# JUNIPER OIL DISTILLATION AND MARKETING PROJECT

WESTERN JUNIPER COMMERCIALIZATION PROGRAM

# FINAL REPORT

December, 1996 - Ver. 2

The Confederated Tribes of the
Warm Springs Reservation of Oregon

Business & Economic Development Branch
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## 1.0 Executive Summary

- During the distillation of thirteen (13) batches of Western Juniper oil (Juniperus Occidentalis) it was found that there was much to learn about the harvesting, preparation and handling of the raw materials if yield is to be maximized. Example: best yields were from materials harvested in the Fall, raising the speculation of seasonal considerations in the tree's oil content.

- With properly prepared materials, and an efficient distillation system, expect oil yield of 0.5% - 0.75% from a zero-pressure system. Though not explored in this project, oil yield could average 1.0% - 1.5% in a 20-40 psi pressure system. Yield from the bole materials would probably be especially enhanced with a pressure system.

- Optimum distillation time was deemed to be 3-4 hours, resulting in a yield of 80% - 90% of the recoverable oil.

- Yield from properly dried materials could be greater than from fresh materials, however fast-drying tends to result in reduced yield since some oil is lost along with the moisture.

- The oil itself is a fine quality oil, rich in monoterpines and bornyl acetate, and with an aroma distinctly similar to the aroma of the fresh tree materials. This is not true of all tree and needle oils.

- The estimated cost of recovered oil (from a zero-pressure system) was $360.00 per gallon for a commercial (volume) operation, and $925.00 per gallon in a small, entrepreneurial operation.

- There are three market sectors to be served, with substantial variance in the prices between them:

  a.) Industrial: $7.50 per pound for wood oil in drum lots.

b.) Commercial:

- Large Commercial Segment; $25.00 per pound (drum lots) for leaf oil for oil blenders and large third party manufacturers of commercial products.

- Specialty Commercial Segment; $60.00 per pound for leaf oil for specialty aroma therapy, naturopathic and specialty scent products, including entrepreneurs doing retail products.

- There is some market interest in the distillate "waters" which come off of the process at a ratio of 300/1 to the oil. The waters may have large volume applications for certain scent cosmetics (e.g., cologne), animal pesticides, and insecticides. The insecticidal/pesticidal qualities of the waters should be explored in a dedicated research project.

- There is no business opportunity for the selling of wood oil to the Industrial sector (@ $7.50 per pound) in competition with Eastern Red Cedar oil (Juniperus Virginiana).

- Serving the Large Commercial segment (@ $25.00 per pound) would only be economically viable at yields in excess of approximately 0.9%.

- Serving the Specialty Commercial Segment is an attractive business opportunity, with a break-even point of 0.5% yield at $60.00 per pound in small (2-10 kgs.) quantities. At a product yield of 1.0%, business viability is assured down to prices as low as $25.00 per pound (see Break-even Sensitivity Analysis).

- There is a substantial business opportunity for entrepreneurs who wish to distill Juniper oil for use in their own specialty retail products, such as hunter's scent mask, mood scent kits, seasonal scents, and other specialty scent products such as soaps and candles. This is, perhaps, the most attractive near-term opportunity even if they had to pay the $60.00 per pound for oil, if it were available. The value-added aspects of blending, packaging and marketing provide very high revenue leverage over the marketing of the raw oil.

- Since Western Juniper oil is not currently available commercially, some investment in market development would be necessary in order to introduce the oil or its end products to the target customers.

## 2.0 Project Initiation, Objectives and Scope

In September of 1994 the Confederated Tribes of Warm Springs presented to the Multi-Region Strategies planning group a proposed research project to distill the essential oils out of Western Juniper trees (species Juniperus Occidentalis) for purposes of determining whether there were viable commercial business opportunities that could be generated from this use of the Western Juniper biomass. This research project was later incorporated into the an overall Western Juniper Commercialization Program, involving a number of other projects seeking to find markets for Western Juniper lumber and products, and was then submitted through the Oregon Economic Development Department as a proposed economic development project.

The proposed Western Juniper Commercialization Program was submitted to Governor Kitzhaber for review, and on April 18th, 1995 the Confederated Tribes of Warm Springs was advised that funding for the Juniper commercialization program, including the proposed oil recovery and marketing project, was approved by the Governor.

On August 29th, 1995 the Confederated Tribes of Warm Springs was advised by the Klamath County Economic Development Association (KCEDA) that they were empowered to authorize the commencement of spending for the oil recovery and marketing project. However, because of the need to execute agreements between the funding and contract administration agencies, and the agencies that would actually do the projects, the agreement between the Confederated Tribes of Warm Springs and KCEDA was not formally executed until April 1996. With the formal contract agreements finalized it was then possible to move ahead with the execution of the project. Copies of the relevant correspondence initiating this are included as Appendix A in this report, for reference.

The objectives of this research were as follows:

1. Determine the economic and process viability of obtaining the essential oils from Western Juniper trees, including the distillation and testing of oil yields from the Juniper leaf, bole wood, and the dried berries.
2. Determine the marketability and market value of the oil obtained from the distillation process by sampling the commercial market for Juniper oils, or oils similar to Juniper, in a variety of their most logical applications.

3. Determine whether business opportunities exist on either a large-scale commercial basis, or an entrepreneurial basis, for the distillation and marketing of Juniper oil, either to the commercial oil markets or, potentially, to end user markets.
4, Identify specific product opportunities toward which the oil could be applied such that entrepreneurs, or small businesses, could consider getting into the markets for these products on a profitable basis.

This project was not intended to be a comprehensive analysis of the many combinations of variables that would be involved in this test including the nature, sex, age, size, etc. of the trees themselves; the nature of the land upon which the Juniper is growing; the altitude and water availability; or the many other variables involved in the harvesting, handling and preparation for use, as well as the many variables of the distillation process itself. To this end it was envisioned that a reasonable test distillation facility would be set up for use in distilling oil from Juniper materials that would be obtained, handled and processed in a nonspecialized way such that most anyone who would want to duplicate this process could readily do so.

## 3.0 Project Implementation Schedule (1996)

| Date | Activity |
|---|---|
| April 30 | Contract between KCEDA and the Confederated Tribes finalized. |
| May 10 | Project management and technical/ marketing support agreements finalized. Equipment site and support agreement with Warm Springs Forest Products Industries (WSFPI) plant finalized. |
| May 17 | Space at WSFPI plant cleared for distillation unit. Distillation unit placed at WSFPI. |
| May 20 | Authorized Juniper cutting area toured, trees cored, selected and marked. Arrangements made with Tribal Natural Resources Branch for harvesting, bagging and hauling crew (for fee). |
| June 6 | First harvesting: two trees harvested. Bagged leaf material, and bole wood, brought to distillation site. |
| June 7, 11 | First distillations: two batches of bagged leaf materials. |
| June 19, 20 | Second harvesting of two trees. Cook two batches of bagged leaf materials. |

| | |
|---|---|
| July 1 | Chip and cook first batches of bagged bole wood. |
| July through November | Continue harvesting and cooking as needed to refine process and obtain oils needed for marketing samples. |
| July 1 | Begin commercial marketing activity. |
| July 30 | Gas chromatography results of leaf oil, boil oil and distillation waters received from Flora Research. |
| August through October | Continue commercial marketing efforts. |
| November 1 | Do extra cookings to obtain additional oil for samples.. |
| November 15 | Prepare sample hunters scent spray bottles to sample retail application market |
| December 1 | Expand retail test marketing of sample spray bottles for general/ seasonal aromatic uses. |
| December 17 | Final report draft completed. |
| December 24 | Final report completed and distributed. |

## 4.0 Distillation Equipment: Installation and Use

The distillation system was obtained under contract from the Essential Oil Co., Lake Oswego, Oregon, which also was retained under contract to provide technical distillation assistance and product marketing services to the commercial essential oils marketplace. An agreement was made with Warm Springs Forest Products Industries (WSFPI) to site the still in a little used portion of their lumber processing plant at Warm Springs, Oregon. The site was accessible by vehicles and heavy equipment, and was generally out of the way of their normal business operations.

The distillation system itself consisted of a retort for holding the raw materials and was capable of handling 250 to 300 pounds of materials when fully charged. The steam outlet from the retort was via an insulated pipe which carried the steam to a condenser and separator which would take the output of the retort, condense the steam into water, and then separate the condensate into oil and the distillate runoff "waters" (which were the secondary byproduct of the process).

Distillation in the retort was accomplished by piping steam to the retort from the plant steam system which supplied steam at approximately 350° and between 125 and 150 pounds per square inch. The condenser required the piping-in of cooling water in order to facilitate the condensing of the steam output from the retort. The separator for separating the oil from the distillate waters obtained the condensate by gravity feed from the condenser. Once in the separator, oil floating on the condensed liquid could be tapped off separate from the distillate waters. The distillate waters were then allowed to feed via a gravity connection to a 20 gallon catch drum which caught the runoff waters from the distillation in progress.

The distillate catch drum was connected via a transfer pump to a 400 gallon holding tank (provided by WSFPI) such that the distillate waters from the catch drum could be pressure-pumped periodically from the catch drum to the holding tank. The plant then provided disposal services for the run-off waters by emptying the holding tank as needed.

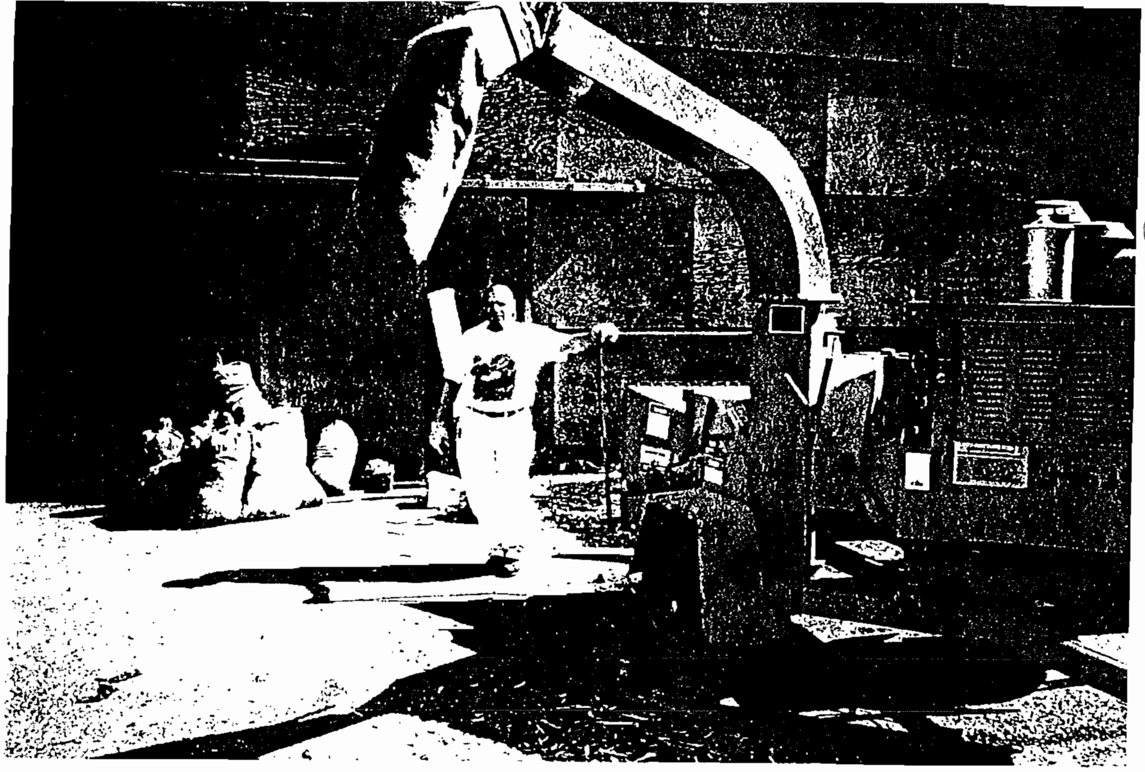
Much of the in-kind costs associated with the project were related to the costs involved in the installation of the equipment and providing the steam, water, supplemental lighting, holding tank and disposal services needed to facilitate operations.

When used for distillation operations the raw materials to be distilled were bagged in burlap bags weighing approximately 15 to 25 pounds each, depending upon the nature of the material being distilled. This was necessary in order to facilitate removal of the distilled materials from the retort after distillation, since the retort needed to be loaded and emptied through the top via the tilting back of a hinged lid.

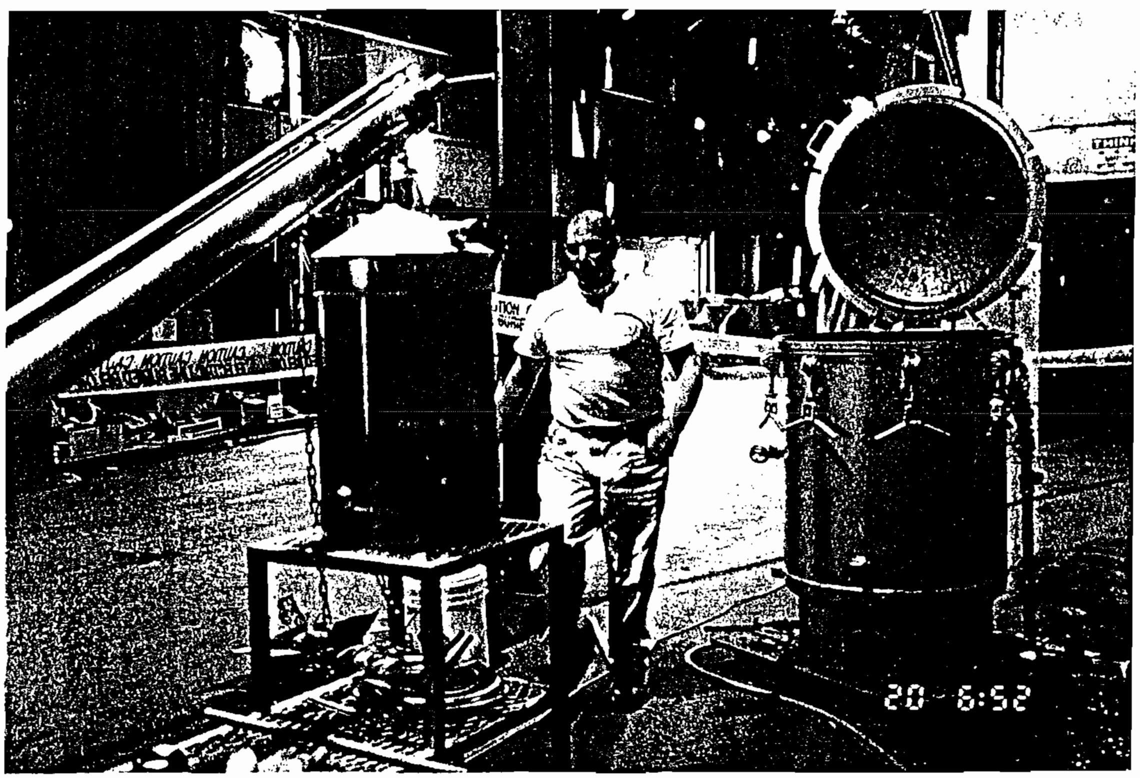
The system operated essentially as a zero pressure system with ambient atmospheric pressure in the retort and condenser. However, there was considerable expertise needed in the management of the volume of steam being applied to the retort during the distillation process, and the amount of cooling water flow to the condenser, in order to balance the throughput of the system such that the condenser was able to effectively condense the quantity of steam coming out of the retort such that there would be no loss of oil through vaporization.

Though the test system was substantial, and was very effective for purposes of this project, it nevertheless was a manually operated system that, when operating, required constant attention and fine tuning by a knowledgeable operator. The Essential Oil Company provided the expertise and the training needed for those involved to effectively run the system and, as time went on, those involved did become more expert in managing the process.

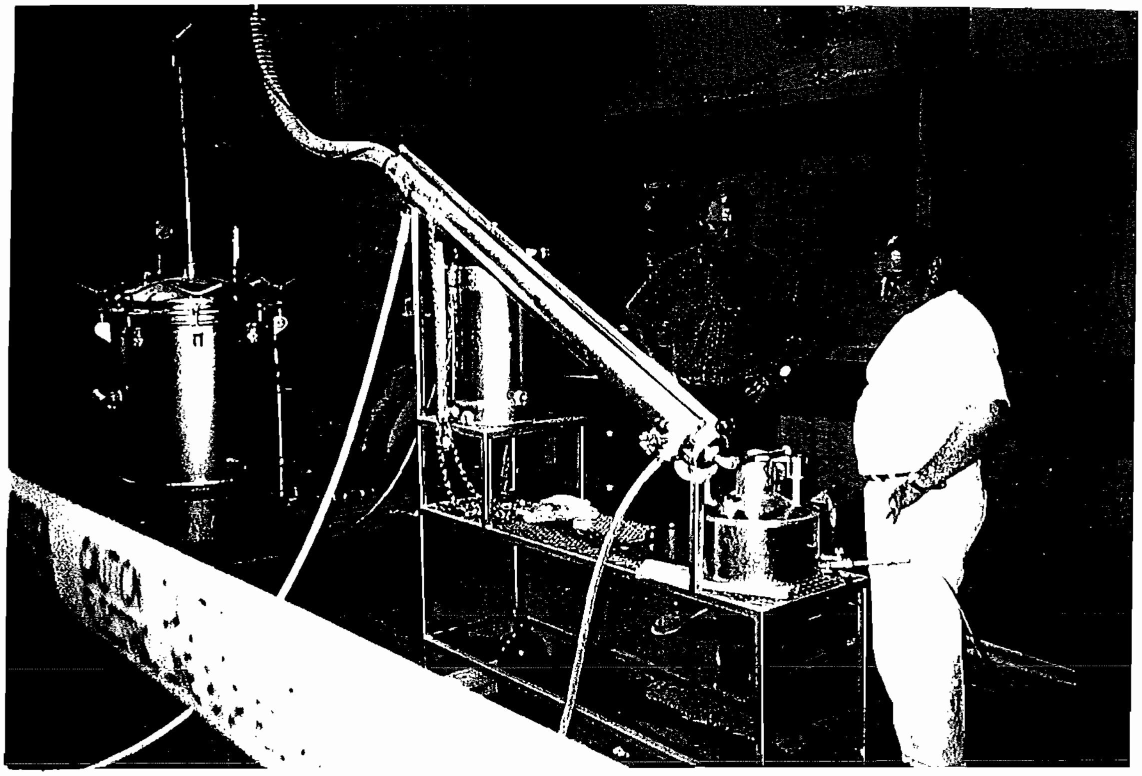
The following pages contain photographs which show the distillation system in place at WSFPI in Warm Springs.



CHIPPING & BAGGING OPERATION

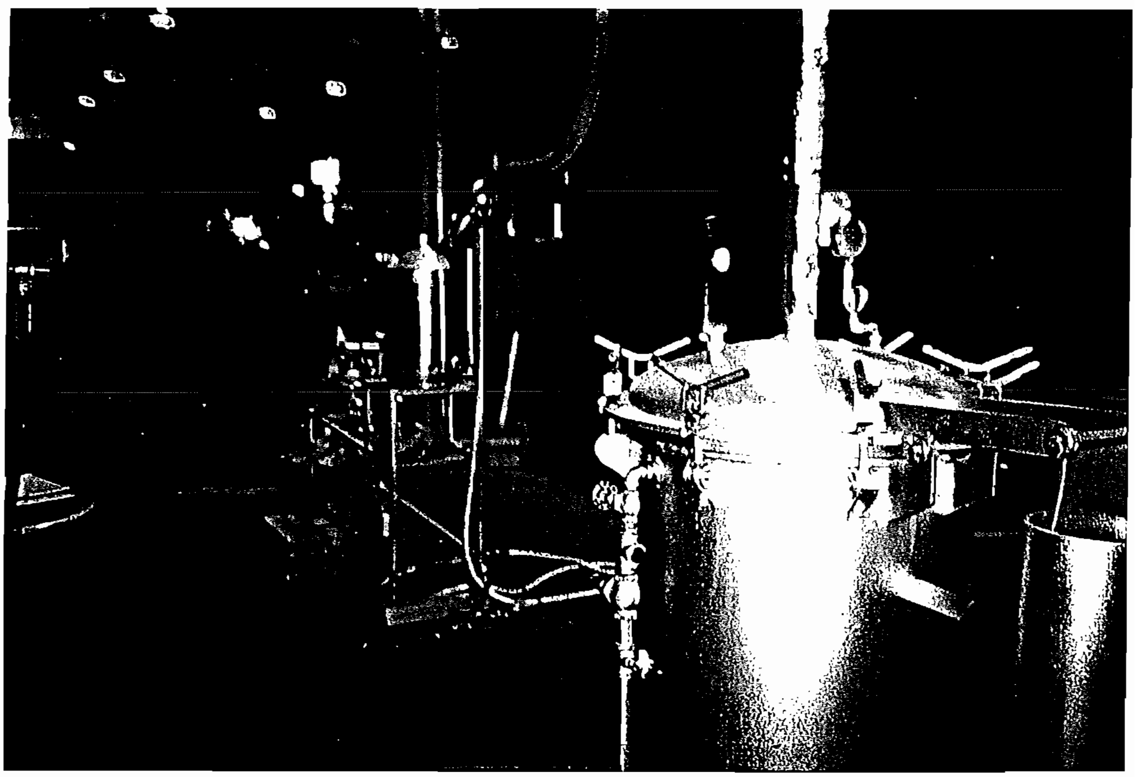



RETORT (OPEN LID) & CONDENSER



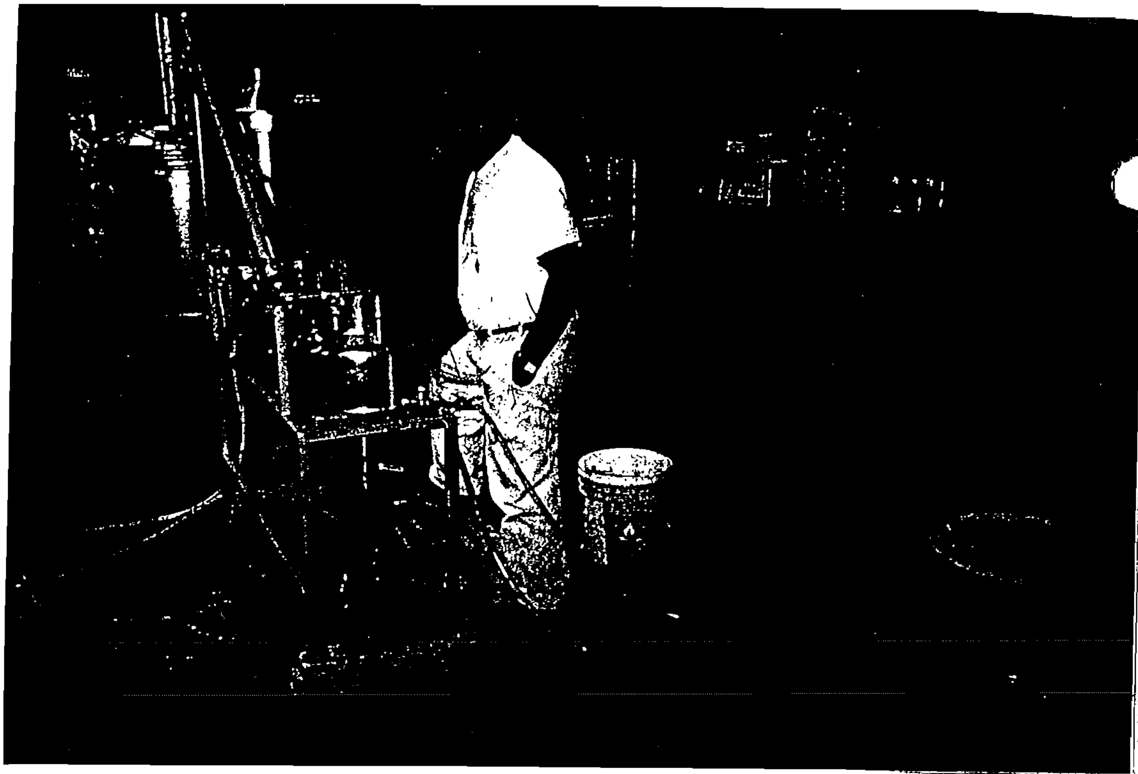
-LEFT TO RIGHT-

RETORT, CONDENSER & SEPARATOR

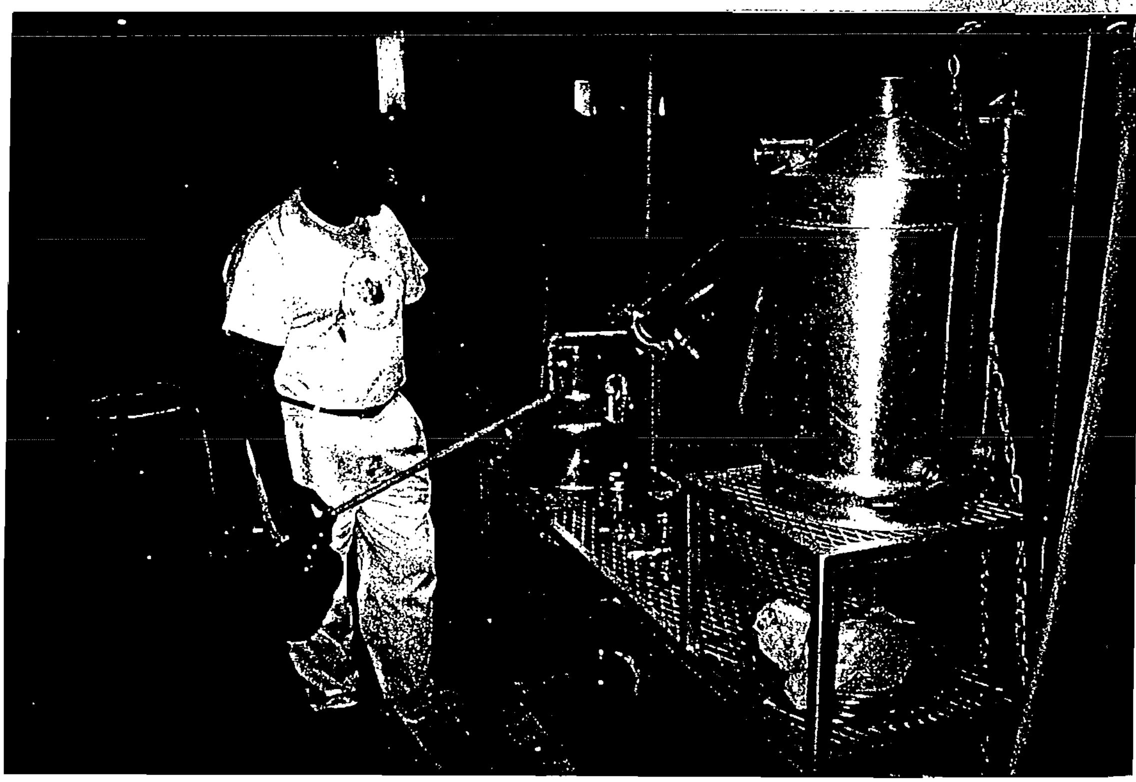


-RETORT (FOREGROUND)

-HOLDING TANK (BACKGROUND)



OPERATING SYSTEM WITH CATCH-DRUM



TAPPING OIL FROM SEPARATOR

## 5.0 Juniper Selection, Harvesting and Processing

**Location** - The Juniper harvest site, as approved by the Tribes' Range and Agriculture Committee, was situated approximately 2 ½ miles S. - S.E. of the Warm Springs community on a terrace, and at an elevation of approximately 2,500 feet, between the Tenino Creek and Seekseequa Creek drainages. The map at the end of this section marks the location of the Juniper harvest area in relation to the town of Warm Springs on the reservation.

