polar fraction revealed considerable activity for most of the extracts (Table 3). However, *J. ashei, J. erythrocarpa* and *J. monosperma* methanol extracts showed reduced activity (Table 3).

Based on the aforementioned series of experiments, several factors seem to be indicated. Not all of the bioactivity could be removed by simple hexane/acetone extraction (Table 2). A non-polar fraction that has a high termiticidal activity exists in all species (Table 3) except *J. californica*. Our investigation of the antitermitic activities of the *J. virginiana* heartwood extractives (McDaniel, C. A., Klocke, J. A. and Balandrin, M. F., unpublished results) indicated that the most toxic components were the sesquiterpene alcohols, cedrol and widdrol. The sesquiterpene hydrocarbons showed considerably less toxicity.

A correlation between the toxicity data generated in this study and the analysis of the major extractive components of the heartwoods of the *Juniperus* species [6] allows some conclusions to be drawn.

A comparison of Tables 2 and 3 indicates that the antitermitic properties of *J. californica* are not contained in the hexane extractable material, but are a result of more polar compounds which are found in the methanol extract. The low yield of steam distillable material found by Adams [6] for this species indicates that the antitermitic components are not likely to be the same as those from *J. virginiana.*

The toxicities of the methanol extracts from the other species may indicate that hexane simply does not completely extract the sesquiterpenes and sesquiterpene alcohols; or they may indicate the presence of additional, more polar toxic components. Further investigations are needed to ascertain the identities of these components.

Due to the large volume of leaves that could be obtained during harvesting, it seemed appropriate to assay the leaf extracts for bioactivity. The hexane leaf extracts of about half of the species had high termiticidal activity (Table 4). It is interesting to note that the two chemical races (based on their volatile leaf oils) of *J. californica*, 'A' and 'B', behaved quite differently in this assay with type 'A' showing little activity and type 'B' displaying considerable activity (Table 4).

TABLE 4. RATE OF SURVIVAL FOR TERMITES FED PAPER CONTAINING HEXANE OR METHANOL EXTRACTS OF UNGROUND JUNIPER LEAVES

Species	Per cent termites surviving at 4 weeks* Hexane extract†	Methanol extract
J. ashei	0 (3.5)	66
J. californica 'A'	84	0 (2.5)
J. californica 'B'	0 (1.5)	0 (2.5)
J. deppeana	88	97
J. erythrocarpa	80	41
J. monosperma	0 (2.5)	0 (3.0)
J. occidentalis	0 (1.5)	83
var. *australis*	0 (2.5)	41
J. osteosperma	88	25
J. pinchotii	96	51
J. scopulorum	36	0 (0.5)
J. virginiana	0 (1.5)	0 (0.5)
Control	100	95

*Values in parentheses are the number of weeks when all the termites in the test had died.

†1 ml of hexane extract (diluted to 10 mg ml^{-1}) was added to each paper pad.

The methanol soluble components from the leaves showed very high termiticidal activity in some of the species and reduced survival in others (Table 4). Only the *J. deppeana* methanol extract had essentially all termites surviving after four weeks. The hexane extract of this species also exhibited low toxicity. *Juniperus ashei, J. californica, J. monosperma, J. occidentalis, J. scopulorum* and *J. virginiana* each had termiticidal activity in one or both of the leaf extracts. Research efforts to date have concentrated on surveys of wood chemicals as sources of termiticides, presumedly because rot and insect resistant woods are often well known and one might expect a long co-evolution of plant chemical defenses against wood-eating insects. The discovery of very active termiticidal components in the leaves appears to be a serendipitous event. Since many tons of leaves can be harvested along with the wood, additional research should be directed toward the isolation and identification of the active components in the leaves.

Experimental

Samples of wood and herbarium vouchers were collected from *J. ashei* (Adams 5007–5009, 9 km west of Ozona,

Crockett Co., TX; 5010–5016, 2 km east of Junction, Kimble Co., TX) *J. californica* 'A' (Adams 5067–5071, 13 km northeast of I40, Granite Mountains, San Bernardino Co., CA) and *J. californica* 'B' (Adams 5072–5076, 30 km southeast of Yucca, Yuma Co., AZ), *J. erythrocarpa* (Adams 4987–4996, 32 km north of Alpine, Jeff Davis Co., TX), *J. deppeana* (Adams 4974–4983, 32 km northwest of Fort Davis, Jeff Davis Co., TX), *J. monosperma* (Adams 5027–5036, 2 km west of Santa Rosa, Guadalupe Co., NM), *J. occidentalis,* (Adams 5077–5086, 8 km west of Juntura, Malheur Co., OR), *J. occidentalis* var. *australis* (Adams 5057–5066, 2 km west of Sonora Junction, Mono Co., CA), *J. osteosperma* (Adams 5047–5056, 25 km west of Monticello, San Juan Co., UT), *J. pinchotii* (Adams 4997–5001, 28 km east of Fort Stockton, Pecos Co., TX; Adams 5002–5006, 10 km west of Sheffield, Pecos Co., TX), *J. scopulorum* (Adams 5037–5046, 5 km east of Clines Corner, Torrance Co., NM) and *J. virginiana* (Adams 5017–5025, 7 km west of Bastrop, Bastrop Co., TX). *Juniperus californica* 'A' and 'B' refer to the two chemical races discovered by Vasek and Scora [7] and reconfirmed by Adams, von Rudloff and Hogge [8] using the leaf volatile oils.

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 laboratory until analysed.

The wood samples were separated into heartwood and bark/sapwood; each subsample was then kept separate. Portions of the heartwood, bark/sapwood and leaves were dried (48 h, 100°) to determine the per cent moisture. Extracts were obtained from fresh heartwood, bark/sapwood and leaves by Soxhlet extraction of each set of materials for 6 h [9]. In each case the first solvent used was hexane and the second (sequential) solvent used was methanol. The material was dried (4 h at 70°) after the hexane extraction to remove the hexane before extraction with methanol [10].

Fresh heartwood sawdusts, fresh bark/sapwood sawdusts and extracted materials were tested on externally undifferentiated termite workers from field-collected colonies of *Reticulitermes flavipes*. Fifty g of sand and 7 ml distilled water were placed into a plastic zipper case. Sawdust samples (1.5 g) were placed in the zipper case along with 100 termites (*R. flavipes*) and kept at 25°. Duplicate samples were run for each species and the bioassays terminated after 4 weeks. Hexane and methanol extracts were placed on filter paper and 25 termites were added. The hexane (Soxhlet) extracts of the heartwoods were initially diluted to a concentration of 10 mg ml^{-1} except for samples from *J. occidentalis*, *J. californica* 'A' and 'B' which contained 5 mg ml^{-1}. At these concentrations, all termites were dead within 3 days for all samples. Therefore, all the extracts were then diluted to 1 mg ml^{-1} except for *J. occidentalis* and *J. californica* 'A' and 'B' which contained 0.5 mg ml^{-1}. Two trials were prepared for each extract: 1 ml extract and 2 ml extract. The methanol extracts were prepared with 10 mg ml^{-1} extracted material and bioassayed as described previously, but with only one bioassay per extract; each filter pad was treated with 1 ml extract. The leaf sample extracts were diluted to 10 mg ml^{-1} for both the hexane and methanol extracts. Test results are reported as the per cent survival after 4 weeks.

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References

1. Guenther, E. (1952) *The Essential Oils.* Vol. 6. Robert E. Kreiger, Huntington, N.Y. (reprint of 1952 edn).
2. Carter, F. L. (1976) *Mater. u. Organismen Beiheft.* **3**, 357.
3. Oda, J., Ando, N., Nakajima, Y. and Inouye, Y. (1977) *Agric. Biol. Chem.* **41**, 201.
4. Smith, H. N. and Rechenthin, C. A. (1964) *USDA Publ. 4-19114.* Soil Conservation Service, Temple, Texas.
5. Clary, W. P. (1974) *J. Range Mgmt* **27**, 387.
6. Adams, R. P. (1987) *Econ. Botany* **41**, 48.
7. Vasek, F. C. and Scora, R. W. (1967) *Am. J. Botany* **54**, 781.
8. Adams, R. P., von Rudloff, E. and Hogge, L. (1983) *Biochem. Syst. Ecol.* **11**, 189.
9. Adams, R. P. and McChesney, J. D. (1983) *Econ. Botany* **37**, 207.
10. Adams, R. P., Balandrin, M. F., and Martineau, J. B. (1984) *Biomass* **4**, 81.