**Soil** - The soil in the harvest area consisted primarily of Type I Lavey-Drybed-Madras bounded by Type II Simas-Ruckles-Antoken, which at this location is a very dry, sandy silt and stony loam. The colored map attached to this section is a general soil map for the Warm Springs Reservation, and an associated soil type legend. The location of the Juniper harvest area is marked for reference.

**Tree Density and Ages** - In the Juniper harvest area the density of trees appeared in general to be approximately 12-20 trees per acre with some substantial variance (more or less) depending on the specific location. Sections which seemed to have mostly larger mature trees also seemed to have fewer younger trees around them. The bulk of the geographic area and space was taken up by relatively mature trees, the average of which appeared to be between 50 and 150 years old. During the course of the study the trees that we took down out of that area all fell within that age range. Mature trees were chosen with the assumption that they would provide the best oil yield. Most of the trees harvested and used for purposes of this study were between 75 and 125 years old. The mature trees typically seemed to vary between 20 and 40 feet high, with a substantial variance in the diameter of the foliage canopy, as well as substantial variance in the density of leaf or foliage on the tree. Most trees in the harvest area appeared to be in reasonably good health. During the course of this study none of the trees that were cut down or core-sampled were materially diseased. They all had very solid boles without any material decay in the interior of the bole.

**Tree Selection** - The trees that were chosen for use in this test were clearly the more mature trees, with robust foliage, and preferably of a deeper or darker green color. Some were chosen that were heavily laden with green berries, and some were chosen that were devoid of berries. Given the level of expertise on site we could not effectively determine the sex of the trees. Upon researching into it further it is our understanding that the sex is difficult to determine at any point in time since the tree changes sex periodically as it goes through its berry- producing and non-berry-producing cycle. Most of the trees that were harvested were cored in order to determine their health through the bole into the heart wood, and all samplings showed healthy trees. More specifics on the characteristics of the first six trees harvested for use is as follows:

| **DATA** | **#1** | **#2** | **#3** | **#4** | **#5** | **#6** |
|---|---|---|---|---|---|---|
| • Age (yrs.) | 65 | 135 | 100 | 160 | 100 | 100 |
| • Height (ft.) | 40 ft. | 35 ft. | 25 ft. | 30 ft. | 25 ft. | 30 ft. |
| • Diameter @ Base (in.) | 24" | 30" | 24" | 28" | 20" | 20" |
| • Est. Percent Heart Wood (%) | 50 | 60 | 50-60 | 50-60 | 40-50 | 40-50 |
| • Berries | Sparse | Sparse | Sparse | Sparse | Heavy | Heavy |

**Harvesting** - Harvesting of the tree and its related materials was done by a crew provided by the Natural Resources Branch of The Confederated Tribes. The Natural Resources Branch, for a variety of reasons, including fire danger, insists that work done on the Reservation be done by folks who are authorized, and who have the proper training, safety equipment, and administration. For this reason the project chose to contract with the Natural Resources Branch for the materials harvesting and hauling activities.

The harvesting operation was fairly standard in that the tree was cut down and de-limbed, after which the ends of the branches containing leaf were nipped off and then gathered up into tightly packed burlap bags. Though the process was somewhat labor intensive, this was necessary because of the need for bagged materials for use in the distillation retort so that the waste materials could be readily removed from the retort after distillation. The bole was cut up into manageable logs, and the bagged leaf and the logs were brought back and deposited under cover near the still site at the WSFPI plant. Typically it would take a three man crew essentially a full work day to get to the site, find the tree to be harvested, cut it down, bag 30-35 bags of limbs, cut up the bole, and then transport it all back to the plant at Warm Springs.

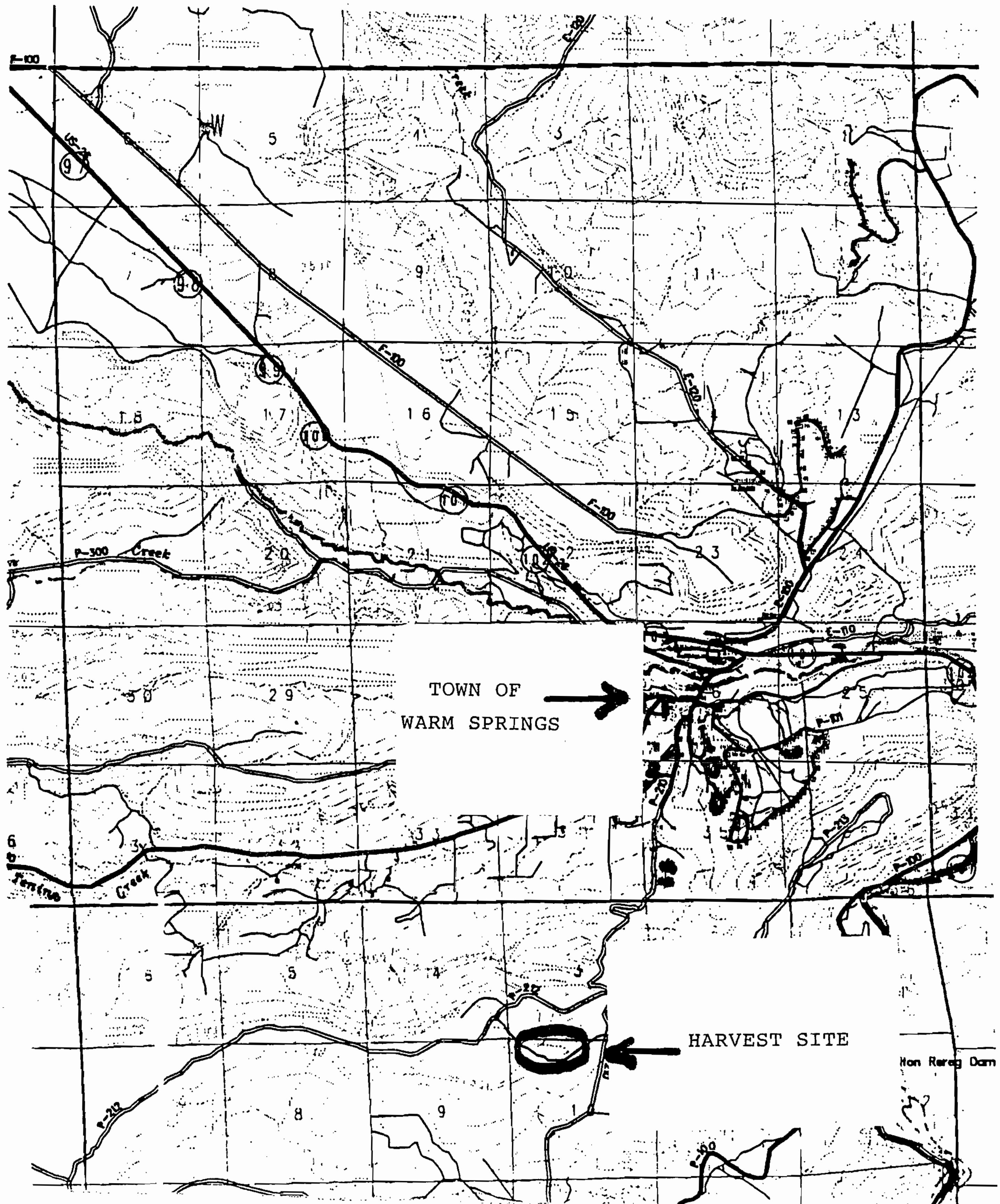
When it was necessary to distill bole wood the logs were split at the distillation site. A rented chipper was brought to the site and the chipping and bagging of the bole was done on site immediately prior to their use in the distillation process. This was necessary because of the tendency of the Juniper to dry very rapidly in the dry Warm Springs climate, particularly during the summer, thereby losing a good deal of its oil and decreasing the yield of the process. In general, it was found that the quicker the Juniper materials were used after harvesting the better the yield, because of this drying and loss factor. More information on this and its effect on the yields is given in the next section on Oil Distillation Process and Results, Section 6.0.

**Waste Disposal** - In keeping with the tribe's Integrated Resource Management Plan for the Reservation, the waste materials from the distillation process were then taken back to the site from which the trees were being harvested, and were distributed across the landscape as ground cover. In this way the spirit of the plan, which calls for the leaving of the branches and boughs at the site of tree harvesting in order to facilitate nutrient restoration into the soils, was accomplished.

See Map T8S R12E

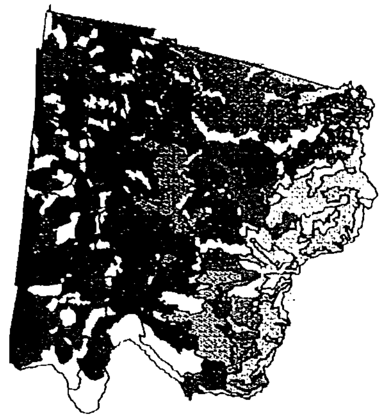
# T9S R12E

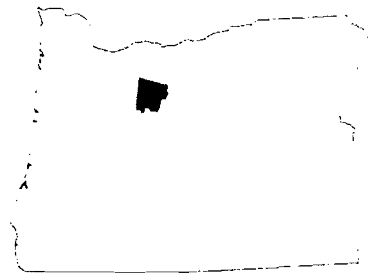
See Map T9S R11E




See T9S R13E

# Warm Springs Indian Reservation





# Legend for General Soil Map

WARM DRY SOILS ON TERRACES

1 Lavey-Drybed-Madras: Moderately deep and very deep, well drained, gravelly silt loams, silt loams and loams that formed in alluvium and loess over colluvium derived from sedimentary rock or tuff.

WARM DRY SOILS ON FOOTHILLS, CANYONS AND MESA

2 Simas-Ruckles-Antoken: Shallow to very deep, well drained, silt loams, very stony loams and extremely cobbly silt loams that formed in residuum and colluvium derived from igneous and sedimentary rock.

3 Bakeoven: Very shallow, well drained, very cobbly loams that formed in residuum derived from igneous rock.

4 Dryhollow: Very deep, well drained loams that formed in residuum and colluvium derived from tephra.

WARM, MOIST SOILS ON CANYONS AND MESAS

5 Kishwalk-Waterbury: Moderately deep and shallow, well drained, very stony loams and extremely stony loams that formed in residuum and colluvium derived from igneous rock.

6 Watama-Rockly: Moderately deep and very shallow, well drained, silt loams and very stony silt loams that formed in residuum derived from igneous rock.

WARM, MOIST SOILS ON FOOTHILLS AND MOUNTAINS

7 Booten-Shiva: Very deep, well drained, loams that formed in residuum and colluvium derived from tephra.

WARM, MOIST SOILS ON OUTWASH PLAINS

8 Tenwalter-Mildam: Shallow and moderately deep, well drained, very cobbly silt loams and silt loams that

9 Prill-Kaskela-Logsprings: Moderately deep to very deep, well drained and moderately well drained, gravelly silty clay loams, clays and loams that formed in residuum and colluvium derived from tuff and sedimentary rock.

10 Mutton-Venator-Oldsferry: Shallow to very deep, well drained gravelly loams and extremely channery loams that formed in colluvium derived from tuff and sedimentary rock.

11 Hehe-Teewee: Moderately deep and deep, well drained, very stony loams and loams that formed in residuum and colluvium derived from igneous rock.

COOL, MOIST SOILS ON MOUNTAINS

12 Smiling-Simnasho-Pipp: Deep and moderately deep, well drained and somewhat excessively drained, sandy loams and very stony sandy loams that formed in residuum and colluvium derived from igneous rock.

COOL, WET SOILS ON MOUNTAINS

13 Hawash-Mackatie: Very deep and deep, somewhat excessively drained and well drained, very cobbly sandy loams and sandy loams that formed in residuum and colluvium derived from igneous rock and volcanic ash.

COLD, WET SOILS ON MOUNTAINS

14 Pinhead: Very deep, somewhat excessively drained, very gravelly sandy loams that formed in residuum and colluvium derived from igneous rock.

15 Jojo-Pinhead: Moderately deep and very deep, somewhat excessively drained, very stony sandy loams and very gravelly sandy loams that formed in residuum and colluvium derived from igneous rock, pyroclastic ash flows, and volcanic ash.

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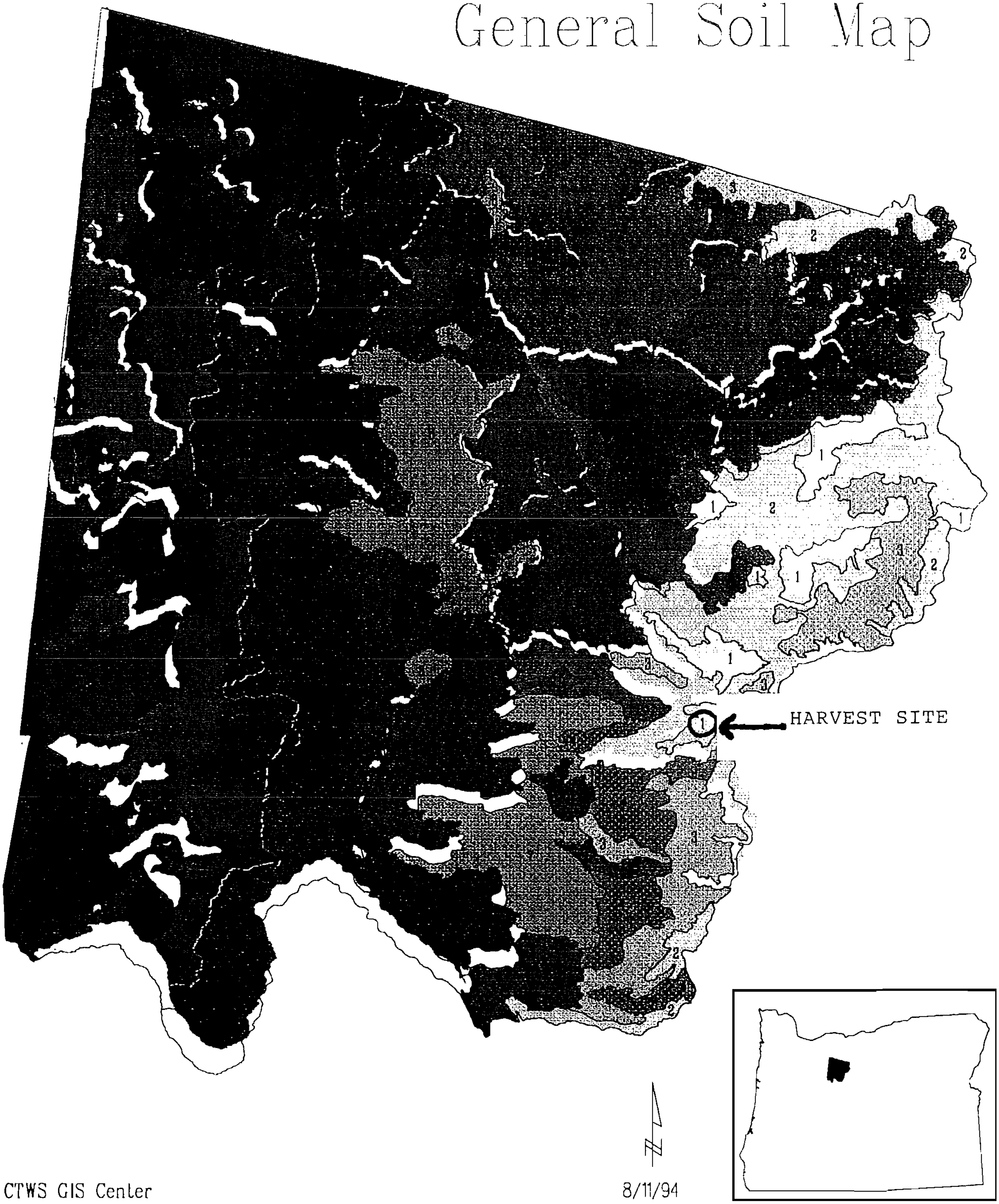
Warm Springs Indian Reservation

# General Soil Map

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## 6.0 Distillation Process and Results

**Materials Preparation** - All the materials to be distilled were placed into sealed burlap bags. The leaf materials were bagged on site at the harvesting area to facilitate their easy transport back to the still site. Bole wood needed for distillation was brought back to the still site in 15" logs where it was chipped and bagged immediately prior to distillation to prevent evaporation losses. As mentioned previously, the bagging was necessary to facilitate the loading of the retort, but most particularly to facilitate the emptying of the retort after the distillation process since the retort was a top-loading device with no facility for removing waste materials out the bottom after the distillation was completed.

After some negative experience, distillation was scheduled either the afternoon of the same day of the harvesting, or the following day, in order to minimize oil loss through evaporation. Information given later in this section will show some of the effects of the loss of oil on yield because of evaporation in the hot and dry summer climate at Warm Springs. When not able to immediately utilize the distillation materials, they were brought inside and kept under cover (out of the sun) until it was time for them to be used. If the materials were left over night they would be wet down with cooling water, or covered with extra burlap bags (also wet), to facilitate evaporative cooling minimizing the oil loss. After the distillation process was completed the bags were removed from the retort with the aid of a long fisherman's gaff because of the high temperature of the materials after distillation. The rate of drying of the materials in the summer climate at Warm Springs was absolutely astounding. We did not make accurate measurements of what the weight loss would be for both the leaf and the logs on a daily or weekly basis, but the weight change of the materials was easily noticeable, and attendant oil loss was reflected in the yields.

This raises the issue of whether or not the materials could be dried in a more controlled environment that would allow water to evaporate from the materials while still maintaining the oil content, in order to give a higher yield for a given weight of materials. We were not able to pursue this within the scope of this project but it would be worth investigating by anyone who might consider getting into the Juniper oil distillation business in the future.

**<u>Still Preparation</u>** - Prior to starting the distillation process it was necessary to purge the steam and water lines in order to insure that clear steam and clean water to the system. This was typically the first step in preparing for a distillation run. Next, the materials in the burlap bags were loaded into the retort and packed in as tightly as possible in order to provide maximum biomass in the retort for the distillation run. When loading the retort with bagged leaf materials in their natural state, the retort would typically hold between 200 and 225 pounds of the material, depending upon the degree of care taken in loading the burlap bags, and in packing the materials tightly in the retort. The same consideration was true when bagging the chipped bole wood, however the velocity of the chip flow out of the chipper would usually insure a fairly tight packing of materials in the burlap bags. Therefore a charge of bole wood in the retort would typically weigh 225 to 275 pounds, on average about 25 or 30 pounds more than if the retort were charged with leaf material.

Prior to loading the retort we found it desirable to preheat the system, particularly the retort, in order to minimize cooking times once the materials were in place. The retort was uninsulated and therefore was subject to considerable heat loss, depending on the ambient air temperature, and wind velocity, on that particular day. To minimize heat loss in the system the retort transfer pipe was insulated, and that seemed to give a more consistent cooking process.

Once fully loaded with the retort lid down and securely fastened, and all other system components properly connected, the steam and cooling water were slowly applied and increased in flow. The optimum point that was being searched for in this process is the point at which there was enough steam passing through the system to heat the materials as rapidly as possible, without spilling out <u>as steam</u> from the condenser bleed-off tube. When that happened it was necessary to either cut back a little bit on the steam or to increase the cooling water flow to the condenser, and this balancing process was the one that it took some experience to master.

During the cooking process, a certain amount of condensate from the steam accumulated in the bottom of the retort. There is a valved tap-off to allow this water to run off. The amount of water in the retort was managed to see whether maintaining <u>some</u> water in the bottom would keep the retort at a more even temperature and therefore increase the rate of oil production. However, it was found that it did not seem to make a material difference in yield whether the water was allowed to accumulate in the bottom of the retort or whether it was allowed to just run.

In the system that was used for test purposes on this project the key system limitation was the size of the condenser. Only a fraction of the steam available (perhaps a 25 psi flow) was utilized before we over-drove the condenser and allowed the live steam to escape from the condenser relief tube, thereby causing a loss in oil yield since the escaping steam would carry its attendant oil along with it. There was plenty of cooling water with which to cool a larger condenser. Therefore, with a larger condenser, one could apply more steam to the retort thereby potentially increasing the rate of oil production and certainly the oil yield for any given period of time.

Typically, once experience was gained with this particular system as to how to bring the retort up to cooking temperature of 210° F., oil production began between 30 minutes and one hour after applying steam, depending upon the type and weight of the materials packed into the retort.

On some of the distillation runs the oil production was measured relative to the cooking time and the results of these measurements are given in the yield tables, following.

**Oil Take Off** - As the system approached cooking temperature (210° F.) steam from the retort would begin to pass through to the condenser and be condensed into a liquid distillate containing both oil and associated waters. This liquid distillate was gravity-fed into a separator which allowed the oil to rise to the top of a separate section of the container, at which point there was a valved take-off outlet from which the oil could be drained off. The run-off waters from the bottom of the separator were then allowed to gravity-drain into the 20-gallon catch drum, from which the waters were periodically pumped into the 400 gallon holding tank. It is important to note that those run off waters were produced at the rate of approximately 300 to 350 gallons of run-off waters for each gallon of oil produced. This is important because in further work on the project, and further market research, it was determined that the waters may have some useful applications, and may have a substantial market value.

**Yield Results** - Following is a table which shows the oil yield from the various batches of materials that were distilled showing the type and weight of materials, the distillation time, the oil yield and any other pertinent information relative to the distillation of that particular batch such as the time between harvesting of the materials and the cooking of the materials. Copies of the Distillation Record for each batch distilled are included in Appendix "B".

## Distillation Table

### Juniper Leaf

| • Batch # | #1 | #2 | #3 | #4 | #7 |
|---|---|---|---|---|---|
| • Batch Weight (Lbs.) | 222# | 216# | 204# | 178# | 252# |
| • Cook Time (hrs.) | 3.0 | 1.0 | 2.5 | 2.5 | 2.0 |
| • Oil Yield (grams) | 325.5 g. | 44.4 g. | 157.2 g. | 197.1 g. | 287.7 g. |
| • Percent Yield (by weight) | 0.32% | 0.045% | 0.170% | 0.244% | 0.250% |
| • Days Since Harvesting | 1 | 5 | 1 | 1 | 1 |
| • Berry Amount | Sparse | Sparse | Heavy Immature | Sparse | Sparse |

## Distillation Table

### Juniper Bole Wood

| • Batch # | #5 | #6 | #8 |
|---|---|---|---|
| • Batch Weight (lbs.) | 257# | 224# | 222% |
| • Cook Time (hrs.) | 4.25 | 2.5 | 3.0 |
| • Oil Yield (grams) | 202.6 g. | 65.8 g. | 175.5 g. |
| • Percent Yield (%) | 0.174% | 0.065% | 0.174% |
| • Notes | Chipped: 4 days prior | Chipped: 4 days prior | Chipped: 1 day prior |

Though a measure of oil yield as a function of time was not rigorously pursued, a few batches were measured to determine oil yield during the cooking process. The results are given in the following table.

## Distillation Table
## Timed Yield Data: Grams of Oil

| Batch # | First Hour | Second Hour | Third Hour | Fourth Hour | Total Grams |
|---|---|---|---|---|---|
| #5 (Bole) | 123.0 g. | 36.5 g. | 35.1 g. | 8.0 g. | 202.6 g |
| #7 (Leaf) | 211.5 g. | 76.4 g. | -- | -- | 287.7 g. |
| #8 (Bole) | N/A | 135.0 g. | 40.5 g. | -- | 175.5 g. |

This data, along with information and observations obtained during the distillation of all batches, generally confirmed the earlier test distillation work done by Kurth and Ross of Oregon State University$_{(1)}$ as follows:

- Two-thirds of the recoverable oil will be obtained during the first two hours of cooking.
- A cooking time of 3-4 hours will yield 80% - 90% of the recoverable oil.
- Cooking longer than 4 hours will only increase oil yield a few percent per hour, and is not economically viable.

For clarity, it should be noted that "cooking time" is measured from when liquid distillate first begins to pass through the condenser, and does not include the time required to heat the retort and distillation materials to cooking temperature. Distillations performed on this particular system usually resulted in a first pass-through of distillate out of the condenser when the retort temperature reached 195° - 200° F. This usually occurred 30 min. - 60 min. after steam was applied to the retort, and varied mostly with the amount of pre-heating done to the system before the cooking process was begun.

Subsequent to the test distillations done in June, July and August, additional batches were distilled in both October and November to provide additional oil and waters at the request of potential customers. It was interesting to note that the oil yield from these batches (leaf; done from trees that had a minimum of immature berries) was substantially greater than from the batches distilled in late spring and early summer. Following is a Distillation Table which shows the distillation results of these later batches.

## Distillation Table

| **Batch #** | **#9** | **#10** | **#11** | **#12** | **#13** |
|---|---|---|---|---|---|
| Material | Leaf | Leaf | Leaf | Leaf | Wood |
| Batch Weight (lbs.) | 265# | 276# | 189# | 166# | 181# |
| Cook Time (hrs.) | 4.0 | 4.0 | 3.0 | 2.5 | 3.25 |
| Oil Yield (grams) | 550 g. | 550 g. | 322 g. | 261 g. | 354 g. |
| Percent Yield (by weight) | 0.46% | 0.44% | 0.375% | 0.346% | 0.42% |

Though this data is scientifically inconclusive, it has resulted in speculation that there is a significant seasonal variation in the oil content of the leaf. Minimum oil when the tree is in the peak of its growth season; maximum oil as the tree approaches dormancy. If true, this act could have a significant impact on the economics of a potential oil-based business, and is an area fur further research. Another possible explanation is that, in the hot, dry summer weather of Central Oregon, the oil evaporates out of the leaf material faster than the tree can replace it.

It is also important to note one change from the original goals of the project: no meaningful work was done on the distillation of dried Juniper berries. This task was eliminated because of the following factors:

- Our experience showed that, at Warm Springs, the Juniper loses much of its oil as it dries.

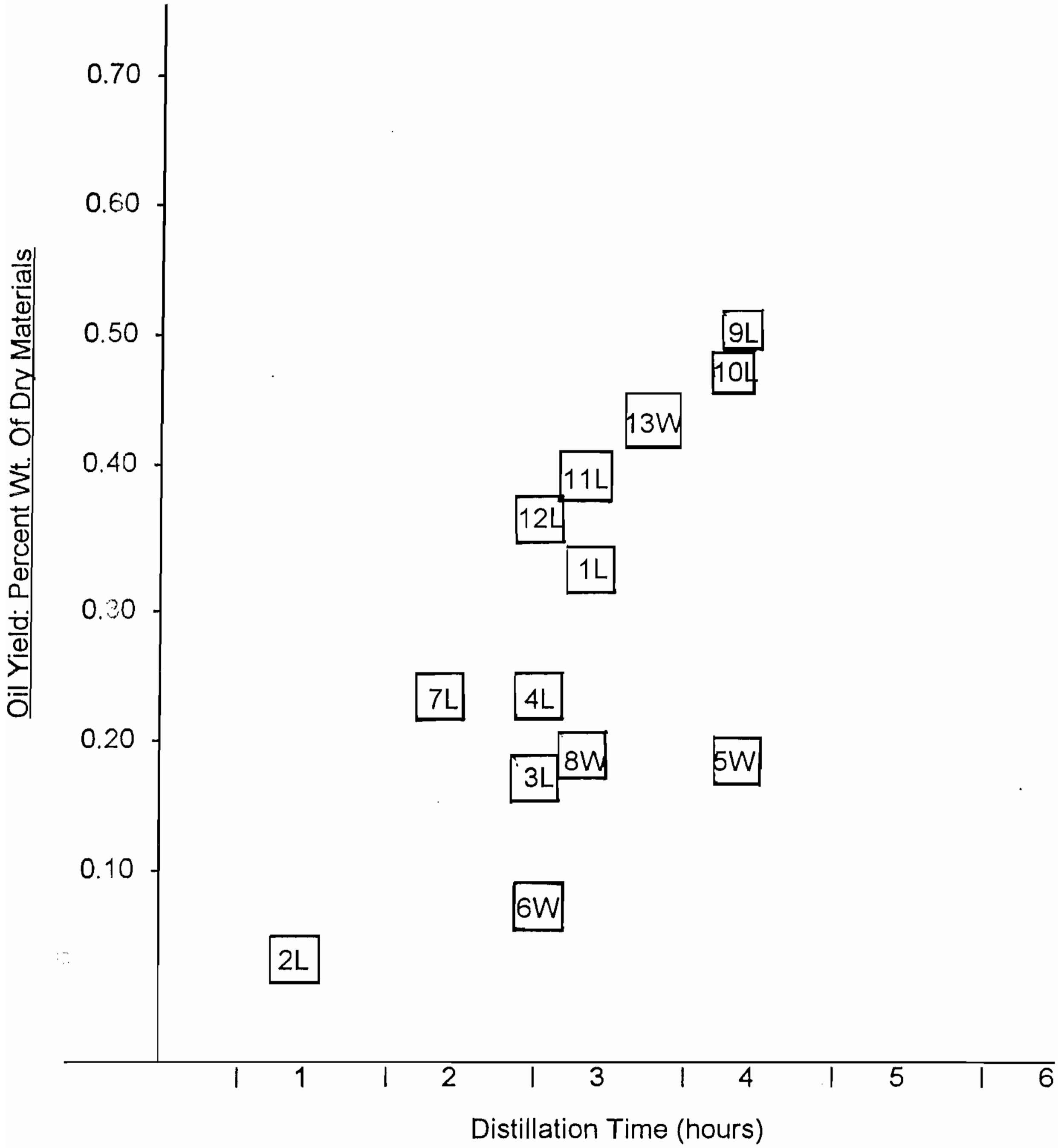
- Green berries, though heavy, contain little oil.

- Few dried berries were visible on he ground or on the trees throughout the period of this project. Our speculation was that they are simply consumed by the local birds and animals.

In a climate different from Warm Springs the dry berries may be more recoverable. However, in the interest of achieving the primary objectives of this project, a judgement was made that the dried berries were not a viable, attractive business resource.

The following Yield vs. Distillation Time chart maps the results of all distillation batches. Note that the highest yields were from the first batch (done in June), and from the last five batches done in October and November. The anomaly of the yield from the first batch (1L) can not readily be explained. Later experience led us to believe that the extraordinarily low yield from leaf batch two (2L) was due to oil loss from drying since the batch was not cooked until about a week after it was harvested, though it was kept under cover during that time. Interestingly, Kurth & Ross$_{(1)}$ also had some unexplained low yields in their work.

**Yield vs. Distillation Time**




L = Leaf
W = Bole Wood

**Comparison with Previous Work** - The report published by Kurth and Ross, Oregon State College, No. C-3, dated 9154 (included in the Bibliography as Exhibit 1) provided the most useable benchmark information with which to compare the resutls obtained in this project. Key comparative observations are as follows:

a.) Though they had a wide range of yields over 30 test distillations (0.24% - 1.82%) their yields were, on average, substantially higher than those of this project (six batches with yield between 0.30% and 0.45%).

b.) Their higher yields were produced in a pressure apparatus (20 psi - 60 psi). This project's test still was a zero-pressure system.

c.) They were using "dry" materials, though no specific information is given in the report on their drying process. During the test proejct it was found that air-drying in the Warm Springs climate (June - Sept.) Materially reduced yield.

d.) They obtained higher yield from materials that were processed to give a smaller particle size than was used in this project.

e.) Their batch sizes, typically 20# - 40# (dry weight) per distillation batch, were much smaller than the average 200# - 225# batches (wet weight) distilled during the project. This raises the issue of wehther larger batches need to be distilled under a different presusre/temprature/time profile in order to maximize yield, because of the nature of the materials.

**Chemical Analyses** - Following are example pages from a gas chromatography analysis report on Juniper leaf oil from batch number WS1JL. The analysis was done by Flora Research of San Juan Capistrano, California, a firm that specializes in gas chromatography analysis of essential oils. Included with the data are graphs that show the relative magnitude of the different chemicals contained in the oil.

Complete copies of all gas chromatography analyses reports are included in Appendix "C" for reference, and includes an analysis of the distillate "waters".

Some of the relevant conclusions from an analysis of the chemical content of the oil are:

a.) It is a colorless or pale greenish-yellowish liquid with a characteristic fresh-basalmic odor and bitter burning taste. Like all Essential oils it must be stored in a cool place, in airtight containers and protected from light.

b.) The oil has more than 70 isolated components. It contains the terpene, pinene, sabinene, limonene (a terpene hydrocarbon) and terpinen-4-ol. The last constituent is said to cause the diuretic action which is mainly loss of water not sodium ions. It does not irritate the tissues in contrast to other terpenes. Juniper also contains borneol, geraniol, and other sesquiterpenes, phenols and esters. The monoterpenes are the main components and account for the antiseptic and antiviral action.

c.) The distillate waters have a high content of boryl acetate and caryophyllene.

d.) The oil is somewhat unique in that its aroma is very similar to the aroma of the living tree.

e.) In the oil that was produced from leaf that was heavily laden with berries, there was elevated sabinene levels (batch number WS3JLB).

f.) The Juniper oil is non-toxic at low dose and non-sensitizing, but irritation may occur on sensitive skin.

g.) The oil can be ingested in moderate doses orally, however, it is not to be used during pregnancy or breast feeding. Juniper is a stimulating diuretic and is not prescribed whenever there is kidney inflamation or irritation. Use for a maximum of four weeks at any one time. Resume administration if indicated after four weeks without use. Prolonged or excessive use can result in renal damage.

# FLORA RESEARCH

## CERTIFICATE OF ANALYSIS

Date: 03-July-1996
Client:Essential Oil Co.

Sample I. D. : Juniperus occidentalis
Description:

The sample was received in a glass 5 ml. vial It was labelled with a label identifying it as Juniper leaf ws 1 jl.

Appearance:

Physical appearance is a clear oil with characteristic smell of juniper/pine oil.

Method of analysis:

High Resolution Capillary Gas Chromatography using dual channel analysis.

Results of analysis:

The analysis reveals an oil rich in monoterpines and bornyl acetate. The oil is unique in its chemistry and offers aroma chemicals similar to both pine oil and juniper.

**I hereby certify that I am an authorized representative of Flora Research and that to the best of my knowledge the following information is true and accurate.**

**Signature:** [signature]

**Title:** **chromatographer** **Date:** Jul 3, 1996

Joe Yesendski
4180 NW Carlton Ct.
Portland 97229

**31921 Camino Capistrano 435 • San Juan Capistrano, CA 92675 • 714.496.8242**

Title :
Run File : C:\STAR\MODULE16\JUNIP001.RUN
Method File : c:\star\juniperm.mth
Sample ID : warm springs.eo

Injection Date: 1-JUL-96 12:23 PM Calculation Date: 3-JUL-96 9:43 AM

Operator : Flora Research jk
Workstation: FLORA
Instrument : Varian Star #1
Channel : B = wax

Detector Type: ADCB (10 Volts)
Bus Address : 16
Sample Rate : 20.00 Hz
Run Time : 60.001 min

*********** Star Chromatography Workstation ******* Version 4.5 ***************

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

| Peak No. | Peak Name | Result () | Ret. Time (min) | Time Offset (min) | Area (counts) | Sep. Code | Width 1/2 (sec) | Status Codes |
|---|---|---|---|---|---|---|---|---|
| 1 | a-pinene | 7.7650 | 3.482 | -0.058 | 2036757 | VV | 1.9 | R |
| 2 | camphene | 1.2072 | 3.919 | 0.039 | 316635 | VV | 1.8 | |
| 3 | B-pinene | 0.4082 | 4.432 | -0.002 | 107069 | VV | 2.2 | |
| 4 | sabinene | 9.7478 | 4.609 | 0.007 | 2556824 | VV | 2.4 | |
| 5 | myrcene | 1.8181 | 5.160 | 0.002 | 476896 | VV | 2.3 | |
| 6 | a-Phellandre | 1.8693 | 5.302 | 0.059 | 490317 | VV | 2.3 | |
| 7 | a-terpinene | 3.0625 | 5.579 | 0.039 | 803281 | VV | 2.5 | |
| 8 | limonene | 4.7498 | 5.981 | -0.060 | 1245860 | VV | 2.9 | R |
| 9 | B-phellandre | 4.3870 | 6.205 | 0.018 | 1150714 | VV | 2.8 | |
| 10 | 1,8 cineol | 0.0072 | 6.292 | 0.029 | 1889 | TS | 0.0 | |
| 11 | g-terpinene | 4.7289 | 7.069 | 0.048 | 1240386 | VV | 3.5 | |
| 12 | p-cymene | 13.5315 | 7.733 | 0.098 | 3549282 | VV | 4.6 | |
| 13 | linalool | 0.4093 | 18.961 | -0.008 | 107361 | VV | 4.6 | |
| 14 | Bornyl aceta | 16.3720 | 20.604 | 0.117 | 4294347 | VV | 10.7 | |
| 15 | B-cary/T-4-o | 7.5964 | 21.685 | -0.118 | 1992517 | VV | 6.8 | R |
| 16 | borneol | 3.1887 | 26.387 | -0.404 | 836385 | VV | 6.5 | R |
| 17 | citronellol | 0.0561 | 29.791 | -0.192 | 14705 | TF | 0.0 | |
| | Totals: | 80.9050 | | -0.386 | 21221225 | | | |

Status Codes:
R - Reference peak

Total Unidentified Counts : 5008638 counts

Detected Peaks: 279 Rejected Peaks: 44 Identified Peaks: 17

Multiplier: 1 Divisor: 1

Baseline Offset: -3 microVolts

Noise (used): 290 microVolts - monitored before this run

Manual injection

Revision Log:

1-JUL-96 1:23 PM: Calculated results from channel B using method:
'C:\STAR\JUNIPERM.MTH'
2-JUL-96 10:07 AM: Calculated results from channel B using method:
'c:\star\juniperm.mth'
2-JUL-96 10:08 AM: Calculated results from channel B using method:
'c:\star\juniperm.mth'
2-JUL-96 10:09 AM: Calculated results from channel B using method:
'c:\star\juniperm.mth'
3-JUL-96 9:22 AM: Calculated results from channel B using method:
'c:\star\juniperm.mth'
3-JUL-96 9:22 AM: Calculated results from channel B using method:
'c:\star\juniperm.mth'
3-JUL-96 9:43 AM: Calculated results from channel B using method:
'c:\star\juniperm.mth'

Original Notes:

Warm Springs Juniper Project Sample WS 1 JL Juniper leaf distilled June 6, 1996.

Appended Notes:

****************************************************************************************

```
Title          :
Run File       : C:\STAR\MODULE16\JUNIP001.RUN
Method File    : c:\star\juniperm.mth
Sample ID      : warm springs.eo

Injection Date: 1-JUL-96 12:23 PM     Calculation Date: 3-JUL-96 9:43 AM

Operator     : Flora Research jk        Detector Type: ADCB (10 Volts)
Workstation: FLORA                      Bus Address   : 16
Instrument : Varian Star #1             Sample Rate   : 20.00 Hz
Channel      : B = wax                  Run Time      : 60.001 min

*********** Star Chromatography Workstation ******* Version 4.5 ***************

Chart Speed =    0.75 cm/min   Attenuation = 256          Zero Offset = 5%
Start Time  =   0.000   min     End Time    = 60.001  min   Min / Tick  = 1.00
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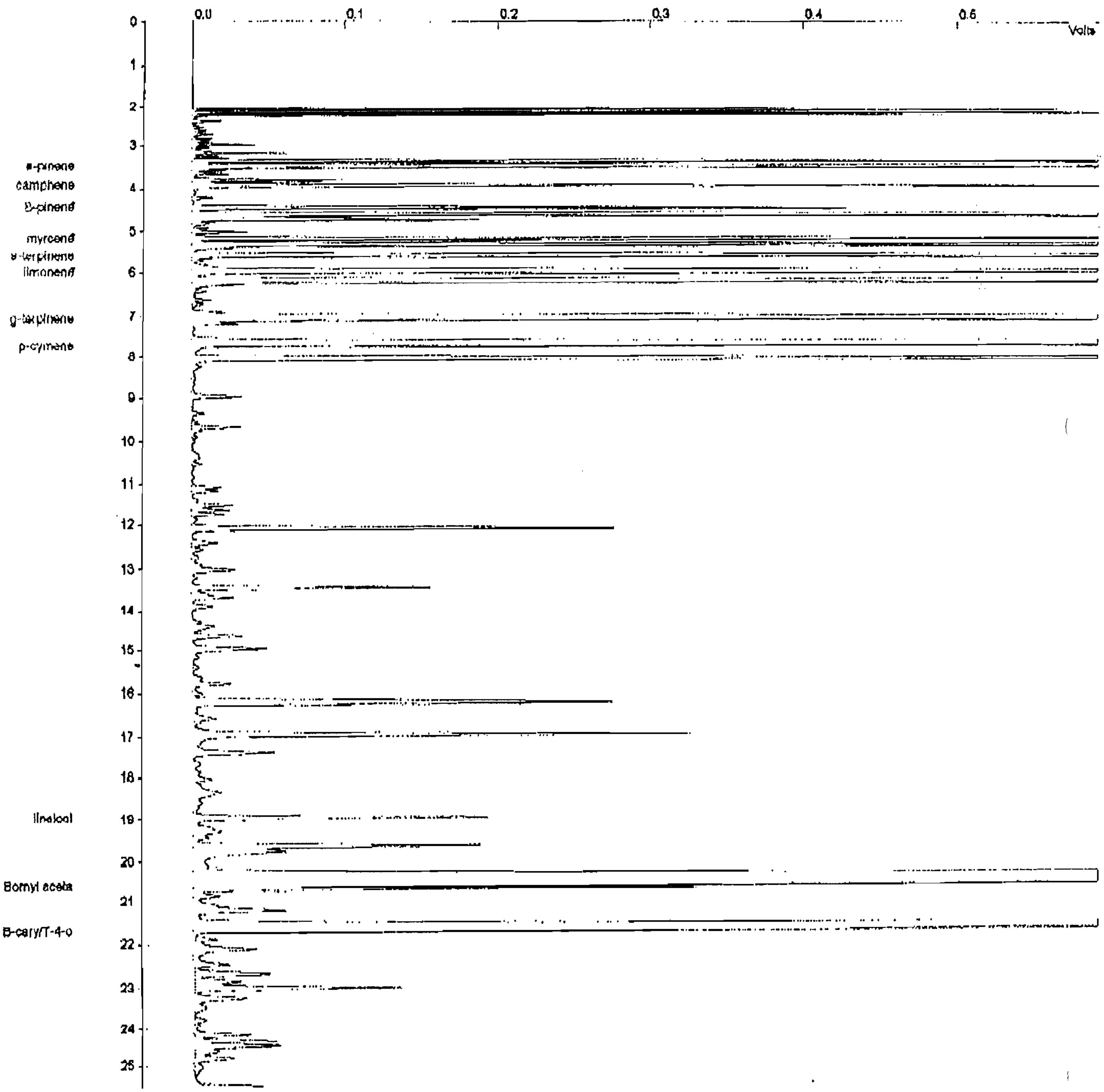


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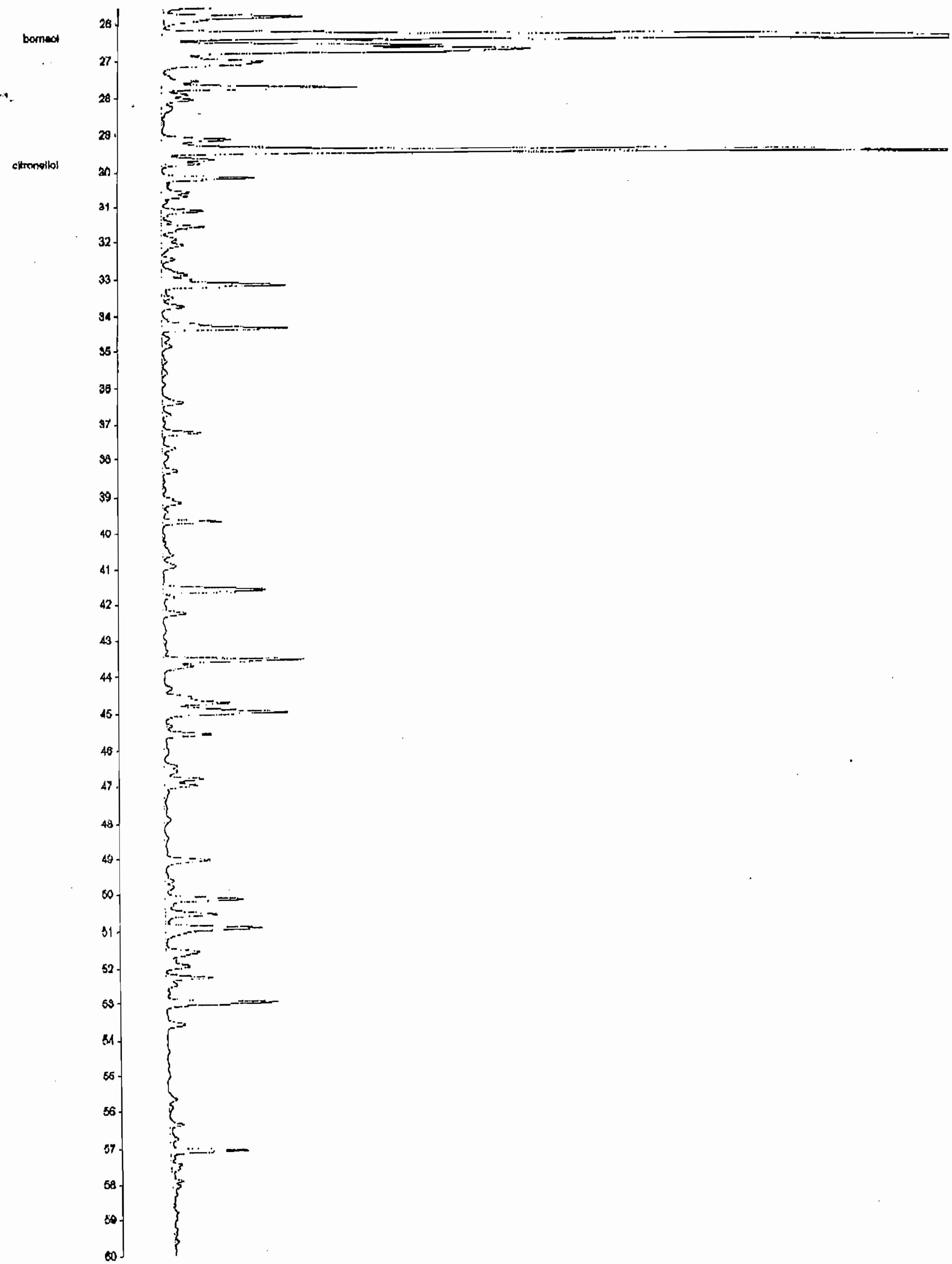
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## Summary: Observations and Conclusions

Project experience with the harvesting, preparation and distillation of Western Juniper materials has resulted in the following broad conclusions, with a high degree of confidence:

- All mature, reasonably healthy trees are candidates for use in an oil extraction process.

- Materials preparation (chipping, chopping, etc.) Is best accomplished at the harvest site.

- The finer the materials are prepared, the quicker oil can be recovered from them in the distillation process. Recommend that bole wood be chipped and leaf materials chopped in a commercial process.

- However, Western Juniper dries quickly and loses significant oil in the process. Recommend that the materials be distilled as soon as possible after harvesting, preferably within 1-2 days in order to obtain maximum yield. Unused materials should be kept in a cool, damp environment to inhibit evaporation of the oil.

- Oil yield by weight from fresh leaf materials will typically be 50% greater than from fresh whole bole materials.

- Trees heavily laden with immature berries will yield significantly less oil from the leaf materials, as compared to leaf from trees which are sparsely berried.

- Maximum economic distillation time is 3-4 hours, depending on the power of steam/retort/condenser equation. Properly tuned, a distillation system should yield 80 oil recovery in three hours.

- From an economic standpoint, distillation of leaf materials seems best because of the higher oil yield; because the materials take less pre-processing; and because the market value is higher.

- When distilling fresh leaf materials from mature trees; in a properly operated zero-pressure system, expect a minimum oil yield of 16 fluid ounces of raw oil from 250 pounds of leaf materials (minimum 128 ounces -- one gallon -- per ton of leaf materials), giving a yield of 0.4%.

- Run-off distillate "waters" should be captured and held for sale. The distillation process produced 300-350 gallons of waters for each gallon of oil produced. There should be a market for these waters.

- With finely chipped or chopped materials, it is possible to pack them too tightly into the retort, thereby resulting in poor steam infiltration away from the steam source.

- The optimum distillation system needs to have matched components for maximum thruput, meaning that the retort, condenser, separator, and steam and water supplies need to be matched in capacity in order to facilitate the maximum yield of oil out of the materials with minimum cooking time.

- Overall, there was much more to be learned about the materials and distillation process than was originally envisioned, and the yields were much lower than expected. Therefore, twice the amount of distillations were required than was originally anticipated.

- Referencing the aforementioned results obtained by Kurth and Ross, three areas of process improvement may be important to providing greater product yield, which is the ultimate key to an oil-based economic business activity:

  a.) Use of a pressurized distillation system (20 psi - 40 psi) may significantly increase oil yield.

  b.) Proper materials drying, such that oil is not lost in the process, may increase yield.

  c.) Reducing the materials to smaller particle size with chopping, hammer-milling, etc., may increase yield.

## 7.0 Production Cost Analysis

As mentioned previously, the distillation unit used for this test project was only capable of handling approximately 250 pounds of materials at a time. Though a distillation system of this size may be adequate for an entrepreneur or small business that is seeking to serve consumer markets with specialty oil products, a larger system, capable of handling a ton (2,000 pounds) of distillation material at a time, would be essential if oil were being produced for the commercial (bulk) oils market.

Based on the experience of this project, and making logical extrapolations for supplying a distillation system of commercial size, the following assumptions are relevant:

- One mature Western Juniper tree will yield 500 pounds of leaf materials and approximately 1,000 pounds of whole bole wood.
- Oil yield from the leaf material and bole wood will be 16 fluid ounces (one pound) from each 250 pounds of material, or one gallon (8 pounds) per ton of material (0.40% yield).
- Oil yield from whole bold wood (chipped) would be 10-12 fluid ounces per 250 pounds of material, or 80 fluid ounces (5 pounds) per ton of material. We would expect that distilling the wood in a pressure system would result in an oil yield equivalent to leaf material.
- Materials for distillation to be prepared (chipped, chopped, etc.) at the harvest site and transported to the still ready for use. Leaf chopped. Bole wood chipped.
- Regardless of the size of the distillation unit, two batches could be processed each day (4 hours per batch), including loading, unloading and cooking time.
- The distillation unit can be handled by one operator.
- A two-man crew can harvest and prepare 6 mature trees per day, with the proper equipment on site, for a commercial operation. This assumes a moveable retort (on wheels) can be brought to the site and loaded there. Assume that the small still (retort) is not moveable.

- A two-man crew can harvest and prepare 4 Juniper trees for use in small-still operations.

- Labor cost is $120 per man day ($15 per labor hour).

- Amortized distillation unit costs are based on a small-still cost of $5,000, and a commercial still cost of $20,000.

- Cost of operating supplies and fuel would be the same regardless of still size, for any unit weight of material processed.

- The market price of wood oil is $7.50 per pound ($60.00 per gallon) bulk.

- The market price of leaf oil is $60.00 per pound ($480.00 per gallon) bulk.

Based on these assumptions, following is a comparative cost model which estimates the cost of processing 2,000 pounds of chopped leaf materials.

## Cost Analysis
## 2000 Pounds Juniper Leaf Distillation

| | 250# Capacity | 2000# Capacity |
|---|---|---|
| Materials Weight | 2000# | 2000# |
| Man-Days Needed to Harvest | 2 | 1 1/3 |
| Cost of Trees | -0- | -0- |
| Retort Batches Needed | 8 | 1 |
| COSTS: | | |
| Harvesting Costs | | |
| - Labor | $240.00 | $160.00 |
| - Amortized Equipment and Transportation | 75.00 | 50.00 |
| Distillation Costs | | |
| - Labor | 480.00 | 60.00 |
| - Amortized Still Cost | 80.00 | 40.00 |
| - Operating Supplies and Fuel | 50.00 | 50.00 |
| Total Product Cost | $925.00 | $360.00 |

**Yield and Commercial Value:**

| | Wood Oil | Leaf Oil |
|---|---|---|
| Oil Yield - One (1) gallon (8 pounds); Commercial value | $ 60.00 | $480.00 |
| Water Yield - 300 gallons; Commercial value @ $1.00 per gallon | 300.00 | 300.00 |
| Potential Commercial Product Value | $360.00 | $780.00 |

The obvious points of interest in this analysis are that:

a.) Small scale operations are not economically viable in providing product to the commercial (bulk wholesale) market.

b.) Even on a large-scale commercial basis, economic viability is dependent upon developing the market for the distillate waters.

c.) The distillate waters from the wood distillations will probably have substantially more commercial value per unit of oil obtained than the oil itself, once the market for the waters is developed.

The key determinant in this economic equation is, of course, the relatively low yield (0.40% - 0.45% under optimum conditions) of oil from the Western Juniper leaf materials in a zero-pressure system as was used for this project.

The economic profile for the distillation of wood oil would be even more unfavorable since:

a.) The bole wood would require additional processing (splitting and chipping).

b.) Oil yield from bole wood would only average 0.75 times that of the leaf (6.0 pounds of oil per ton of material).

c.) The current market price for equivalent competitive wood oil (Eastern Red Cedar-Juniperus Virginiana) has a current commercial market price of only $7.50 per pound ($60.00 per gallon).

d.) Oil yield from the wood may be greater if the heartwood were boxed out of the log and used in the processes, however this would also add cost to materials preparation.

## Process Yield vs. Product Value

| | | Product Value (w/o waters) | | Product Value (with waters) | |
|---|---|---|---|---|---|
| Oil Yield (Percent) | Oil Yield (Lbs.) | Leaf | Wood | Leaf | Wood |
| 0.35% | 7.0# | $420 | $52 | $720 | $352 |
| 0.4% | 8.0# | $480 | $60 | $780 | $360 |
| 0.5% | 10.0# | $600 | $75 | $900 | $375 |
| 0.6% | 12.0# | $720 | $90 | $1,020 | $390 |
| 0.7% | 14.0# | $840 | $105 | $1,140 | $405 |
| 0.8% | 16.0# | $960 | $120 | $1,260 | $420 |
| 0.9% | 18.0# | $1,080 | $135 | $1,380 | $435 |
| 1.0% | 20.0# | $1,200 | $150 | $1,500 | $450 |
| 1.25% | 25.0# | $1,500 | $187 | $1,800 | $487 |
| 1.50% | 30.0# | $1,800 | $225 | $2,100 | $525 |

**Economic Leverage Points** - Looking at the cost analysis for the distillation of fresh (wet) leaf materials, following are factors which could materially change the cost (and therefore profitability) profile of the oil recovery process:

a.) Yield would likely be improved by the use of a pressurized distillation unit, as was the experience noted in the work of Kurth and Ross at OSU, referencing item (1) in the enclosed bibliography.

Yield could possibly also be improved by drying the materials according to a proper drying schedule that would not cause attendant oil loss in the process. Additionally, preparing the materials into smaller particles by chopping, hammer-milling, etc., could result in an improved yield as is also noted in Kurth and Ross' work.

The effects of oil yield improvement on the product-value of the process are startling, as given in the following table:

On a commercial basis the business break-even point for leaf oil (w/o waters) is at a yield of 0.45% - 0.50%, as shown in Section 9.0, Business Opportunity Conclusions. Therefore, even small changes in yield could radically change the business attractiveness of this opportunity. If yield on the order of 1.0% - 1.5% could be achieved, as experienced by Kurth and Ross with a pressurized system, business viability would be certain, even with substantial swings in market price.

b.) Labor costs for harvesting and hauling could be somewhat reduced with a more efficient harvesting and materials preparation system, however labor efficiencies wouldn't have nearly the effect on business profitability as would the improvement of product yield.

c.) All other cost elements have only a relatively minor effect on the potential economic viability of the process.

## 8.0 Market Test Results

The methodology for doing the market test on the Juniper oil included the determining of target market applications, the development of a market segmentation scheme, the development of a list of potential customers in the market, market sampling, an analysis of similar and "competitive" products that are on the market today, and the distillation of market feedback to determine market interest and potential pricing of the Juniper oil in the target market segments.

The Essential Oil Company of Lake Oswego, Oregon, was contracted in this project to provide the test distillation still, technical distillation services, and test marketing services. The Essential Oil Company is in the business of providing many different types of essential oils to brokers, blenders and manufacturers throughout the world. Hence they have the product and market knowledge necessary to do a reasonable market test of the Juniper oil for purposes of determining its market value and potential as the basis of a business enterprise.

### Target Applications

A list of the best possible target applications for the oils of Juniperus Occidentalis is as follows. This list was the basis for developing a target customer list of firms that provide either raw oil or blended oils to companies that produce the end products in these application areas, or in some cases are the companies that in themselves produce the end products and take them to either the wholesale or retail markets.

- Aromatherapy
- Mood scent kits
- Room fresheners
- Scent masks
- Insect repellants
- Soaps and candles
- Cosmetics, fragrances, lotions and cremes
- Naturopathic (possible application: antibacterial/antiviral applications)

## Market Sampling

Small samples of essential oils, and in some cases the distillate waters, distilled during the course of the Juniper oil project were sent to prospective buyers of such materials for appraisal. In some cases, results of the gas chromatography testing were also disclosed. Feedback from the potential customers on the applicability of the oils or waters to their products and markets was solicited.

After analyzing the potential markets it was determined that, for purposes of this market test the potential target markets broke down into the following major secotrs:

- Industrial Sector: firms that supply essential oils and related blending chemicals, including artificial scents, to product manufacturers in bulk form, typically 400 pound (45 gallon) drums. Since these firms supply a great number of manufacturers that use essential oils in their products, our particular interest here was in testing whether any of them would know of a market requirement for which the Juniper oil or waters might have a unique fit. These firms are typically large distillers of essential oils, and/or are brokers and wholesale distributors of oils produced internationally or by other speciality chemical firms.

- Commercial Sector: The commercial sector breaks down into two distinctly different kinds of customers:

  1.) Large Commercial Segment. The companies in this segment are not necessarily always distillers but are principally volume oil brokers and distributors for oil in drum lots to oil blenders and large third party manufacturers of commercial products. Most of the commercial products produced by their customers ultimately end up at the retail distribution level for consumers.

  2.) Specialty Commercial Segment. These firms are distributors and sometimes blenders of a wide variety of essential oils, often on a regional basis. Their customers generally purchase in relatively small (2 kg. to 10 kg.) lots, and they are generally producing specialty products in aroma-therapy, naturopathic medicine, and specialty scent products for selected niche markets, including entrepreneurs doing specialty scent products at a local or regional level. These firms typically have a broad technical, product and marketing expertise, and can assist their customers in the defining and developing of new scent products. Some of these firms also do a certain amount of business in the large commercial segment for some of the more common generic oils. The Essential Oil Company is one of the firms that is in both of these market segments.

3.) For purposes of this project another market segment was delineated for analysis and that was the Entrepreneur/Retail Segment, defined for purposes of this project to include local Oregon entrepreneurs who might consider distilling relatively small quantities of oil which they would use themselves to create retail products for local and regional distribution, typically in either aromatherapy or the specialty scent products market.

The list of potential industrial and commercial customers used for purposes of market sampling is consdered proprietary to the Essential Oil Company because of the highly competitive nature of this industry. Hence the list is not included for general distribution in this report but will be sent under proprietary acknowledgement to the program manager (Larry Swan) and the project administreator (Candice Richard at KCEDA) for their files. Requests for access to this list should be made through either Larry Swan or Candice Richard, who can then forward the request to Robert Seidel of the Essential Oil Company in Lake Oswego, Oregon.

## Competitive Products

Other manufacturers and distributors were contacted regarding the availability and pricing of competitive or similar oils, particularly those of the cedar, spruce, pine, fir and hemlock families. Following is the list of oils available in the commercial marketplace of some of the products which might be considered similar and/or competitive to Western Juniper oils.

- Oil of balsam fir: $10.00 per pound (50 pound drums)
- Oil of White Pine: $30.00 per pound (50 pound drums)
- Oil of black spruce: $30.00 per pound (50 pound drums)
- Oil of Western Red Cedar leaf: $20.00 - $25.00 per pound (400 pound drums)
- Oil of Western Yellow Cedar: $60.00 per pound (45 gallon drums)

- Oil of Pacific Silver Fir: $67.00 per pound (45 gallon drums)
- Oil of Douglas Fir: $72.00 per pound (45 gallon drums)
- Oil of Western Hemlock: $180.00 per pound (45 gallon drums)
- Oil of Cypress: $58.00 per pound
- Oil of Juniper berry: $75.00 per pound
- Oil of Eastern Red Cedar wood: $7.50 per pound (400 pound drums)

The above list represents pricing for various "needle" oils.

**Market Results**

Following are the results and conclusions from the feedback obtained from the test marketing activities.

- Industrial Sector: This sector deals with bulk production and distribution of various oils, including needle oils. In that regard the bulk of their current business is for the oil of Eastern Red Cedar which carries a market price of $7.50 a pound at the industrial distribution level. They are generally uninterested in talking to potential vendors of a relatively unique oil like the Western Juniper for which their is no established volume market, and for which the end use products have generally not yet been defined. Given the fact that the oil from the Western Juniper is not remarkably different from that distilled from the Eastern Red Cedar (Juniperus Virginiana) these potential customers see it as a product competitive with the Eastern Red Cedar and therefore are only interested in talking about it at a competitive price of $7.50 a pound. Another limiting factor in dealing with this kind of customer is their tendency to only want to spend time working with distillers that are already in production and from which there is already a projected volume output that they can consider and buy. We were also limited in addressing these potential customers by the sizes of the samples we were able to give them in that a small sample for their purposes would have been a kilogram. These potential customers, therefore, were not considered viable potential target customers in the near term.

- Commercial Sector - Large Customers. The feedback from customers serving the large commercial sector segment was mixed in that they also saw the oil from the bole wood of the Juniper as being essentially the same as, and competitive with, the oil of the Eastern Red Cedar which is currently on the market. They are only interested in purchasing that oil at a competitive market price (less than $10 a pound in 100 pound drums). One of the characteristics of the potential customers in this segment are that they attempt to buy at as low a price as possible and then take a middle man's markup. They are, however, a principle distribution source for the list of needle oils given above and sell those needle oils at the prices and lot sizes given in the above list. Larger volume users can obtain discounts from them on these prices of as much as 50%. There was particular interest expressed in the oil from the Juniper leaf, with indications from some of them that they would expect to sell that product for $60 to $75 a pound to specific small target users once the market were developed for the oil. However, the estimate of price that they would be willing to pay for the oil, which would represent the distillers price to them, would be approximately $25 a pound.

- Specialty Commercial. Many of the customers in this segment are the customers for the large commercial segment brokers and distributors, and many of the firms in this segment are also blenders and provide technical product development services along with being an oil supplier. It is these potential customers that would be the market for the Juniper leaf oil at an estimated $60 a pound in relatively small quantities, typically 2 kg. and 10 kg. per order, with a 20 kg. order being a large order. These firms typically are specialty products distributors, and are often themselves in the business of blending and marketing products for the retail consumer markets, particularly in the aroma therapy marketplace.

  Included in the Specialty Commercial segment are the small local entrepreneurs who do specialty aroma therapy and scent products, principally for local and regional distribution. These small firms would also be prospective customers in small volume, typically at the 1 to 5 kgm. per order size, but which can be marketed to via catalog, direct mail, Internet, and general distribution vehicles.

## Conclusions

There is a high degree of confidence that there would be a market for the Juniper leaf oil at prices ranging from $25 a pound to $75 a pound for the leaf oil, depending on the sector and the segment being served. The volume of oil required to serve this market is highly uncertain at this point because it involves the development of new scent products and the development of the markets for those products. However, as part of this project, a few dozen one ounce scent spray bottles of 20% oil solution, in alcohol, were prepared to test customer acceptance of them as a hunter scent mask product. Later, the test was broadened to include the use of that same product as a room scent, and as a scent for artificial Christmas wreathes, Christmas trees, etc. A few gift shops specializing in those seasonal products were sampled and oil spray samples were left with them. The response to both the hunter scent and the use of the same spray scent bottle as a room or seasonal scent were both very good. These products would make good entrepreneurial business opportunities.

## 9.0 Business Opportunity Conclusions

Commercial (Bulk) Products: In developing a business opportunity analysis on a commercial (bulk wholesale sales) basis, the following assumptions are in order.

- The analysis for bulk production and marketing of leaf oil (without the marketing of the distillate runoff waters) will be the baseline. Other cost and revenue assumptions will be estimated relative to leaf oil on an appropriate business basis.

- Cost for the production of a given volume of both leaf and wood oil is the same, given all tradeoffs.

- Marketing and selling costs are estimated at 20% of revenue for all saleable products.

- G&A, and other expenses, are fixed at 20% of leaf oil revenue, and are assumed the same for a similar volume of wood oil.

- Marginal cost and revenue estimates for the sale of the distillate waters are considered to be marginal to the oil costs, and are estimated accordingly.

- Each 0.10% yield is equivalent to 2 pounds of yield (1 quart: 32 fluid ounces) on a distillation yield of 2000 pounds.

- The market price of leaf oil is $60.00 per pound. The market price for wood oil is $7.50 per pound.

- Assumes the business is dedicated to the distillation and marketing of Juniper Oil, and that oil distillation is not a secondary by-product of other Juniper processing operations.

Using a standard production unit of one ton (2,000 pounds) of materials as the standard production unit for cost estimates, following are the profitability profiles for both leaf and wood oil, at different process yields.

## Profitability Profile: Leaf Oil

| | Oil Yield | | | | | |
|---|---|---|---|---|---|---|
| | 0.3% | 0.4% | 0.6% | 0.8% | 1.0% | 1.5% |
| Product (lbs) | 6# | 8# | 12# | 16# | 20# | 30# |
| Revenue | $360 | $480 | $720 | $960 | $1,200 | $1,800 |
| Costs | | | | | | |
| Production | $360 | $360 | $360 | $360 | $360 | $360 |
| Marketing and Sales | 72 | 96 | 144 | 192 | 240 | 360 |
| G&A, and Other | 96 | 96 | 96 | 96 | 96 | 96 |
| Total Costs | $528 | $552 | $600 | $648 | $696 | $816 |
| Gross Profit (loss) | ($168) | ($72) | $120 | $312 | $504 | $984 |
| Gross Profit (%) | (47%) | (15%) | 17% | 32% | 42% | 55% |

## Profitability Profile: Wood Oil

| | Oil Yield | | | | | |
|---|---|---|---|---|---|---|
| | 0.3% | 0.4% | 0.6% | 0.8% | 1.0% | 1.5% |
| Product (lbs) | 6# | 8# | 12# | 16# | 20# | 30# |
| Revenue | $45 | $60 | $90 | $120 | $150 | $225 |
| Costs | | | | | | |
| Production | $360 | $360 | $360 | $360 | $360 | $360 |
| Marketing and Sales | 9 | 12 | 18 | 24 | 30 | 45 |
| G&A, and Other | 96 | 96 | 96 | 96 | 96 | 96 |
| Total Costs | $465 | $468 | $474 | $480 | $486 | $501 |
| Gross Profit (loss) | ($420) | ($408) | ($384) | ($360) | ($336) | ($276) |
| Gross Profit (%) | (993%)------------------------------------------------------->(123%) | | | | | |

A profitability analysis of marketing the runoff distillate runoff waters, at $1.00 per gallon, once the market for this produce were fully developed, would be as follows, at a standard yield of 300 gallons of waters for each ton of materials processed.

**Profitability Profile: Waters**

| | |
|---|---|
| Product (gallons) | 300 gal. |
| Revenue | $300 |
| Costs | |
| Production (10%) | $ 30 |
| Marketing & Sales (20%) | 60 |
| G&A, Other (5%) | 15 |
| Total Costs | $105 |
| Gross Profit | $195 |
| Gross marginal Profitability | 65% |

For an established business selling both oil and waters, the profitability profiles would be as follows:

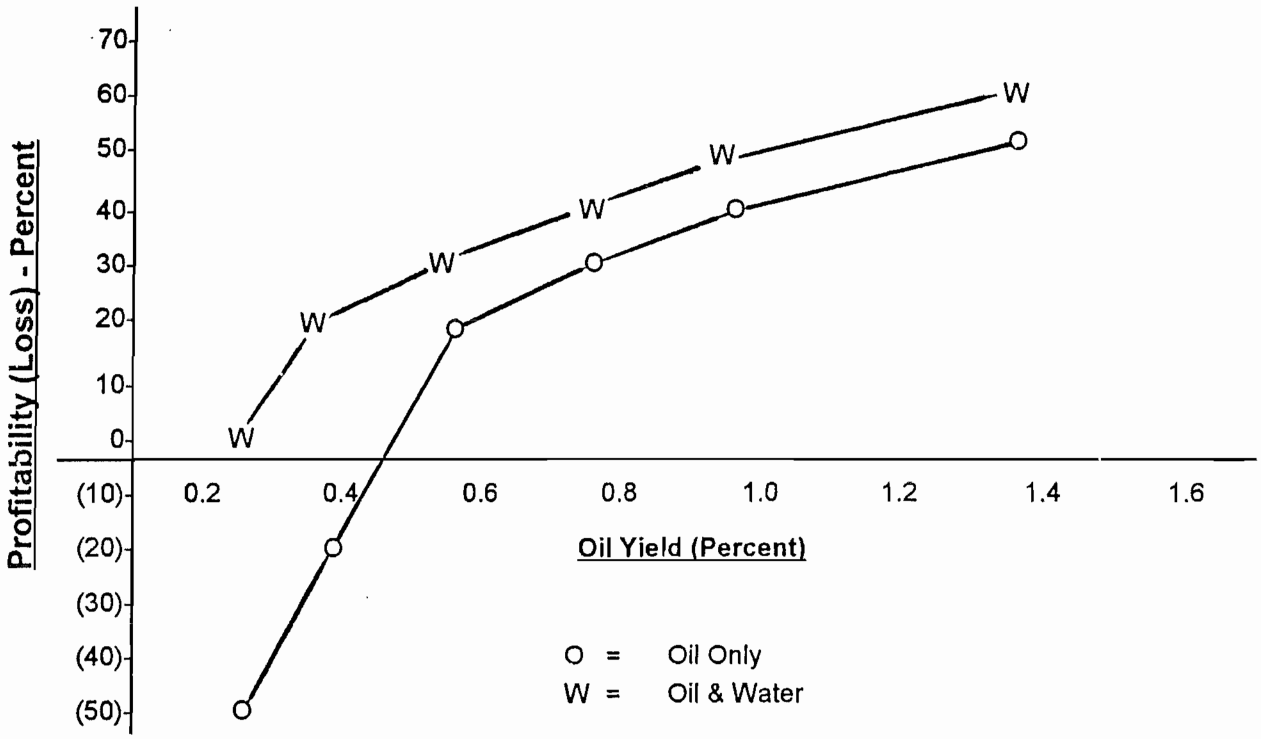
## Profitability Profiles: Oils and Waters

| | Oil Yield | | | | | |
|---|---|---|---|---|---|---|
| | 0.3% | 0.4% | 0.6% | 0.8% | 1.0% | 1.5% |
| LEAF OIL | | | | | | |
| Total Revenue | $660 | $780 | $1,020 | $1,260 | $1,500 | $2,100 |
| Gross Profit (oil) | ($168) | ($72) | $120 | $312 | $504 | $984 |
| Gross Profit (waters) | $195 | $195 | $195 | $195 | $195 | $195 |
| Total Gross Profit | $27 | $123 | $315 | $507 | $699 | $1,179 |
| Gross Profit (%) | 4% | 16% | 31% | 40% | 47% | 56% |
| WOOD OIL | | | | | | |
| Total Revenue | $345 | $360 | $390 | $420 | $450 | $525 |
| Gross Profit (oil) | ($420) | ($408) | ($384) | ($360) | ($336) | ($276) |
| Gross Profit (waters) | $195 | $195 | $195 | $195 | $195 | $195 |
| Total Gross Profit | ($225) | ($213) | ($189) | ($165) | ($141) | ($81) |
| Gross Profit (%) | (Loss)------------------------------------------------------------------> | | | | | |

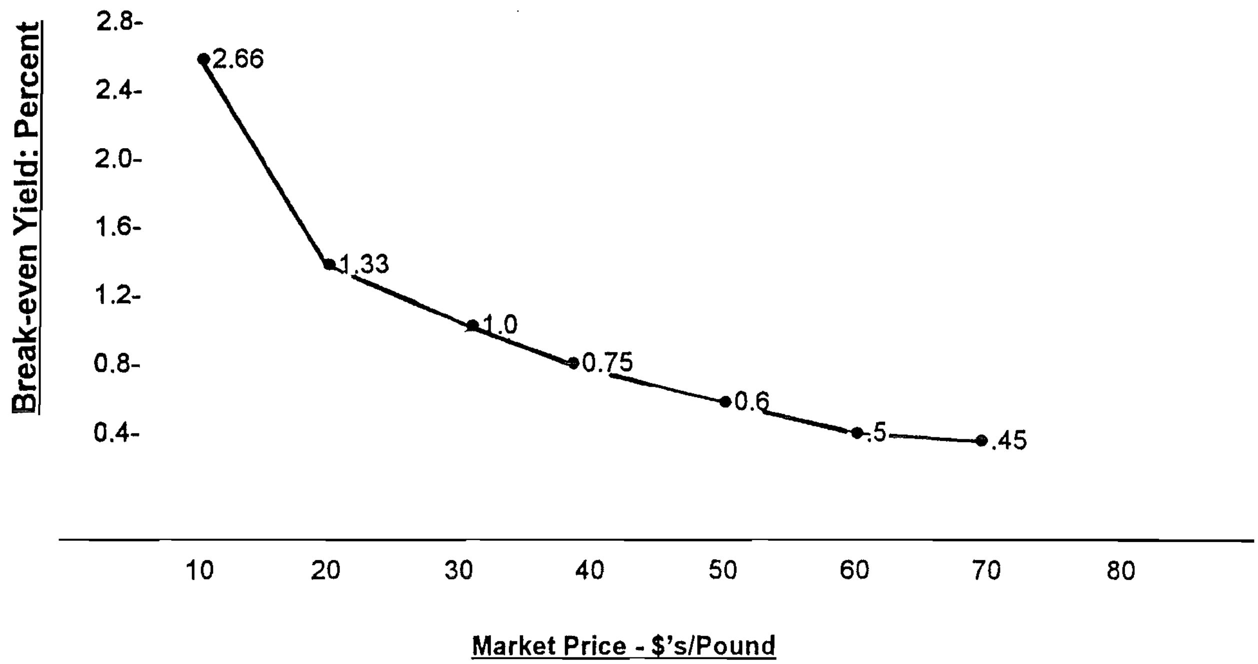
These analyses show the difficulty of making a profitable commercial business out of the distillation of oil from Juniper wood on a dedicated business basis, even if the waters from the process are marketed. However, the distillation of Juniper leaf oil shows promise, but is much dependent on the estimated market price for the oil ($60.00 per pound) for which there is not an established market -- or a truly competitive product -- today (see Section 8.0, Test Market Results.)

Following is a graph which shows the changing profitability of leaf oil as process yield changes, with and without the marketing of the associated distillate "waters".

**Profitability vs. Yield: Leaf Oil**



## Break-even Sensitivity: Commercial Leaf Oil
## Break-even Yield vs. Market Price




## Commercial Conclusions:

- The critical determinants driving the viability of a Juniper oil business in the commercial marketplace are:
  - Product price
  - Process yield.

- This project has shown that, even given reasonable extrapolations for economies of scale, at the current market price of $7.50 per pound (competitive with Virginia Red Cedar -- Juniperus Virginiana oil), and with maximum anticipated product yields of 1.5%, the business would still not be profitable. Both price and yield would both have to increase 50% - 100% in order to make this a viable business opportunity.

- An except to the above statement would be a case where a distiller could set up in conjunction with an existing Juniper processing operation and use (free) readily available waste materials. Processing (distillation) and other costs would remain the same, however $210 of production costs (per 2,000 pound batch) could be saved by not having to harvest and transport Juniper materials. Though this would help, it would only change the wood profile as follows:

| | Oil Yield | | | | | |
|---|---|---|---|---|---|---|
| | 0.3% | 0.4% | 0.6% | 0.8% | 1.0% | 1.5% |
| Gross Profit | | | | | | |
| Oil Only | ($210) | ($198) | ($174) | ($150) | ($126) | ($66) |
| Oil and Waters | ($15) | ($3) | $21 | $55 | $69 | $129 |

If a commercial market could be found for the distillate waters, then this scenario takes on a modest business attractiveness at the upper end of the anticipated yield spectrum.

- Using price/positioning estimates based on the markets for other needle oils, the business potential for Juniper leaf oil is attractive, particularly if yield can approach those achieved by Kurth and Ross in a pressure distillation system (1.0% - 2.0%). The break-even point for leaf oil is at a yield of approximately 0.5%, that almost achieved in the projects small, zero-pressure distillation apparatus. This without considering the potential for revenue from sale of the distillate "waters".

- If estimates of leaf-oil process yield and market price are reasonably correct, and if the distillate waters can be marketed, then the distillation of leaf oil becomes a very attractive business opportunity with gross profit of 42% at the 1.0% yield level, and 55% at the 1.5% yield level.

**Entrepreneurial/Retail**

Market sampling has shown that there is opportunity to enterprising entrepreneurs to make a business of distilling Juniper, and possibly other aromatic botanicals, for use in their own specialty retail products (see Section 8.0 - Test Marketing Results). The products for this opportunity would be pre-packaged oils and other specialty "scent" products for home, commercial, aroma therapy and other "mood" applications, as well as for the hunter's scent - mask market.

For this business opportunity only a modest-size distillation unit would be required (the 250 pound capacity still used in this project is a good example). The key to business viability comes from using oil to create a large volume of small, pre-packaged retail products from a small volume of oil. Oils are often packaged down to 1/4 oz. sizes (or less) for a pure oil, and other spray-scent products are a mixture of oil and alcohol in a 5% or 10% (1/20 or 1/10) oil/alcohol solution. This provides enormous product and revenue leverage from small amounts of oil. It does require, however, that the entrepreneur be prepared to do the packaging, marketing and selling of the products (at wholesale) to the appropriate customer (chain stores; gift shops, etc.). A business analysis for this opportunity follows:

## Key Assumptions

- Distillation process yield will be that used in determining a standard expected yield from a small, zero-pressure still as was used in this project -- meaning an expected minimum yield of 0.40%.
- Harvesting, materials preparation and distillation costs will be the same as estimated previously in this report -- $925.00 per 2,000 pounds of material processed.
- Either the leaf oil or wood oil will be equally useable for these products (however, leaf oil may be somewhat preferable).
- Wholesale value of the pre-packaged products is 50% of the retail price.
- Based on market investigations, retail prices for comparable "pure" oil scent products is: $8.00 - $10.00 per 1/4 ounce of the oil; $4.00 to $6.00 per ounce for 10% solution pre-packaged spray scent products.
- Packaging, marketing and selling costs are 30% of revenue for the pure oil, and 40% of revenue for scent sprays because of the additional materials, blending and packaging costs.

- The greater of these estimates will be used for purposes of this analysis because of the uniqueness and quality of the Western Juniper oil-based products.

**Profitability Profile: Packaged Retail Products**

| | Product | |
|---|---|---|
| | 1/4 oz. Pure Oil | One Ounce Scent Spray |
| Raw Oil Product | 1.0 gallon | 1.0 gallon |
| Packaged Sale Items | 512 bottles | 1,280 spray ounces |
| Wholesale Price | $5.00 | $3.00 |
| Revenue | $2,560 | $3,840 |
| Costs | | |
| Production | 925 | 925 |
| Packaging, Marketing & Sales | 768 | 1,536 |
| Other Business Exp. | 256 | 384 |
| Total Costs | $1,949 | $2,845 |
| Gross Profit | $ 611 | $ 995 |
| Gross Profit (%) | 24% | 26% |

If the oil were commercially available at $480 per gallon wholesale, as estimated in the commercial analysis, rather than having an entrepreneur distill it themselves at a cost of $925 per gallon, the profitability of a retail products business would look as follows.

## Profitability Profile: Packaged Retail Products Using Purchased Commercial Oil

| | Product | |
|---|---|---|
| | 1/4 oz. Pure Oil | One Ounce Scent Spray |
| Revenue | $2,560 | $3,840 |
| Costs | | |
| Purchased Oil | 480 | 480 |
| Packaging, Marketing & Sales | 768 | 1,536 |
| Other Bus. Exp. | 256 | 384 |
| Total Costs | $1,504 | $2,400 |
| Gross Profit | $1,056 | $1,440 |
| Gross Profit (%) | 41% | 38% |

## Retail Conclusions:

- There is a very high revenue leverage in developing specialty niche products for retail markets.

- Modest investment would be required (est. $10,000 - $20,000) and a low-cost access to Juniper would be required.

- There is no direct equivalent competitive products available today. The Western Juniper essential oils market remains largely untapped at the retail level. A unique market position is available for enterprising entrepreneurs.

- Business profitability is adequate (est. 25%) for product pioneers who do their own distilling in small batches. Enhanced profitability (40%) would come from being able to purchase the needed raw oil on the "bulk" commercial market.

- As is true with many products, the business leverage comes from the business acumen needed to define and serve retail markets with new products. The key to doing this, however, will be marketing and distribution savy, and the working capital needed to support these activities in the early stages of the business.

- To complement the basic oil-based scent products a variety of low-cost, high-margin products such as scent rings, candle-heated vaporizers, etc., can be distributed along with the scent products. In addition, once an oil supply is available, items such as scent soaps and candles can be produced to specification by third-party manufacturers and distributed at high margin to the aroma markets.

- In summary, there is a window of opportunity for entrepreneurial ventures in supplying retail scent products based on Western Juniper oil derivatives.

## Appendix "A"
## Project Documentation and Contract

- COIC Project Approval Notice - 4/18/95
- KCEDA Spending Authorization Letter - 8/29/95
- Project Schedule
- Tribal "In-Kind" Cost Summary

CENTRAL OREGON
INTERGOVERNMENTAL
COUNCIL

*Everywhere Central Oregon Works.*

April 18, 1995

Bob Finch, Economic Development Department
Confederated Tribes of the Warm Springs Reservation
P.O. Box 1359
Warm Springs, Oregon 97761

Dear Bob,

This letter is to confirm that the "Juniper Commercialization" multi-region, Regional Strategy project was approved by Governor Kitzhaber on April 10, 1995. The "Juniper Commercialization" project received the full funding amount, $109,300, requested. This will enable the South Central Oregon Regional Strategy Board to commit $18,300 to the Warm Springs Tribe's "Juniper Distillation and Marketing" project. This commitment of Regional Strategy funds is to be matched with funding and in-kind commitment from the Tribes of $23,500. Of that total, $12,500 is a cash match and $11,000 is in-kind match.

The multi-regional projects approach is complicated. The South Central region must establish Intergovernmental Agreements with the other supporting regions, sign a contract with the state, and sign contracts with the individual project elements of which yours is one of six. We are working towards a June deadline to complete these tasks and have the funds available for projects. Please call if you have any questions.

Sincerely,

Drew Foster
Senior Economic Development Planner
South Central Oregon Regional Strategy staff

REDMOND
P.O. Box 575
1135 W. Highland
Redmond OR 97756

BEND
2410 NE Twin Knolls Dr
Suite A
Bend, OR 97701

MADRAS
35 S E C Street
Suite A
Madras, OR 97741

PRINEVILLE
411 W 3rd Street
Prineville, OR 97754

KLAMATH

KLAMATH COUNTY ECONOMIC DEVELOPMENT ASSOCIATION

August 29, 1995

Joseph Yesenofski, Management Consultant
Economic Development of The Confederated Tribes of Warm Springs
P.O. Box 1359
Warm Springs, Oregon 97761

Dear Joe:

This letter shall constitute authorization to commence spending for the "Juniper Commercialization" multi-region, Regional Strategies project that was approved by Governor Kitzhaber on April 10, 1995, provided that authorization of the grant to the Confederated Tribes of Warm Springs has been approved.

A formal contract will be provided under separate cover. Expenses for the project incurred after the award date (April 10, 1995) may be eligible for reimbursement, provided that the expenses are reimbursable under the provisions of the grant contract.

I will be administering the grant contract through the office of KCEDA. Feel free to contact me with any questions that arise. I look forward to working with you and the members of The Confederated Tribes of Warm Springs.

Sincerely,

Jason M. Statuta

Jason M. Statuta
Executive Projects Coordinator

cc: Larry Swan, Winema National Forest
L.H. Senn, Executive Director KCEDA
Jackie Yoder, OEDD

(503) 882-9600 • FAX (503) 882-7648 • 125 SOUTH 6TH ST. • P.O. BOX 1777 • KLAMATH FALLS, OR 97601

# WESTERN JUNIPER COMMERCIALIZATION PROJECT
# WORK PLANS AND SCHEDULE

## *Oil Distillation and Marketing Research*

Purpose: Test business value of using western juniper berries, bough, branches, and bole wood for distillation of commercial juniper oil.

Project Work Schedule: Attached. Begin 3/15/96 - Finish ~~11/15/96~~ 12/31/96

| TASK | Begin Date | Completion Date | Deliverables* | Pay Schedule** |
|---|---|---|---|---|
| 1) Complete arrangements for renting or leasing still & install still; 2) Complete arrangements for juniper harvest (including berries), chipping & transportation; | 3/15/96 | 4/15/96 | 1)Letter***documenting completion of arrangements for still and any needed changes. | |
| 3) Complete sample material collection for test distillation; 4)Complete test distillations & obtain sample oil: | 4/15/96 | 5/30/96 | 1)Letter documenting completion of collection of material & test distillation. Include discussion about process, problems encountered, and outcomes; 2) Sample viles of oil from each set of materials. | KCEDA will pay 40% total contract amt. after deeming deliverables ok |
| 5)Complete oil analyses; 6) Complete marketing pkg., send samples to potential customers, & obtain mkt. feedback. | 5/15/96 | 7/15/96 | 1)Letter documenting completion of oil analyses; 2)Copy of marketing package; 3) Copy of mkt. feedback; 4)Set of samples sent to customers; 5)Copy of Mailing List | KCEDA will pay 30% total contract amt after deeming deliverables ok |
| 7) Complete 2nd set of test distillations & obtain sample oil; including Potential Sales Reps. | 5/30/96 | 6/30/96 | 1) Letter documenting completion of collection of material & test distillation. Include discussion about process, problems encountered, and outcomes; 2) Sample viles of oil from each set of materials; 3) list of Potential Sales Reps; 4) Any other mkt. feedback. | |
| 8) Expand test marketing effort with second set of oil samples. | 5/30/96 | 8/30/96 | 1) Letter documenting action; 2) Mailing List; 3) Report Distribution List | KCEDA will pay 20% total contract amt. after deeming deliverables ok |
| 9) Accumulate final test marketing results, prepare project documentation, publish & distribute report | 10/1/96 | 10/31/96 | 1) Draft of report, KCEDA will return comments within 10 working days; 2) Final Copy by 10/31/96. | KCEDA will pay 10% total contract amt. after deeming deliverables ok. |
| 10) Submit Regional Strategies Program Final Report | 10/31/06 | 11/15/96 | | |
| 11) Submit job creation report. | 10/31/96 | 11/15/96 | | |

*To be delivered to KCEDA within two calendar weeks of completion dates.

**KCEDA shall receive up to five working days for review of deliverables, before check is cut.

***Letter(s) should include current In-Kind and Cash Contributions.

The Confederated Tribes of Warm Springs

JUNIPER OIL DISTILLATION PROJECT

Tribal "In-Kind" Costs

Through June 30th, 1996 (One-Time Costs)

| | Cost |
|---|---|
| • CTWS Tribal Management: Contract development and finalization; Presentations; obtain CTWS approvals (16 hours @ $50.00/hour | $ 800.00 |
| • Natural Resources: Project Assessment and Management plan; Site tours and analysis; Committee(s) approvals processes (20 hours @ $50.00/hour) | 1,000.00 |
| • Natural Resources: Site tours with contractors; Site selection; Tree selection; Harvest plan and resource commitments (12 hours @ $50.00 per hour) | 600.00 |
| • Warm Springs Forest Products | |
| - Facilities engineering, waste disposal and planning (12 hours) | 600.00 |
| - Pipe in and valve steam and water | 450.00 |
| - Provide waste disposal tank and disposal commitment | 1,000.00 |
| - Hook-up of steam and water to still | 225.00 |
| - Install power and extra lights | 350.00 |
| Total In-Kind Costs to 6/30/96 | $ 5,025.00 |

June 1st - September 30th, 1996 (On-Going Costs over 5 months)

| | |
|---|---|
| • Business and Economic Development: | |
| Project Administration (10 hours) | $ 500.00 |
| Clerical and Accounting Support | 500.00 |
| • Natural Resources: Harvesting crew supervision and administration (24 hours) | 1,200.00 |
| - 2-Trucks for 6 days @ $100/day | 600.00 |
| - Tree value: 16 Trees @ $25.00 each | 400.00 |
| • WSFP (Plant Site) | |
| - Facilities @ $100/mo | 500.00 |
| - Plant floor space: 1,000 $ft^2$ @ $0.50/foot/mo. | 2,500.00 |
| Total On-Going Cost (5 months) | $ 6,200.00 |

TOTAL PROJECT IN-KIND COSTS

| | |
|---|---|
| ONE-TIME COSTS THROUGH 6/30/96 | $ 5,025.00 |
| ON-GOING COSTS: 6/1/96 through 10/31/96 | 6,200.00 |
| TOTAL PROJECT IN-KIND COSTS | $ 11,225.00 |

## Appendix "B"

- Original distillation Records

# Distillation Record

Date: 6/7/96

Batch #: WS-1-JL

Location: Warm Springs, Oregon

Material: Western Juniper Leaf (immature berries)

Source: Dry Hollow 2500' elev

Weight: 222 lbs

Form: As harvested, not chopped -branches to 1/4"

Start Time 10:15AM

Finish Time: 1:15PM

Total Distillation Time: 3 Hrs

Yield: 325.5 grams

Percent Yield: 0.32%
(=Weight of finished oil/weight of raw material)

---

NOTES:

Two Trees were cut 6/6/96- branches to 3/8"
#1 Approximately 65yrs old
#2 Approximately 135yrs old

Branches baged in burlap on site. 16 bags leaf representing approx 40% of total green leafy material

First water 45 minutes-195Degrees F
Steady flow at 1hr 206 degrees F
Resulting oil is clear amber colored- possibly due to resins- Distilled too long!?

The Essential Oil Company PO Box 206 Lake Oswego, OR 97034 503-697-5992

# Distillation Record

Date: 6/11/96

Batch #: WS-2-JL

Location: Warm Springs, Oregon

Material: Western Juniper Leaf

Source: Dry Hollow 2500' elev

Weight: 216lbs

Form: As harvested, not chopped -branches to 1/4"

Start Time 3:45PM

Finish Time: 4:45PM

Total Distillation Time: 1 Hrs

Yield: 44.4 grams

Percent Yield: 0.045%
(=Weight of finished oil/weight of raw material)

## NOTES:

Branches cut from 135yr old tree 5 days ago
Bagged in burlap in the field- stored inside "distillery"
Retort cleaned with 200 proof alcohol for 1/2hr. This also serves to preheat equipment.

Preheating effective-193 degrees F at 15 minutes-first waters

Yield Quite low. Suspect loss to evaporation after five days since cutting.
Oil Clear/amber

# Distillation Record

Date: 6/20/96

Batch #: WS-3-JLB

Location: Warm Springs, Oregon

Material: Western Juniper Leaf heavy imm. berries

Source: Dry Hollow 2500' elev

Weight: 204lbs

Form: As harvested, not chopped -branches to 1/4"

Start Time 9:35AM

Finish Time: 12:05PM

Total Distillation Time: 2.5hrs

Yield: 157.2 grams

Percent Yield: 0.170%
(=Weight of finished oil/weight of raw material)

---

NOTES:

Preheated still to 2310 degrees F
First waters 9:45
Oil yellow/golden color
Leaf cut 24hrs prior to distillation

# Distillation Record

Date: 6/20/96

Batch #: WS-4-JL

Location: Warm Springs, Oregon

Material: Western Juniper Leaf tree#2

Source: Dry Hollow 2500' elev

Weight: 178lbs

Form: As harvested, not chopped
-branches to 1/4"

Start Time 3:15PM

Finish Time: 5:45PM

Total Distillation Time: 2.5hrs

Yield: 197.1 grams

Percent Yield: 0.244%
(=Weight of finished oil/weight of raw material)

NOTES:

Oil Lighter in color than previous batch with berries (WS 3 JLB)

Oil yellow

# Distillation Record

Date: 7/1/96

Batch #: WS-5-JW

Location: Warm Springs, Oregon

Material: Western Juniper Wood

Source: Dry Hollow 2500' elev

Weight: 257lbs

Form: Chips-Bole wood not barked

Start Time 7:45AM

Finish Time: 12:00PM

Total Distillation Time: 4.25 hrs

Yield: 202.6 grams (1hr-123gms,2hrs-36.5gms,3hrs-35.1gms,3.5hrs-8gms)

Percent Yield: 0.174%
(=Weight of finished oil/weight of raw material)

NOTES:

Material chipped 6/27/96 Tree #2-150yrs old
Insulated vertical and horizontal steam outflow
Oils after 3hrs are extremely viscous and slightly darker in color
Oils appear yellowish in color

# Distillation Record

Date: 7/1/96

Batch #: WS-6-JW

Location: Warm Springs, Oregon

Material: Western Juniper Wood

Source: Dry Hollow 2500' elev

Weight: 224lbs

Form: Chips-Bole wood not barked

Start Time 2:30PM

Finish Time: 5:00PM

Total Distillation Time: 2.5hrs

Yield: 65.8 grams

Percent Yield: 0.065%
(=Weight of finished oil/weight of raw material)

---

NOTES:

Retort hot to start from batch 2hrs prior
196 degrees F in 10 minutes-first waters
Wood chipped and bagged 4 days before cooking
Tree #2 approx 80yrs old
Yield quite low- oil is much darker
Oil is amber in color

# Distillation Record

Date: 7/31/96

Batch #: WS-7-JL

Location: Warm Springs, Oregon

Material: Western Juniper Leaf

Source: Dry Hollow 2500' elev

Weight: 252lbs

Form: Branches to 1/4"

Start Time 2:00PM

Finish Time: 4:00PM

Total Distillation Time: 2hrs

Yield: 287.7 grams (1hr-211.5 gms, 2hr-76.4 gms)

Percent Yield: 0.250%
(=Weight of finished oil/weight of raw material)

---

NOTES:

First hour approx 9oz oil-golden yellow
Second hour- approx 3oz almost amber

Cut in AM distilled 3-4hrs after cutting

# Distillation Record

Date: 8/1/96/96

Batch #: WS-8-JW

Location: Warm Springs, Oregon

Material: Western Juniper Leaf

Source: Dry Hollow 2500' elev

Weight: 222lbs

Form: Chipped

Start Time 9:50AM

Finish Time: 12:50PM

Total Distillation Time: 3hrs

Yield: 175.5 grams (2hrs-135.0 gms,3hrs-40.5gms)

Percent Yield: 0.174%
(=Weight of finished oil/weight of raw material)

---

NOTES:

Tree cut 24 hrs prior
Chipped, bagged and cooked
Small rounds stacked under cover in the mill
Oil at 2hrs golden yellow
Preheating and soaking chips seems to be of value in releasing oil

# Distillation Record

Date: 9/25/96

Batch #: WS-9-JL

Location: Warm Springs, Oregon

Material: Western Juniper Leaf

Source: Dry Hollow 2500' elev

Weight: 265 lbs

Form: As harvested, not chopped -branches to 1/4"

Start Time 1:30PM
first flow 2:30 195 degrees F

Finish Time: 5:30PM

Total Distillation Time: 4 Hrs

Yield: 550 grams approximated from volume

Percent Yield: 0.46%
(=Weight of finished oil/weight of raw material)

---

NOTES:

96 year old tree

The color of Burgundy wine (unfortunately contaminated)

# Distillation Record

**Date:** 9/26/96

**Batch #:** WS-10-JL

**Location:** Warm Springs, Oregon

**Material:** Western Juniper Leaf

**Source:** Dry Hollow 2500' elev

**Weight:** 276 lbs

**Form:** As harvested, not chopped -branches to 1/4"

**Start Time** 9:00PM

**Finish Time:** 1:00PM

**Total Distillation Time:** 4 Hrs

**Yield:** 550 grams approximated from volume

**Percent Yield:** 0.44%
(=Weight of finished oil/weight of raw material)

---

NOTES:

128 year old tree
Gatered and bagged 9/25/96 afternoon
The color of Burgundy wine (unfortunately contaminated)

# Distillation Record

Date: 11/8/96

Batch #: WS 11 JL

Location: Warm Springs

Material: Juniper leaf.

Source: Dry Hollow

Weight: 189 lbs

Form: Cut & bagged in field

Start Time: 9:30

First water. 10:30

Finish Time: 1:30.

water over - 11:10

Total Distillation Time:

Yield: 322 gms -

Percent Yield: .375%

(=Weight of finished oil/weight of raw material)

NOTES:

18
16
14
14
18
15
12
17
15
10

12
15
13

120
56
176
13
189

Steam on 9:30

# Distillation Record

Date: 11/8/96

Batch #: WS 12 JL

Location: Warm Springs

Material: Leaf Juniper

Source: Dry Hollow

Weight: 166 lbs

Form: Cut & bagged.

Start Time: 2:00

Finish Time: 4:30

Total Distillation Time:

Yield: 261 gms

Percent Yield: .346%
(=Weight of finished oil/weight of raw material)

NOTES:
Bag Weights

14 16
15 12
14 17
13
13 75364 gms
14
12
10
13

120
46
166

# Distillation Record

Date: 11/15/96

Batch #: WS 13 JLW
Juniper leaf water.

Location: Warm Springs

Material: Western Juniper leaf

Source: Dry Hollow

Weight: 363 lbs.

Form: Cut & bagged. 1/4" stem.

Start Time: 930 Am – 2:00
2:30 – 4:30

Finish Time: 4:30 pm.

Total Distillation Time: 6.5 hrs. (2 batches) (3.25 hrs. each)

Yield: 707.5 gms.

Percent Yield: .42%
(=Weight of finished oil/weight of raw material)

---

NOTES:

20 363
14 454
14 1452
20 1815
20 .1815
12 1452
10 16480 oz
14 .004
18
16
8
166
16
182

15
20
17
12
16
17
16
14
18
18
18
181
182
363

440 gms
267.5
707.5 gms.

# Appendix "C"
## Gas Chromatography Analyses

- Batch WS1JL: Juniper Leaf Oil
- Batch WS3JLB: Juniper Leaf Oil with Berries
- Batch WS4JL: Juniper Leaf Oil
- Batch WS4JL (Water): Juniper Leaf Waters

# FLORA RESEARCH

## CERTIFICATE OF ANALYSIS

Date: 09-July-1996
Client:Essential Oil Co.

Sample I. D. : Juniperus occidentalis 3
Description:
The sample was received in a glass 5 ml. vial It was labelled with a label identifying it as Juniper leaf ws 3 jlb.
Appearance:
Physical appearance is a clear oil with characteristic smell of juniper/pine oil.

Method of analysis:
High Resolution Capillary Gas Chromatography using dual channel analysis.

Results of analysis:

The analysis reveals an oil rich in monoterpines and bornyl acetate. The oil is unique in its chemistry and offers aroma chemicals similar to both pine oil and juniper.

**I hereby certify that I am an authorized representative of Flora Research and that to the best of my knowledge the following information is true and accurate.**

**Signature:**

**Title: chromatographer** **Date:** 9- July - 96

**31921 Camino Capistrano 435 • San Juan Capistrano, CA 92675 • 714.496.8242**

Title : Juniper leaf and immature berries essential oil
Run File : C:\STAR\MODULE16\JUNIP002.RUN
Method File : C:\STAR\JUNIPERM.MTH
Sample ID : ws3jlb.eo

Injection Date: 9-JUL-96 10:07 AM Calculation Date: 9-JUL-96 11:07 AM

Operator : Flora Research jk
Workstation: FLORA
Instrument : Varian Star #1
Channel : B - wax

Detector Type: ADCB (10 Volts)
Bus Address : 16
Sample Rate : 20.00 Hz
Run Time : 60.001 min

*********** Star Chromatography Workstation ******* Version 4.5 ***************

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

| Peak No. | Peak Name | Result () | Ret. Time (min) | Time Offset (min) | Area (counts) | Sep. Code | Width 1/2 (sec) | Status Codes |
|---|---|---|---|---|---|---|---|---|
| 1 | a-pinene | 9.3400 | 3.470 | -0.070 | 2618686 | VV | 2.5 | R |
| 2 | camphene | 0.8422 | 3.897 | 0.034 | 236131 | VV | 1.9 | |
| 3 | B-pinene | 0.4779 | 4.407 | -0.004 | 133985 | VV | 2.4 | |
| 4 | sabinene | 11.2418 | 4.587 | 0.012 | 3151908 | VV | 3.0 | |
| 5 | myrcene | 1.6956 | 5.125 | -0.000 | 475395 | VV | 2.2 | |
| 6 | a-Phellandre | 0.6526 | 5.259 | 0.049 | 182977 | VV | 2.2 | |
| 7 | a-terpinene | 2.5307 | 5.538 | 0.034 | 709553 | VV | 2.4 | |
| 8 | limonene | 4.3888 | 5.939 | -0.102 | 1230506 | VV | 2.8 | R |
| 9 | B-phellandre | 2.1289 | 6.145 | 0.001 | 596882 | VP | 2.4 | |
| 10 | 1,8 cineol | 0.0171 | 6.246 | 0.026 | 4789 | TS | 0.0 | |
| 11 | g-terpinene | 4.1311 | 7.011 | 0.037 | 1158255 | PB | 3.2 | |
| 12 | p-cymene | 6.4207 | 7.640 | 0.056 | 1800207 | BB | 3.6 | |
| 13 | linalool | 0.2102 | 18.866 | 0.005 | 58941 | VV | 4.8 | |
| 14 | Bornyl aceta | 19.4243 | 20.545 | 0.173 | 5446064 | VV | 12.0 | |
| 15 | B-cary/T-4-o | 5.2717 | 21.563 | -0.240 | 1478035 | VV | 6.3 | R |
| 16 | borneol | 1.0017 | 26.647 | -0.144 | 280861 | VV | 12.1 | R |
| 17 | citronellol | 0.2094 | 30.515 | -0.020 | 58708 | VV | 0.0 | |
| | Totals: | 69.9847 | | -0.153 | 19621883 | | | |

Status Codes:
R - Reference peak

Total Unidentified Counts : 8415476 counts

Detected Peaks: 261 Rejected Peaks: 33 Identified Peaks: 17

Multiplier: 1 Divisor: 1

Baseline Offset: -36 microVolts

Noise (used): 340 microVolts - monitored before this run

Manual injection

Revision Log:

9-JUL-96 11:07 AM: Calculated results from channel B using method:
'C:\STAR\JUNIPERM.MTH'

Original Notes:

Juniper leaf ws3jlb June 20, 1996. It is a mix of immature berries and leaf.

*****************************************************************************

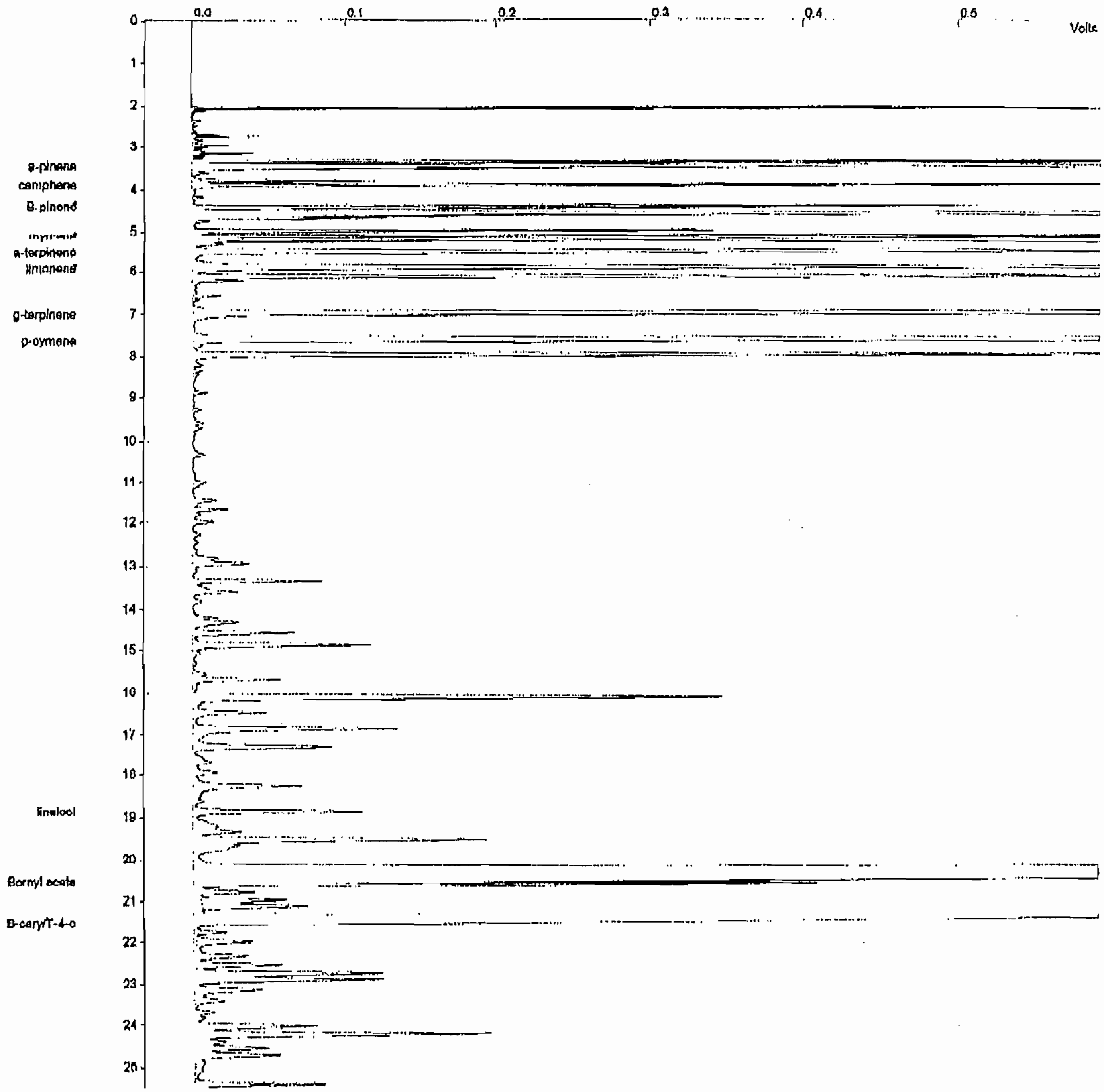
```
Title         : Juniper leaf and immature berries essential oil
Run File      : C:\STAR\MODULE16\JUNIP002.RUN
Method File   : C:\STAR\JUNIPERM.MTH
Sample ID     : ws3jlb.eo

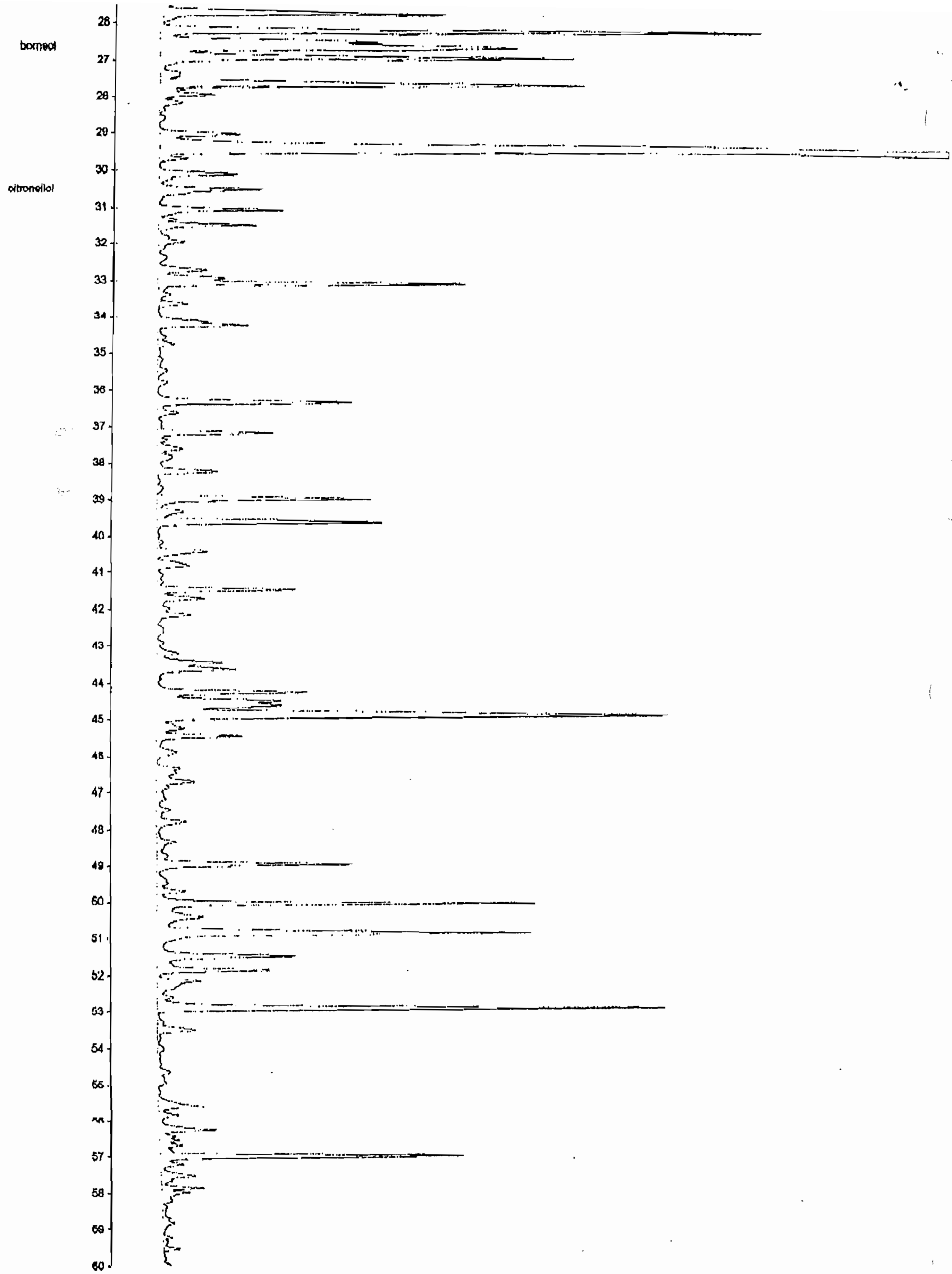
Injection Date: 9-JUL-96 10:07 AM    Calculation Date: 9-JUL-96 11:07 AM

Operator    : Flora Research jk       Detector Type: ADCB (10 Volts)
Workstation: FLORA                    Bus Address  : 16
Instrument : Varian Star #1           Sample Rate  : 20.00 Hz
Channel     : B = wax                 Run Time     : 60.001 min

*********** Star Chromatography Workstation ******* Version 4.5 ***************

Chart Speed =    0.75 cm/min   Attenuation = 256           Zero Offset = 5%
Start Time  =   0.000   min     End Time    = 60.001  min   Min / Tick  = 1.00
```




borneol
citronellol
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FLORA RESEARCH

## CERTIFICATE OF ANALYSIS

Date: 09-July-1996
Client:Essential Oil Co.

Sample I. D. : Juniperus occidentalis 4
Description:
The sample was received in a glass 5 ml. vial It was labelled with a label identifying it as Juniper leaf ws 4 jl.
Appearance:
Physical appearance is a clear oil with characteristic smell of juniper/pine oil.

Method of analysis:
High Resolution Capillary Gas Chromatography using dual channel analysis.

Results of analysis:

The analysis reveals an oil rich in monoterpines and bornyl acetate. The oil is unique in its chemistry and offers aroma chemicals similar to both pine oil and juniper.

**I hereby certify that I am an authorized representative of Flora Research and that to the best of my knowledge the following information is true and accurate.**

**Signature:** [signature]

**Title:** chromatographer **Date:** 9-July-96

31921 Camino Capistrano 435 • San Juan Capistrano, CA 92675 • 714.496.8242

Print Date: Tue Jul 09 12:24:20 1996

Title : Juniper Leaf essential oil ws4jl
Run File : C:\STAR\MODULE16\JUNIP003.RUN
Method File : C:\STAR\JUNIPERM.MTH
Sample ID : ws4jl.eo

Injection Date: 9-JUL-96 11:12 AM    Calculation Date: 9-JUL-96 12:12 PM

Operator : Flora Research jk
Workstation: FLORA
Instrument : Varian Star #1
Channel : B = wax

Detector Type: ADCB (10 Volts)
Bus Address : 16
Sample Rate : 20.00 Hz
Run Time : 60.001 min

*********** Star Chromatography Workstation ******* Version 4.5 **************

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

| Peak No. | Peak Name | Result () | Ret. Time (min) | Time Offset (min) | Area (counts) | Sep. Code | Width 1/2 (sec) | Status Codes |
|---|---|---|---|---|---|---|---|---|
| 1 | a-pinene | 7.2853 | 3.474 | -0.066 | 1460926 | VV | 1.7 | R |
| 2 | camphene | 0.8696 | 3.914 | 0.046 | 174392 | VV | 1.9 | |
| 3 | B-pinene | 0.3036 | 4.424 | 0.007 | 60879 | VV | 2.3 | |
| 4 | sabinene | 9.8431 | 4.592 | 0.009 | 1973840 | VV | 2.3 | |
| 5 | myrcene | 1.8200 | 5.144 | 0.010 | 364966 | VV | 2.3 | |
| 6 | a-Phellandre | 0.7325 | 5.280 | 0.061 | 146883 | VV | 2.3 | |
| 7 | a-terpinene | 2.8963 | 5.558 | 0.044 | 580806 | VV | 0.0 | |
| 8 | limonene | 4.0086 | 5.951 | -0.090 | 803840 | VV | 2.6 | R |
| 9 | B-phellandre | 2.4401 | 6.165 | 0.009 | 489324 | VP | 2.5 | |
| 10 | 1,8 cineol | 0.0063 | 6.271 | 0.039 | 1270 | TS | 0.0 | |
| 11 | g-terpinene | 4.7568 | 7.030 | 0.043 | 953886 | PV | 3.1 | |
| 12 | p-cymene | 8.6800 | 7.669 | 0.071 | 1740607 | VV | 3.6 | |
| 13 | linalool | 0.1703 | 18.910 | 0.023 | 34149 | VV | 4.9 | |
| 14 | Bornyl aceta | 22.0542 | 20.551 | 0.152 | 4422553 | VV | 10.8 | |
| 15 | B-cary/T-4-o | 5.9073 | 21.592 | -0.211 | 1184602 | VP | 6.0 | R |
| 16 | borneol | 1.0828 | 26.685 | -0.106 | 217137 | VV | 8.1 | R |
| 17 | citronellol | 0.1081 | 30.543 | -0.036 | 21673 | TF | 0.0 | |
| | Totals: | 72.9649 | | 0.005 | 14631733 | | | |

Status Codes:
R - Reference peak

Total Unidentified Counts : 5421372 counts

Detected Peaks: 270    Rejected Peaks: 49    Identified Peaks: 17

Multiplier: 1    Divisor: 1

Baseline Offset: -11 microVolts

Noise (used): 220 microVolts - monitored before this run

Manual injection

Revision Log:

9-JUL-96 12:12 PM: Calculated results from channel B using method:
'C:\STAR\JUNIPERM.MTH'

Original Notes:

Juniper leaf essential oil ws4jl June 20, 1996 from 80 year old tree. (ws1jl from 135 year old tree)

*************************************************************************************

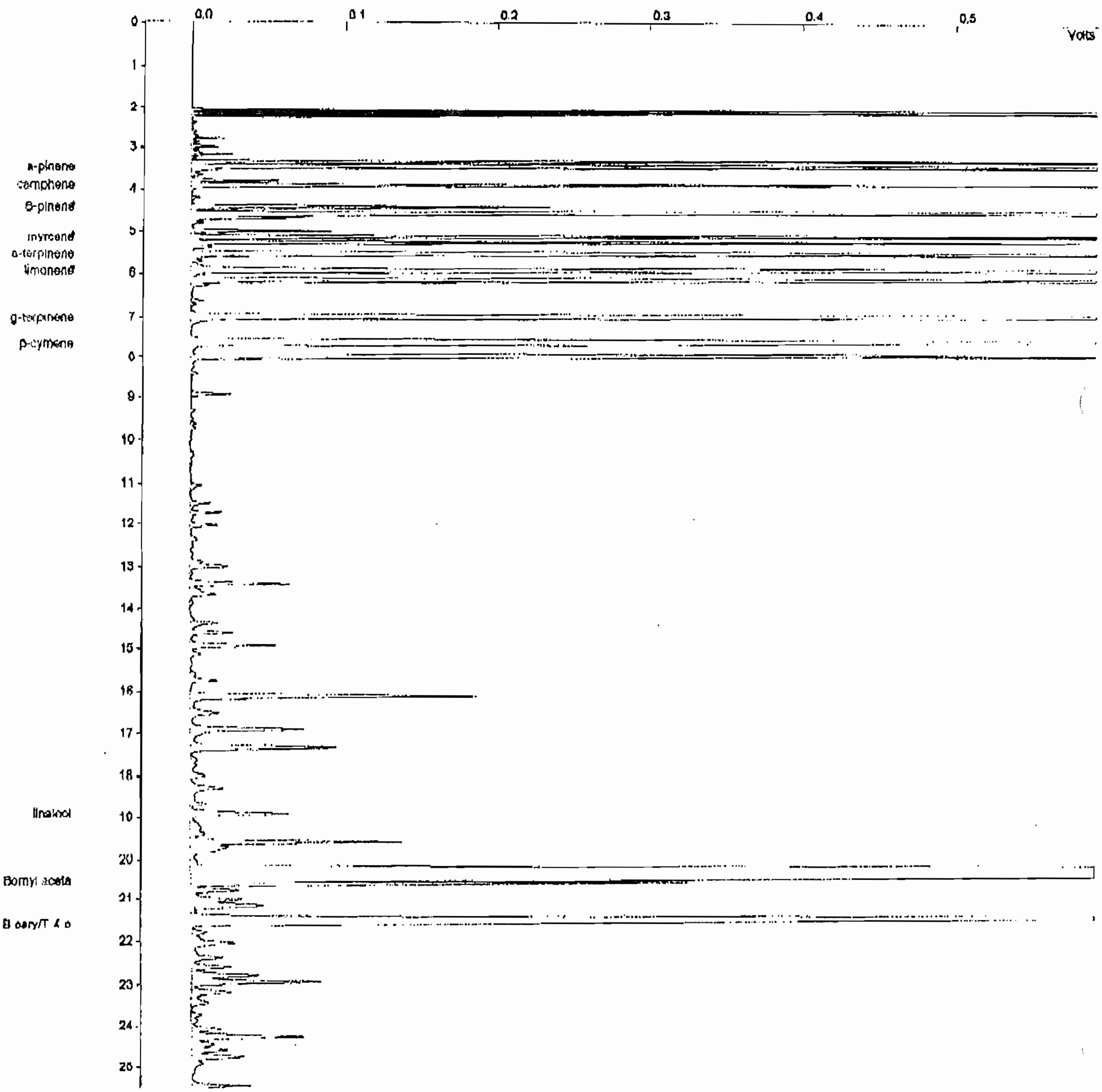
```
Title          : Juniper Leaf essential oil ws4jl
Run File       : C:\STAR\MODULE16\JUNIP003.RUN
Method File    : C:\STAR\JUNIPERM.MTH
Sample ID      : ws4jl.eo

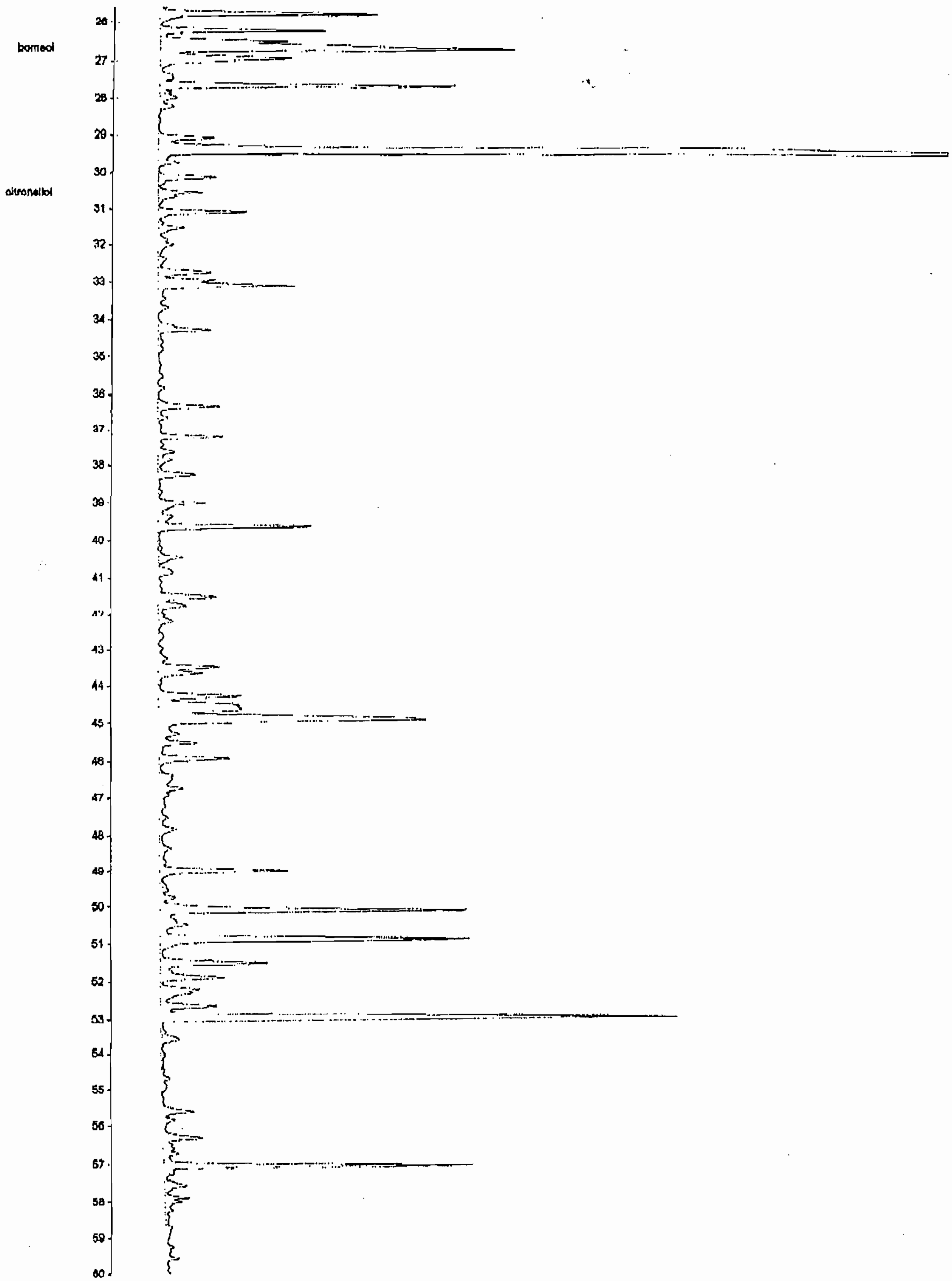
Injection Date: 9-JUL-96 11:12 AM    Calculation Date: 9-JUL-96 12:12 PM

Operator    : Flora Research jk      Detector Type: ADCB (10 Volts)
Workstation: FLORA                   Bus Address  : 16
Instrument : Varian Star #1          Sample Rate  : 20.00 Hz
Channel     : B = wax                Run Time     : 60.001 min

*********** Star Chromatography Workstation ******* Version 4.5 ***************

Chart Speed =    0.75 cm/min   Attenuation = 256          Zero Offset = 5%
Start Time  =   0.000   min     End Time    = 60.001  min   Min / Tick  = 1.00
```





borneol
citronellol
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# FLORA RESEARCH

## CERTIFICATE OF ANALYSIS

Date: 09-July-1996
Client:Essential Oil Co.

Sample I. D. : Juniperus occidentalis distillation water
Description:
The sample was received in a glass 5 ml. vial It was labelled with a label identifying it as Juniper leaf ws 4jl water.
Appearance:
Physical appearance is a clear watery oil with characteristic smell of juniper/pine oil.

Method of analysis:
High Resolution Capillary Gas Chromatography using dual channel analysis.

Results of analysis:

The analysis reveals a water that is very high in bornyl acetate and caryophyllene.

I hereby certify that I am an authorized representative of Flora Research and that to the best of my knowledge the following information is true and accurate.

**Signature:**

**Title:** chromatographer

**Date:** 9-July-96

31921 Camino Capistrano 435 • San Juan Capistrano, CA 92675 • 714.496.8242

Title : Juniper Leaf Distillation Water
Run File : C:\STAR\MODULE16\JUNIP009.RUN
Method File : c:\star\juniperm.mth
Sample ID : ws4jl water.eo

Injection Date: 9-JUL-96 3:05 PM Calculation Date: 9-JUL-96 4:08 PM

Operator : Flora Research jk
Workstation: FLORA
Instrument : Varian Star #1
Channel : B = wax

Detector Type: ADCB (10 Volts)
Bus Address : 16
Sample Rate : 10.00 Hz
Run Time : 60.002 min

*********** Star Chromatography Workstation ******* Version 4.5 ***************

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

| Peak No. | Peak Name | Result () | Ret. Time (min) | Time Offset (min) | Area (counts) | Sep. Code | Width 1/2 (sec) | Status Codes |
|---|---|---|---|---|---|---|---|---|
| 1 | Ethanol tid | | 2.630 | | | | | M |
| 2 | a-pinene | | 3.540 | | | | | M |
| 3 | camphene | | 3.938 | | | | | M |
| 4 | B-pinene | | 4.493 | | | | | M |
| 5 | sabinene | | 4.660 | | | | | M |
| 6 | myrcene | | 5.217 | | | | | M |
| 7 | a-Phellandre | | 5.302 | | | | | M |
| 8 | a-terpinene | | 5.600 | | | | | M |
| 9 | limonene | | 6.041 | | | | | M |
| 10 | B-phellandre | | 6.248 | | | | | M |
| 11 | 1,8 cineol | | 6.324 | | | | | M |
| 12 | g-terpinene | | 7.085 | | | | | M |
| 13 | p-cymene | 4.3443 | 7.615 | -0.086 | 1579 | BB | 2.8 | |
| 14 | linalool | | 19.077 | | | | | M |
| 15 | Bornyl aceta | 48.6948 | 20.298 | -0.303 | 17702 | BB | 5.0 | |
| 16 | B-cary/T-4-o | 35.5451 | 21.528 | -0.275 | 12922 | BB | 5.0 | |
| 17 | borneol | | 26.791 | | | | | M |
| 18 | unknown | 8.4962 | 29.400 | -0.040 | 3089 | BB | 5.5 | |
| 19 | citronellol | | 30.605 | | | | | M |
| 20 | | 2.9196 | 50.942 | 0.000 | 1061 | BB | 5.7 | |
| | Totals: | 100.0000 | | -0.704 | 36353 | | | |

Status Codes:
M - Missing peak

Total Unidentified Counts : 1061 counts

Detected Peaks: 17 Rejected Peaks: 12 Identified Peaks: 19

Multiplier: 1 Divisor: 1

Baseline Offset: 5 microVolts

'oise (used): 330 microVolts - monitored before this run

Manual injection

Data Handling: Reference peak not identified correctly
Data Handling: Default to A%
Data Handling: Non-reference peak not identified
Data Handling: Non-reference peak not identified
Data Handling: Non-reference peak not identified
Data Handling: Non-reference peak not identified
Data Handling: Non-reference peak not identified
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Data Handling: Non-reference peak not identified
Data Handling: Non-reference peak not identified
Data Handling: Non-reference peak not identified
Data Handling: Non-reference peak not identified
Data Handling: Non-reference peak not identified
Data Handling: Non-reference peak not identified

Revision Log:

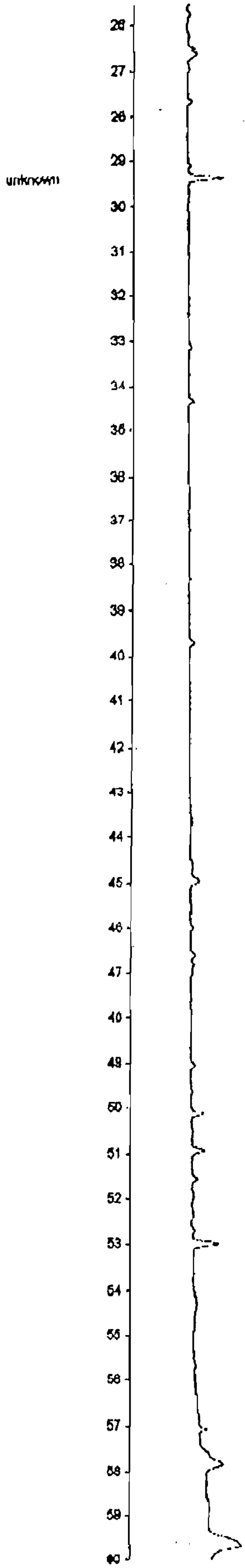
9-JUL-96 4:05 PM: Calculated results from channel B using method:
'C:\STAR\JUNIPERM.MTH'
9-JUL-96 4:08 PM: Calculated results from channel B using method:
'c:\star\juniperm.mth'

Original Notes:

still water from ws4jl 6/20/96 second run.

Appended Notes:

*******************************************************************************



unknown

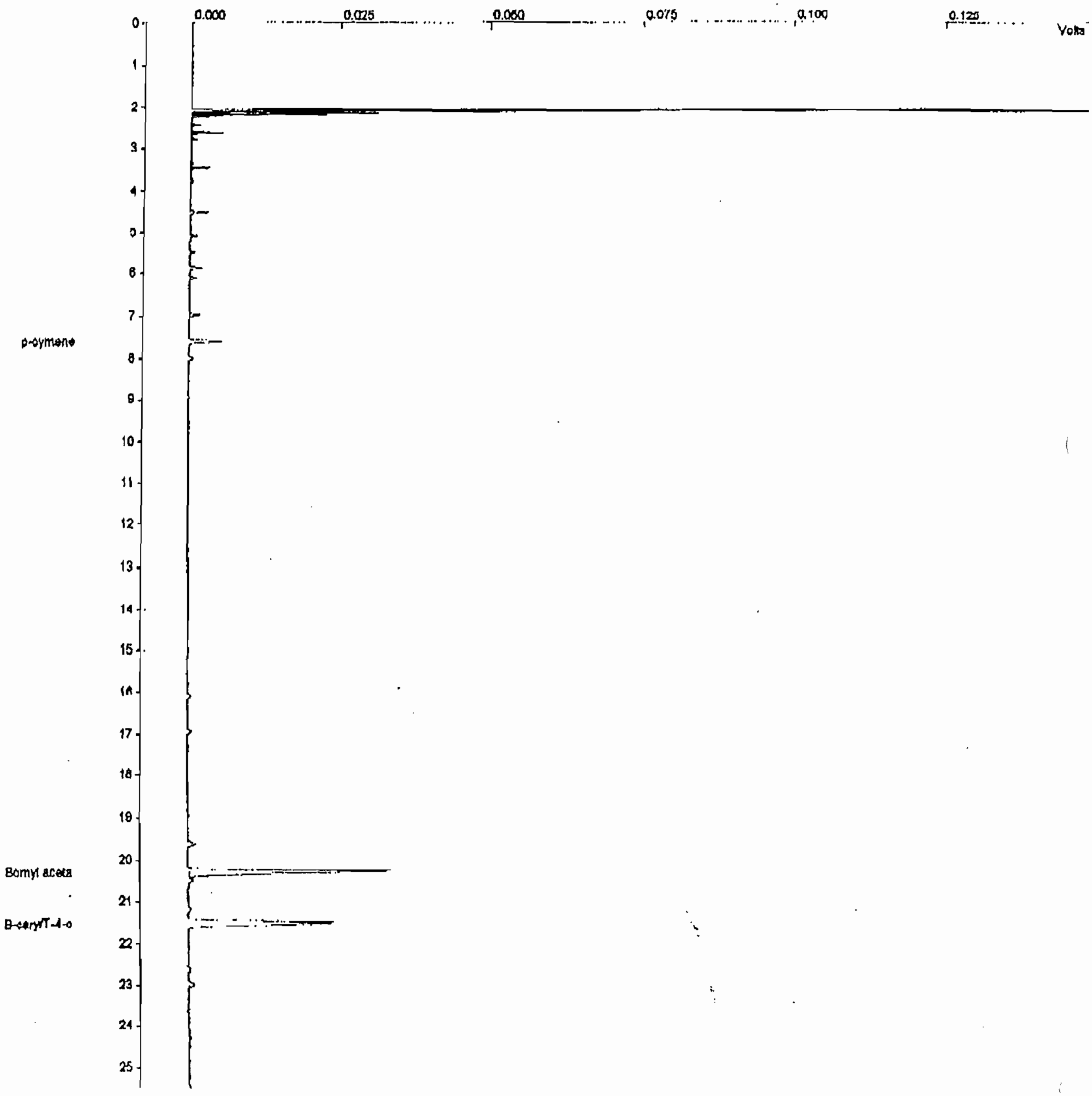
Title : Juniper Leaf Distillation Water
Run File : C:\STAR\MODULE16\JUNIP009.RUN
Method File : c:\star\juniperm.mth
Sample ID : ws4jl water.eo

njection Date: 9-JUL-96 3:05 PM    Calculation Date: 9-JUL-96 4:08 PM

Operator : Flora Research jk    Detector Type: ADCB (10 Volts)
Workstation: FLORA    Bus Address : 16
Instrument : Varian Star #1    Sample Rate : 10.00 Hz
Channel : B = wax    Run Time : 60.002 min

*********** Star Chromatography Workstation ******* Version 4.5 ***************

Chart Speed = 0.75 cm/min    Attenuation = 64    Zero Offset = 5%
Start Time = 0.000 min    End Time = 60.002 min    Min / Tick = 1.00



## Bibliography

(with copies)


(1) E.F. Kurth and J.D. Ross (1954), "Volatile Oil From Western Juniper", Report #C-3: Oregon Forest Products Laboratory, Oregon State University.

(2) Robert P. Adams (1987), "Investigation of Juniperus Species of the United States for New Sources of Cedarwood Oil": Economic Botany, New York Botanical Garden.

(3) H.L. Gholz (1979), "Structure and Productivity of Juniperus Occidentalis in Central Oregon": School of Forestry, Oregon State University.

(4) Robert P. Adams (1987), "Yields and Seasonal Variation of Phytochemicals from Juniperus Species of the United States": Biology Department, Baylor University.

# Volatile Oil from Western Juniper

By

E. F. Kurth

J. D. Ross

Report No C-3

April 1954

OREGON FOREST PRODUCTS LABORATORY

State Board of Forestry and School of Forestry,

Oregon State College, Cooperating

Corvallis

TABLE OF CONTENTS

SUMMARY

Thirty-two charges of wood from western juniper trees were steam distilled. The volatile oil was recovered, and its composition and yield determined.

The wood was reduced to various-size particles by chipping and by hammer-milling. Some charges contained bark; some were of wood only.

Low steam pressures (under 10 psi) and hammer-milled chips gave oil yields of higher quality and greater volume.

Oil recovery averaged around 1.4 per cent, of which about 15-40 per cent was cedrol.

VOLATILE OIL FROM WESTERN JUNIPER

by

E. F. Kurth

J. D. Ross

## INTRODUCTION

In large areas of central and eastern Oregon the principal tree species is western juniper (Juniperus occidentalis Hook.). The principal uses for the wood are confined at present to fence posts, novelties, and fuel. Most large trees are decayed internally by fungus attack, making difficult the recovery of lumber or pencil stock.

In an effort to find high-value products from the species which would permit its economic utilization, an investigation of its properties and constituents was initiated at the Oregon Forest Products Laboratory.

The present report is a study of steam distillation of the volatile oils in the wood.

Previous studies of western juniper wood included an investigation in 1947 of the amount and composition of the extractives in the wood.[1] This investigation showed yields of 0.9-1.25 per cent of volatile oil (based on the weight of dry wood), with the main constituents being cedrol and cedrene. These materials are also present in commercial cedarwood oil recovered from eastern redcedar heartwood (J. virginiana) and species of juniper (J. ashei Buchholz, J. flaccida Schlecht., and J. monosperma Sarg.) growing in Texas and Northern Mexico. Other extractives found in western juniper wood were fatty and resin acids, phytosterol, resenes, and a catechol phlobaphene.

A further study of the volatile-oil content of juniper trees was made in the fall of 1951 on trees growing on moist ground along stream beds, dry ground, and arid lava-ash-type soil east and south of Prineville, Oregon.[2] In this investigation, five trees about 16 inches in diameter two feet above the ground were felled and sampled for each type soil; cross-sectional disks were then cut from the butt logs and the top logs to obtain information on the distribution of the oil within the tree trunk and under different growing conditions. The yield of volatile oil from these trees was determined in the Laboratory and is given in Table 1.

Table 1. Yield of Volatile Oil from Juniper Trees
Growing on Different Types of Soil.

| Soil type | Volatile oil | |
|---|---|---|
| | Butt[1] | Top[2] |
| | Per cent[3] | Per cent[3] |
| Moist | 2.43 | 1.12 |
| Dry | 2.35 | 1.01 |
| Arid lava-ash | 2.01 | 1.14 |

[1] Two feet above ground level.
[2] Six-inch top diameter.
[3] On an oven-dry weight basis.

The data show that the yield of volatile oil from all of the trees was similar; the yield of oil from the wood at the 2-foot above-ground height varied from 2.01 to 2.43 per cent, whereas that from the top of the trees varied from 1.01 to 1.14 per cent. The average yield at the bottom of the trees was 2.26 per cent and that from the top of the trees was 1.09 per cent, giving an average yield for top and bottom disks of 1.73 per cent.

In June 1952, the John D. Walsh Company, New York City, advised the Laboratory that the price of cedrol was from 3.50 to 4.00 dollars a pound and requested that crude and refined samples of the oil steam-distilled from western juniper wood be submitted to them for market evaluation. In this connection, fresh lots of juniper wood were obtained, the wood was then chipped or hogged, and the chipped or hogged material was steam-distilled at various steam pressures and for different periods of time to ascertain the optimum conditions of oil recovery. Samples of the oil were submitted for market evaluation and the use of the oil-free steamed wood residue was explored for fiberboard production. The purpose was to establish whether an integrated operation combining oil recovery with use of the spent wood residue for fiberboard production would be economically feasible.

## EXPERIMENTAL PROCEDURE

The first lot of juniper wood was collected by Laboratory personnel in the Prineville, Oregon, area during the latter part of May 1953. Living trees were felled and cut into 4-foot lengths. These were hand-barked, slabbed, and chipped into conventional pulp-size chips. Where necessary they were further sized by passing through a chip breaker. A part of the chips was further processed by hammer-milling to ascertain the effect of particle size on the rate of oil distillation.

The influence of particle size on oil yield was explored on a small scale in a Clevenger apparatus. The results on 1/2-inch wood chips, hammer-milled chips, and Wiley-milled chips passing a 1/2-inch screen are shown in Table 2. It was observed that large chips gave substantially lower yields of oil than did fine material.

Table 2. Effect of Particle Size on Oil Yield in a Clevenger Apparatus.

| Material | Time of distillation | Volumetric yield | Gravimetric yield |
|---|---|---|---|
| | Hours | Per cent[1] | Per cent[1] |
| 1/2" chips | 20 | 0.63 | 0.40 |
| Hammer-milled chips | 30 | 1.08 | 0.85 |
| Wiley-milled chips | 30 | 1.20 | 0.92 |

[1] Dry wood basis.

The Laboratory's pulp digester, with some modifications, was used for the large-scale steam distillations of juniper wood. Steam was admitted at the bottom of the digester, passed up through the bed of wood chips, and the vapors were vented from the top of the digester through a 50-foot spiral of 3/8-inch copper tubing contained in a 10-gallon metal can through which cooling water was passed.

## First Series of Distillations

The condensate was collected in a 6-liter aspirator bottle filled with 1/4-inch Raschig rings, water, and hexane as shown in Figure 1. The oil dissolved in the hexane, which was drained off at the end of a run and fractionally distilled to leave the crude oil. Some of the oil was lost along with some hexane through incomplete separation of the hexane from the water layer.

In operation, the digester was preheated for 15-30 minutes with the cover off, then loaded with wood from which a composite sample had been taken for moisture determination. The digester was then closed, inverted, and the condenser connection attached. Condenser cooling water was started and steam admitted to the charge according to a set pressure regulated by a temperature controller attached to the digester. Rate of vapor discharge was regulated by manual control of a valve in the vent line. Condensed vapors were conducted through the hexane trap, and waste water was collected and weighed as the distillation progressed. At the end of the distillation, usually 4-6 hours, the condenser was shut off and the condensate temperature allowed to rise in order to flush out any oil trapped in the condenser. After this, the hexane was drained off and distilled in a laboratory apparatus to leave the crude oil. After standing overnight, the waste water was skimmed with paper to recover traces of oil; this was then added to the main sample of oil.

Data for all runs are compiled in Table 3. The yields of oil increased with increase in steam pressures. Some of the yields were rather irregular; this may be attributed to (1) channeling of steam through the charge, (2) differences in proportions of heartwood and sapwood in the charges, (3) possible retention of oils in the condenser from one run to another and (4) incomplete separation of the oil from the aqueous condensate.

Rate of vapor venting was not investigated to any extent because of its irregularity and the inability to control it satisfactorily. It appears customary to use considerably more than theoretical amounts of steam in volatile oil distillations, so this practice was followed (3, Volume I, 159-166).

In runs 10, 11, 12, the hexane trap was drained at the end of the first hour, the third hour, and at the end of the run. The crude oil was recovered and an approximate distillation rate established. The results of these runs are shown in Table 4. These values do not appear reliable, probably because of appreciable quantities of oil held up in the condenser. The values for rate of distillation shown in Figure 4 are considered more representative.

Table 3. Data from Steam Distillation of Western Juniper Wood; First Series.

| Run | Material | Wet wt of charge | Moisture content | Dry wt of charge | Steam pressure | Distillation time | Wt of condensate | Wt of crude oil | Oil yield |
|---|---|---|---|---|---|---|---|---|---|
| | | Lb | Per cent[1] | Lb | Psi | Hr:Min | Lb | Lb | Per cent[2] |
| 1 | Chips[3] | 20 | 53.3 | 9.3 | 0 | 3:05 | Discarded | | — |
| 2 | Chips | 57 | 55.5 | 25.3 | 5 | 6:00 | 214 | 0.06 | 0.24 |
| 3 | H.M. Chips[4] | 60 | 42.1 | 34.6 | 35 | 4:45 | 392 | 0.386 | 1.11 |
| 4 | H.M. Chips | 60 | 49.5 | 30.3 | 15 | 6:15 | 358 | 0.114 | 0.38 |
| 5 | H.M. Chips | 54 | 43.2 | 30.6 | 60 | 4:10 | 453 | 0.446 | 1.46 |
| 6 | H.M. Chips | 56 | 42.8 | 32.0 | 5 | 6:00 | 402 | 0.271 | 0.85 |
| 7 | Chips | 56 | 43.2 | 31.7 | 25 | 6:00 | 492 | 0.334 | 1.05 |
| 8 | H.M. Chips | 50 | 49.2 | 25.4 | 5 | 6:00 | 254 | 0.111 | 0.44 |
| 9 | H.M. Chips | 50 | 34.0 | 33.0 | 40 | 6:00 | 356 | 0.60 | 1.82 |
| 10 | H.M. Chips | 45 | 36.9 | 28.4 | 40 | 5:45 | 427 | 0.35 | 1.23 |
| 11 | H.M. Chips | 41 | 37.5 | 25.5 | 20 | 5:45 | 422 | 0.37 | 1.45 |
| 12 | H.M. Chips | 39 | 31.5 | 26.6 | 0 | 6:20 | 241 | 0.201 | 0.76 |
| 13 | Chips | 64 | 34.5 | 42.0 | 25 | 5:15 | 376 | 0.396 | 0.945 |
| 14 | Chips | 60 | 33.7 | 39.7 | 45 | 5:35 | 368 | 0.302 | 0.76 |
| 15 | Chips | 42 | 32.8 | 28.2 | 40 | 6:10 | 439 | 0.312 | 1.11 |
| 16 | Chips | 52 | 16.5 | 43.4 | 60 | 8:00 | 519 | 0.464 | 1.07 |
| 17 | H.M. Chips | 34 | 18.3 | 27.8 | 60 | 8:00 | 596 | 0.209 | 0.75 |

1 On the wet basis.

2 On the oven-dry basis.

3 Chips -- Conventional 1/2-inch pulp chips.

4 H.M. Chips -- Hammer-milled chips.

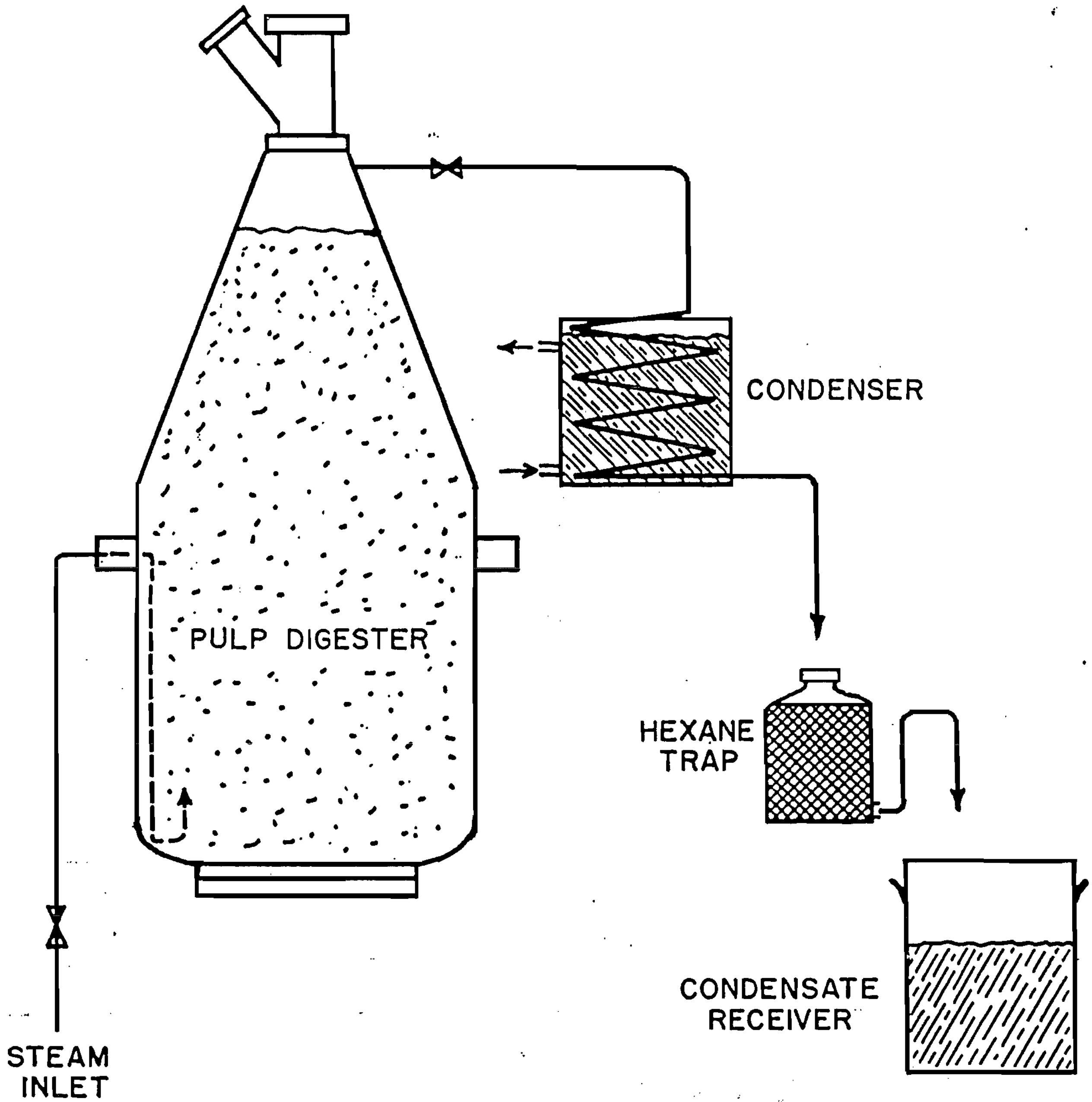



FIGURE I. STEAM DISTILLATION APPARATUS.

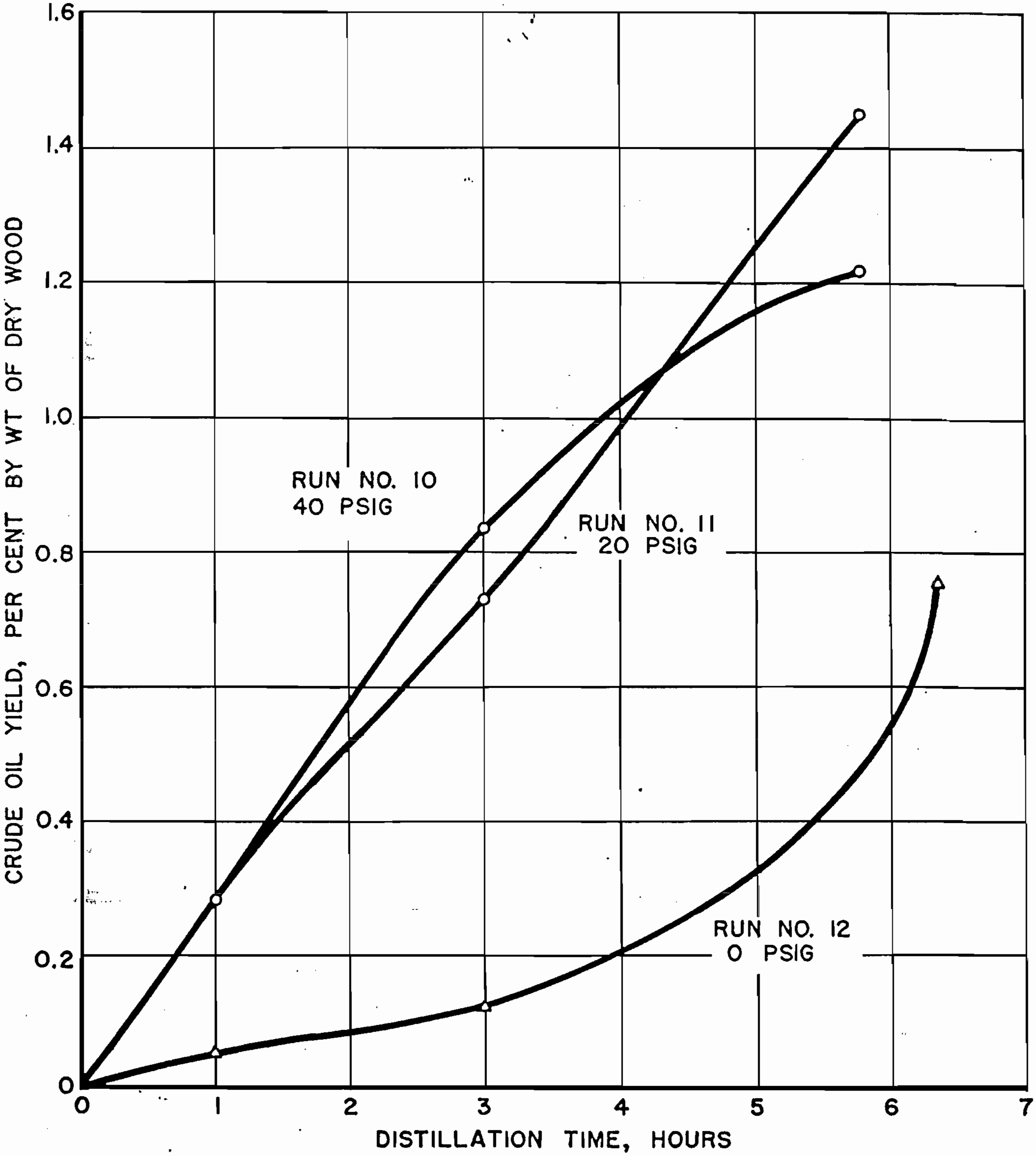



FIGURE 2. RATE OF STEAM DISTILLATION OF CRUDE JUNIPER-WOOD OIL.

Table 4. Rate of Steam Distillation.

| Run | Dry wood | Steam pressure | Oil recovered 0-1 hr | Oil recovered 1-3 hr | Oil recovered end | Over-all yield |
|---|---|---|---|---|---|---|
| | Lb | Psi | Lb | Lb | Lb | Per cent[1] |
| 10 | 28.4 | 30 | 0.082 | 0.155 | 0.113 | 1.23 |
| 11 | 25.5 | 20 | 0.073 | 0.114 | 0.18 | 1.45 |
| 12 | 26.6 | 0-1 | 0.0154 | 0.0187 | 0.168 | 0.76 |

[1] Oven-dry weight basis.
Rate curves for these runs are shown in Figure 2.

Runs 2, 7, 13, 14, 15 were made on whole chips, primarily to supply material for fiberboard tests. The yields appeared to be slightly lower than from hammer-milled chips, although the difference was not so great as was expected. No determination of distillation rate was made on whole chips, since this distillation appeared to be slower than that of the hammer-milled chips.

Examination of the crude oil

The variation in oil yields indicated that there might be some difference in oil composition obtained by various steam temperatures. Samples of crude oil were therefore vacuum distilled to determine any substantial difference in composition.

In each case, 50 grams of crude oil were vacuum distilled at 10 mm pressure. Material balances for these distillations are shown by Table 5.

Table 5. Material Balances for Vacuum Distillations.

| Oil from run -- | Distillate | Residue | Loss | Recovery |
|---|---|---|---|---|
| | Grams | Grams | Grams | Per cent[1] |
| 5 (60 psi) | 40.59 | 4.17 | 5.24 | 81.5 |
| 6 (5 psi) | 42.13 | [illegible] | [illegible] | [illegible] |
| 8 (5 psi) | 40.93 | 3.29 | [illegible] | [illegible] |
| 9 (40 psi) | [illegible] | 3.03 | [illegible] | [illegible] |

By observation of the temperature and the nature of the distillate, it was possible to estimate the yields of cedrene and cedrol. In each case, the cedrol fraction appeared to come over at 100 deg C and above. The low-pressure runs yielded an oil having higher cedrol content than that from high-pressure runs. Since the total oil yield was higher for high-pressure runs, the over-all cedrol yield was higher for these pressures.

This situation is indicated by a rough calculation of the cedrol-fraction yields shown in Table 6. For this approximation, specific gravities of 0.94 were used for all fractions, and 100 deg C was taken as the division between cedrol and cedrene.

Table 6. Approximate Calculation of Cedrol Content of Various Oils from Vacuum-distillation Data.

| Run | Steam pressure | Low-boiling fraction | | High-boiling fraction | | Cedrol content | Cedrol yield |
|---|---|---|---|---|---|---|---|
| | | Volume | Weight | Volume | Weight | | |
| | Psi | Ml | Grams | Ml | Grams | Per cent[1] | Per cent[2] |
| 5 | 60 | 26.0 | 24.4 | 14.0 | 13.21 | 35.0 | 0.51 |
| 6 | 5 | 23.5 | 22.1 | 20.5 | 19.3 | 46.5 | 0.395 |
| 8 | 5 | 13.0 | 12.2 | 28.0 | 26.3 | 68.2 | 0.30 |
| 9 | 40 | 23.5 | 22.1 | 16.0 | 15.0 | 40.5 | 0.736 |

[1] Based on weight of crude oil.
[2] Based on dry weight of wood.

While this calculation is admittedly approximate, it does indicate that the low-pressure oils were richer in cedrol, but high pressures gave better over-all yield of cedrol.

Three samples of juniper oil were submitted to the John D. Walsh Company for evaluation. The samples and their properties are shown in Table 7. This company reported that all three samples showed high cedrol contents, but that they had an odor significantly different from that obtained from the <u>mexicana</u>

(*ashei*) or *virginiana* species of juniper. Specifically, the oil was reported to have a greasy, unpleasant odor which repeated distillation did not remove.

Table 7. Properties of Volatile Oil Samples Sent to the John D. Walsh Co.

| Sample | Refractive index at 20° C. | Ester (cedryl acetate) content | Alcohol content |
|---|---|---|---|
| | | Per cent | Per cent |
| 1. Crude oil | 1.4988 | 10.3 | 48.1 |
| 2. Vacuum-distilled oil | 1.5075 | 5.4 | 45.2 |
| 3. Cedrol fraction | 1.5125 | 10.4 | 84.4 |

## Second Series of Distillations

Additional work was undertaken with the purpose of eliminating the greasy odor from the oil, ascertaining the suitability of mechanical fiber prepared from the steamed wood residue, and obtaining further information on the best operating conditions for oil recovery. Another lot of juniper wood was collected in October 1953 which contained some heartwood rot. The hexane trap previously used to recover the oil was discarded, and a water trap installed. This trap, shown in Figure 3, permitted continuous observation and measurement of the oil as it collected and thus the rate of distillation could be calculated.

Operating procedure was the same as before. The digester was preheated, loaded with a weighed charge, and steam passed through at controlled temperature and rate for the desired length of time. Samples of the charge were oven-dried to determine dry weight of the charge. The oil, recovered with some water, was placed in a separatory funnel until a clear separation was obtained, then removed and weighed.

The variables involved were (1) particle size of wood, (2) digester steam pressure, (3) rate of steam flow, (4) distillation time, and (5) presence or absence of bark in the charge.

Only two particle sizes were used in the new series of runs, since the spent material was to be used for board products. These were (1) standard 1/2-inch pulp chips suitable for fiber production and (2) hammer-milled chips for particle-board production.

The operating steam pressure was varied from 10 to 50 psi. Calculations, included in the appendix, on theoretical steam requirements for distillation of cedrol, indicated that 50-60 psi was probably the practical maximum pressure.

The rate of steam flow was reduced considerably for this work over that previously used. The rate of steaming is relatively unimportant, except from the economic standpoint, since the theoretical consumption is necessarily exceeded several-fold in practice. While the theoretical steam consumption for distillation of cedrol is about 20-25 pounds of steam per pound of oil at 50-60 pounds per square inch, the actual consumption in practice for recovery of cedarwood oil is around 100 lb/lb oil (3, Vol 1, p. 159). This figure was approached in some of the runs in this series.

The time of distillation was not investigated to any extent in this series of runs, for the new oil trap permitted visual observation of the rate of oil recovery. Therefore, it was possible to cut off a distillation when oil recovery became small.

The charges were steamed both with and without bark. It is questionable whether much distinction can be made in the two types of charges, for the juniper logs were rather difficult to peel and there was some bark (especially inner bark) left in the irregularities of the logs. It is probable that any commercial operation will use both wood and bark because of the difficulty in barking the twisted and irregular shapes that commonly occur in juniper logs.

Some 15 runs were made; a tabulation of pertinent data is shown in Table 8.

Figure 4 shows the relationship between the rate of oil recovery and the time of distillation.

The first 5 runs were made with hammer-milled wood, the remaining 10 runs on chips. It will be observed that the yields and distillation rates are higher for the smaller particle sizes. This difference is more pronounced at lower steam pressures and tends to diminish at higher pressures.

It appeared that the presence of bark in the charge reduced the oil yield slightly; however, the experimental results were too erratic to draw a definite conclusion.

As previously observed, higher yields and faster distillation were achieved with higher steam pressures. It appeared, however, that at any pressure, the bulk of the oil was recovered within the first 3-4 hours.

The steam consumption, as far as oil recovery was concerned, was relatively unimportant. The minimum rate of steaming in this case was dictated by the amount of flow necessary to keep the condenser open. It was possible to operate with a rate of about 100-150 pounds of steam per pound of oil, which conforms to commercial practice. Run 13 used a high excess of steam. There was no apparent difference in the characteristics of the run with the exception that the oil was more difficult to trap because of dispersion in the large amount of water. The steam consumption also became high and somewhat unrealistic when the distillation was continued beyond a practical length of time.

<u>Examination of the crude oil</u>

The crude oil, as recovered, was a dark-red, opaque liquid. The odor was somewhat harsh, suggestive of the odor of the foliage or bark. There was no apparent difference in odor between oil from bark-free and bark-included charges.

Table 8. Data from Steam Distillation of Western Juniper Wood; Second Series.

| Run | Material[1] | Wet wt of wood | Moisture content | Dry wt of wood | Steam pressure | Distillation time | Wt oil recovered | Yield | Steam/oil |
|---|---|---|---|---|---|---|---|---|---|
| | | Lb | Per cent[2] | Lb | Psi | Hours | Lb | Per cent | Lb/lb |
| 1 | 6 hm - wob | 57.5 | 44.0 | 32.1 | 50 | 6 1/2 | 0.53 | 1.65 | 240 |
| 2 | 5 hm - wb | 52.0 | 46.0 | 28.1 | 50 | 5 3/4 | 0.353 | 1.26 | 246 |
| 3 | 6 hm - wob | 44.0 | 37.5 | 27.4 | 50 | 3 | 0.398 | 1.46 | 126 |
| 4 | 5 hm - wb | 48.0 | 43.0 | 27.4 | 50 | 3 3/4 | 0.34 | 1.24 | 246 |
| 5 | 5 hm - wb | 40.0 | 37.0 | 25.0 | 10 | 7 | 0.345 | 1.38 | 385 |
| 6 | 3 chips-ns-wob | 39.0 | 45.0 | 21.4 | 10 | 7 | 0.187 | 0.88 | 700 |
| 7 | 3 chips-ns-wob | 49.0 | 36.5 | 31.0 | 50 | 7 | 0.358 | 1.16 | 328 |
| 8 | 2 chips-ns-wb | 42.0 | 43.0 | 23.9 | 30 | 7 | 0.230 | 0.96 | 505 |
| 9 | 4 chips-s-wob | 44.0 | 41.5 | 25.8 | 30 | 4 | 0.274 | 1.07 | 224 |
| 10 | 1 chips-s-wb | 39.0 | 41.5 | 22.8 | 50 | 3 1/3 | 0.176 | 0.78 | 305 |
| 11 | 4 chips-s-wob | 44.0 | 38.5 | 27.0 | 50 | 3 | 0.313 | 1.16 | 144 |
| 12 | 4 chips-s-wob | 46.0 | 40.5 | 27.4 | 50 | 3 | 0.30 | 1.10 | 146 |
| 13 | 4 chips-s-wob | 41.0 | 39.5 | 24.8 | 10 | 8 | 0.15 | 0.61 | 2050 |
| 14 | 2 chips-ns-wb | 49.0 | 37.5 | 30.5 | 50 | 4 | 0.39 | 1.28 | 118 |
| 15 | 7 trash chips | 33.0 | 35.0 | 21.5 | 10 | 4 1/2 | 0.172 | 0.80 | 495 |

[1] hm -- hammer-milled
wob -- without bark
wb -- with bark
ns -- not screened
s -- screened

[2] On a wet-weight basis.

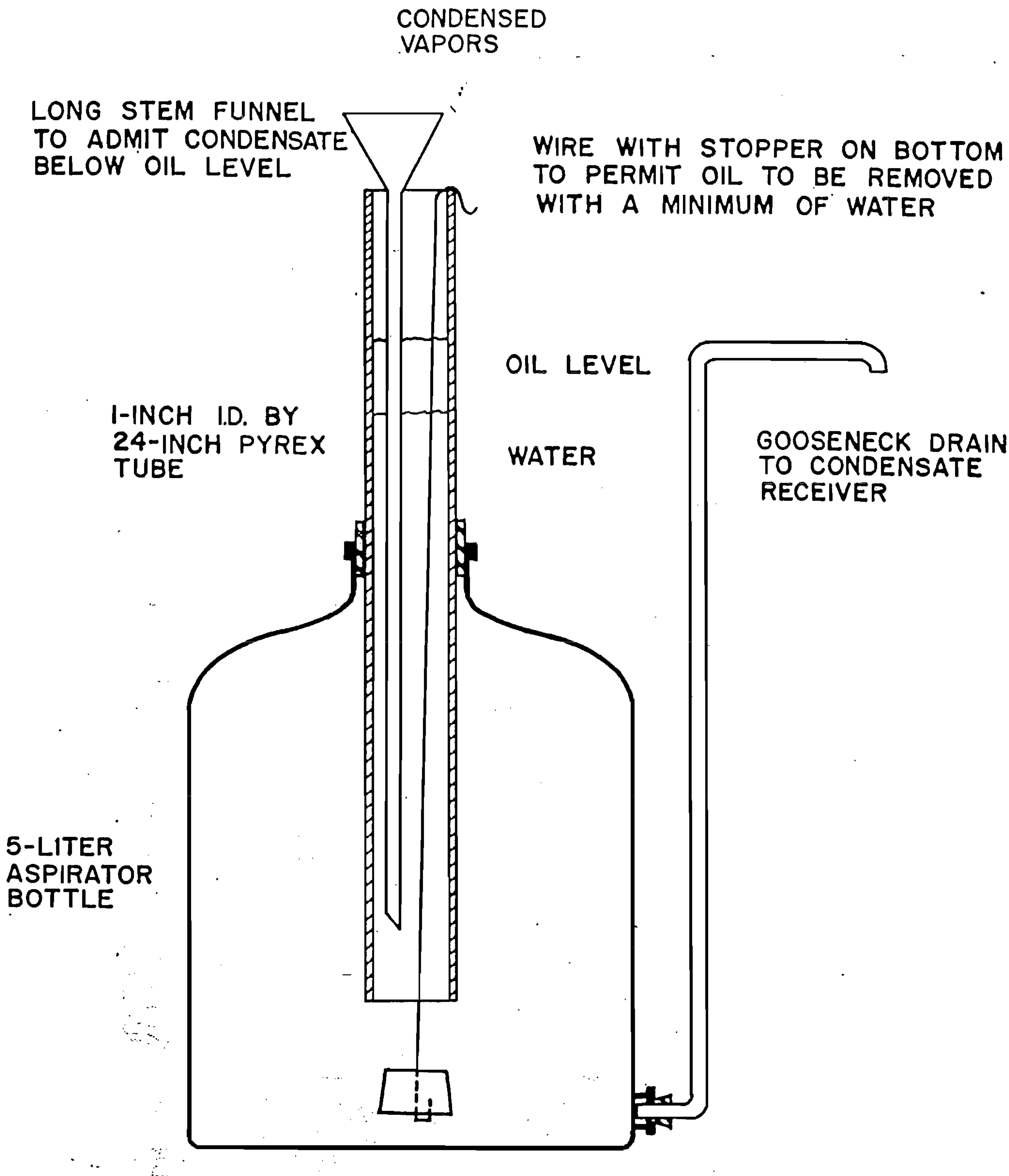



FIGURE 3. SKETCH OF OIL TRAP.

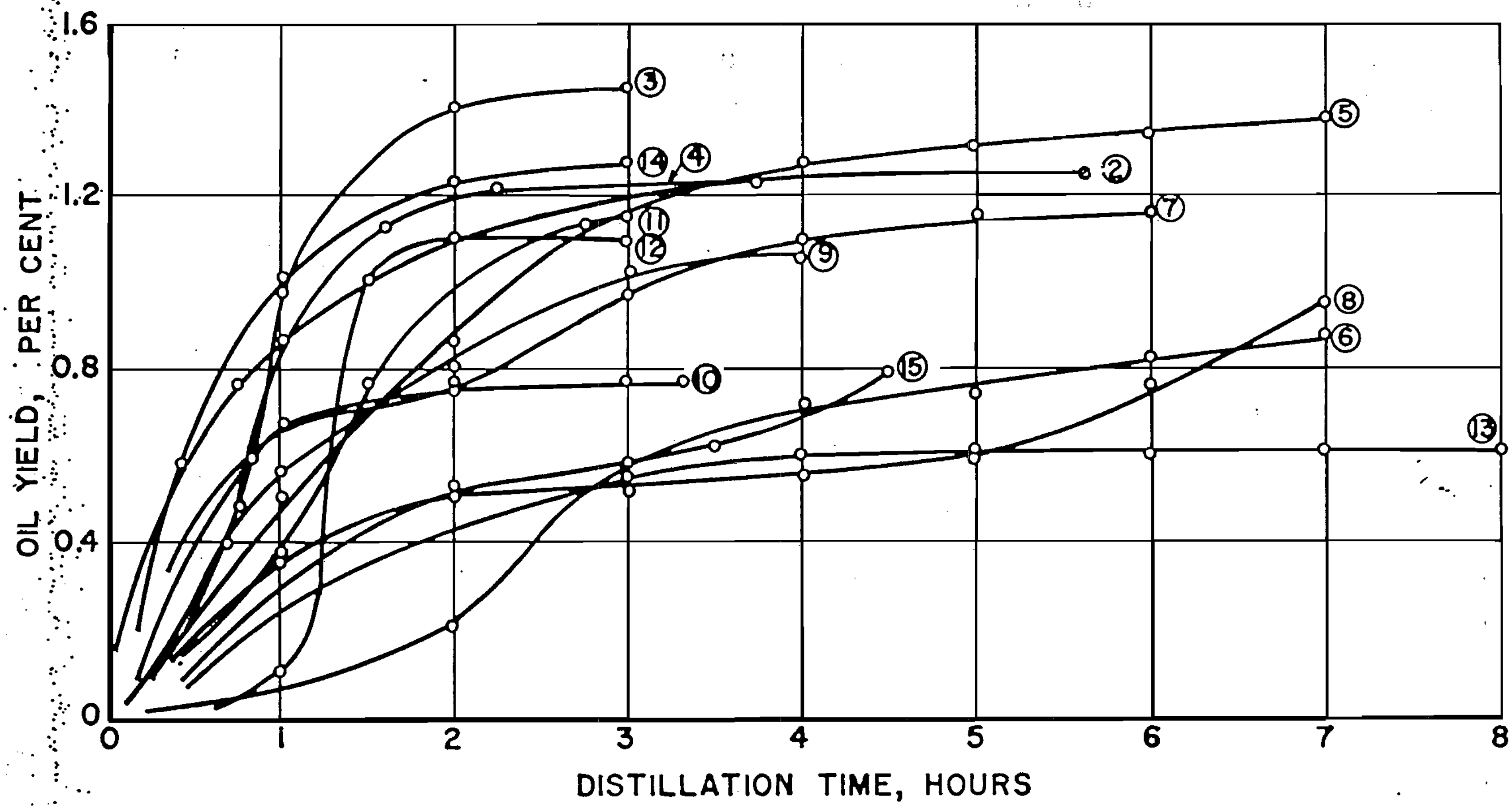



FIGURE 4. RATE OF OIL RECOVERY DURING STEAM DISTILLATION OF WESTERN JUNIPER WOOD; ON A DRY-WEIGHT BASIS.

A number of samples, when exposed to air for several days, developed a dark blue-green color, apparently because of copper contamination from the condenser. This color was removed by treatment with citric acid, as described by Guenther p. 311 Vol I.[3]

Oils obtained from runs using 10 psi steam pressure and less deposited cedrol crystals on standing overnight. Samples of oil from runs 5 and 6 reacted in this manner. Filtration of the samples showed 17.4 per cent of the weight of oil deposited as cedrol in run 5, and 10 per cent from run 6. The other low-pressure runs, 13 and 15, did not give crystalline cedrol. Run 13 used a high excess of steam which resulted in partial emulsification of the oil and water which seemed to inhibit crystallization. Run 15 used waste wood with considerable bark, and this did not deposit crystalline cedrol.

The oils from high-pressure runs, when treated by the Raybak process (Guenther p. 300 Vol I) yielded about 20-25 per cent of the weight of oil as crystalline cedrol.

A number of random treatments of the oil were tried in an effort to improve the odor of the oil as requested by the John D. Walsh Co., 32 Broadway, New York 4, New York.

Samples treated in the following manner were sent to this firm for examination:

1. Crude oil as recovered, not treated.
2. Oil extracted with citric acid to remove heavy metals.
3. Oil extracted with (1) citric acid and (2) with hot, saturated, sodium bisulfite solution.
4. Oil refluxed with 5 per cent sodium hydroxide solution.
5. Oil extracted with 10 per cent hydrochloric acid.

According to the Walsh Company, the above treatments did not result in any noticeable improvement in the odor of the oil. It therefore appeared that the oil from western juniper had somewhat different properties from the other cedarwood oils. This suggested two further avenues of research if this project were continued. The oil could either be modified to duplicate the properties of established cedarwood oils, or product research could be carried out to find uses for the western juniper oil in its natural condition.

## REMARKS

The following remarks can be made regarding operating conditions for volatile oil recovery:

1. For maximum oil yields at the most favorable rate, distillation at 50-60 psi is recommended.

2. If oil recovery is the only purpose, hammer-milled wood will be better than chips because of the higher oil yield and faster recovery.

3. The use of steam pressures under 10 psi gave oils that deposited crystalline cedrol on standing. It was possible to recover 10-15 per cent of the weight of the oil as solid cedrol by filtration. The cedrol was quite pure and could easily be recrystallized from hot ethanol if higher purity was desired. This product had a pleasant odor.

4. Oils from high-pressure runs, when treated by the Raybak method, yielded 20-25 per cent cedrol. It was more difficult to purify this cedrol, however, than that from low-pressure runs.

5. Minimum steam consumption for the range of pressures investigated appeared to be 100-150 pounds per pound of oil recovered.

The following generalizations outline probable economics of juniper oil production:

1. Average oil recoveries from dry juniper wood will be 1.3-1.5 per cent, or [redacted].

2. If operating pressures under 10 psi are used for steam distillation, the oils will naturally deposit cedrol crystals on standing. The yield of crystalline material is roughly 15 per cent of the oil, so cedrol

recoveries would be roughly 3.9 to 4.5 pounds per ton of dry wood. At the quoted price of from $3.50 to $4.00 per pound, this would amount to a gross value of from $13.60 to $18.00 per ton of dry wood. This is exclusive of the liquid portions of the oil, mainly cedrene.

3. If higher steam pressures are used for distillation, the amount of cedrol recoverable is increased, although the crystallization process is more difficult. Using an average yield of 25 per cent from the oil, the potential recovery would be from 6.5 to 7.5 pounds per ton of dry wood. The gross return would be from $22.70 to $30.00 per ton of wood. Again, the liquid fraction of the oil remains as a byproduct.

4. If the crude oil is vacuum fractionated, the maximum cedrol yield is obtained, roughly 40 per cent of the oil. This would make a gross return of from $36.50 to $48.00 per ton of dry wood for the cedrol alone.

5. If crude oil is the end product of the process, the possible return would be from $13.00 to $15.00 per ton of wood. This is based on current market prices for cedarwood oils which are about 50¢ per lb.

6. The cost of steam is quite low for the processing. Steam costs would probably be about 50¢ per 1000 pounds of steam. Since the estimated consumption is 3000 pounds per ton of wood, the cost would be only $1.50 per ton of wood. The steam cost would be further reduced if spent wood could be used as boiler fuel.

7. No attempt has been made to estimate the cost of procuring juniper wood since the nature of the logging operation would influence the cost considerably.

It is concluded therefore that recovery of crude oil as the end product is probably the least profitable of the possible processes. The recovery of crystalline cedrol offers more promise since the cedrol commands a good price, and the liquid fraction of the oil remains as a byproduct which should have some substantial value.

The use of the spent wood for fiber production would also enhance the economics of the process.

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# APPENDIX

Data in this section are included to provide means of calculating theoretical steam requirements for distillation of cedrol. Indications were that 50-60 psi was probably the practical maximum pressure.

Theoretical Calculations for Steam Distillation of Cedrol and Cedrene:

Table 9. Vapor-pressure Curves for Cedrene and Cedrol, Plotted by Method of Dühring (Figures 5, 6).

| Material | Boiling point | Vapor pressure | Temp at which water has same vapor pressure |
|---|---|---|---|
| | Deg C | Mm Hg | Deg C |
| Cedrene | 262 | 750 | 99.6 |
| | 130 | 17 | 19.5 |
| | 102 | 3.5 | - 3.6 |
| Cedrol | 291 | 760 | 100 |
| | 135 | 5 | 1.2 |

(Guenther, pp. 118, 285, Volume II)

Table 10. Calculation of Theoretical Steam Required for Distillation of Cedrol.

| Operating Pressure | | Temperature | | Vapor pressure of water | Temp from Dühring plot | Vapor pressure of cedrol |
|---|---|---|---|---|---|---|
| Psig | Lb abs | Deg F | Deg C | Mm Hg | Deg C | Mm Hg |
| 0 | 14.7 | 212 | 100 | 760 | - 27 | 0.6 |
| 10 | 24.7 | 239 | 115 | 1275 | - 13 | 1.7 |
| 20 | 34.7 | 259 | 126 | 1790 | - 4 | 3.4 |
| 30 | 44.7 | 274 | 135 | 2310 | 1.2 | 5.0 |
| 40 | 54.7 | 286 | 141 | 2830 | 8 | 8.05 |
| 50 | 64.7 | 297 | 148 | 3340 | 12.5 | 10.9 |
| 60 | 74.7 | 307 | 153 | 3860 | 16 | 13.6 |
| 80 | 94.7 | 324 | 162 | 4900 | 21 | 18.7 |
| 100 | 114.7 | 338 | 171 | 5930 | 26.5 | 25.9 |

Calculating theoretical steam required to distill cedrol, using conventional steam-distillation equation:

$$\frac{\text{Weight water}}{\text{Weight cedrol}} = \frac{Pw\ (M.W._w)}{Pc\ (M.W._c)}$$

$MW_w$ = 18 (Molecular weight of water)

$MW_c$ = 222.4 (Molecular weight of cedrol)

$P_w$ = vapor pressure of water

$P_c$ = vapor pressure of cedrol

Partial pressure of cedrol was neglected in the lower range.

At pressures up to 100 psi, steam required is as follows:

| Pressure | Steam required |
|---|---|
| Psi | Lb/lb cedrol |
| 0 | 102.5 |
| 10 | 60.6 |
| 20 | 42.5 |
| 30 | 37.3 |
| 40 | 28.3 |
| 50 | 24.7 |
| 60 | 22.9 |
| 80 | 21.1 |
| 100 | 18.4 |

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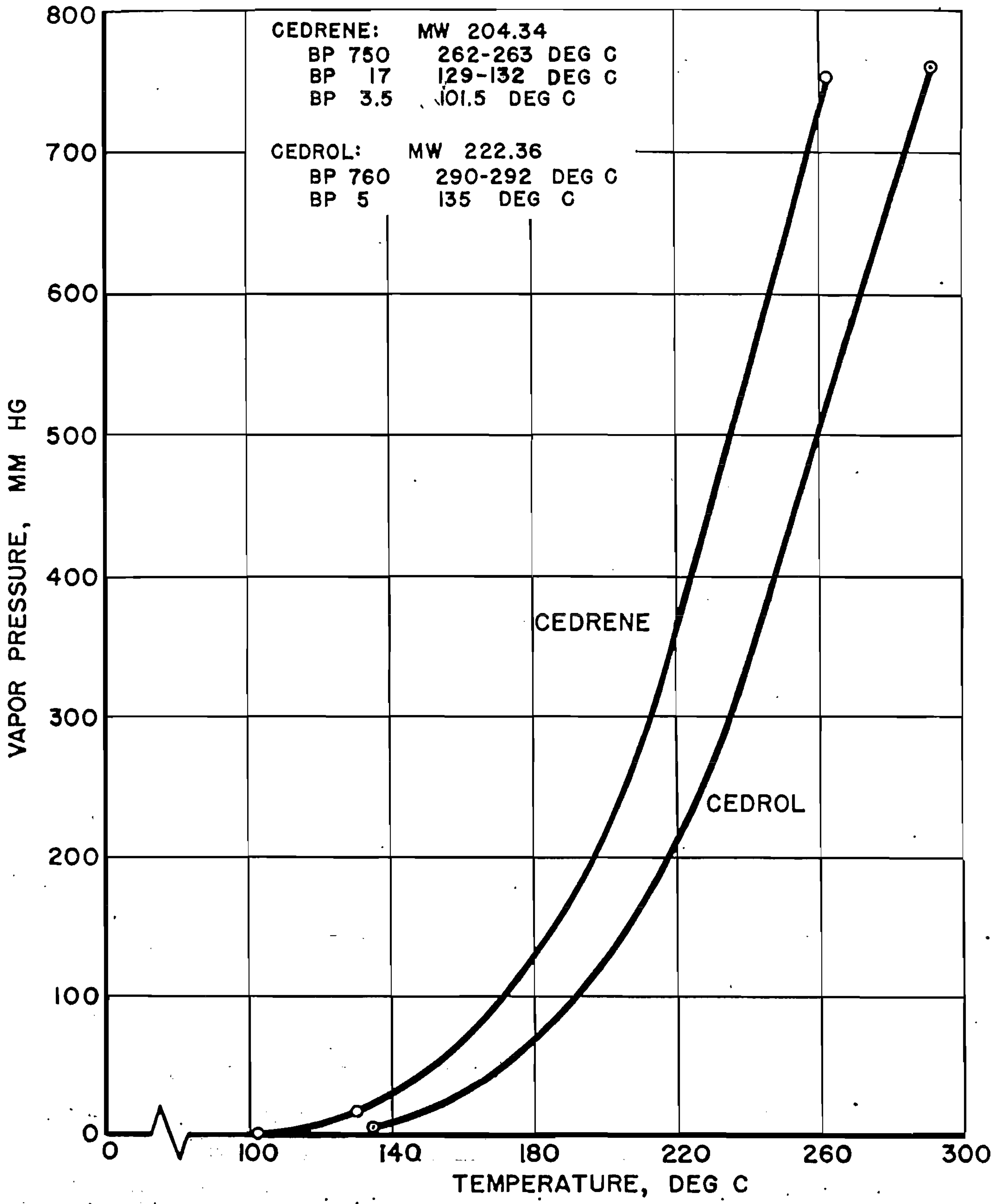


FIGURE 5. VAPOR PRESSURES OF CEDROL AND CEDRENE (GUENTHER, II, PP. 118, 285).

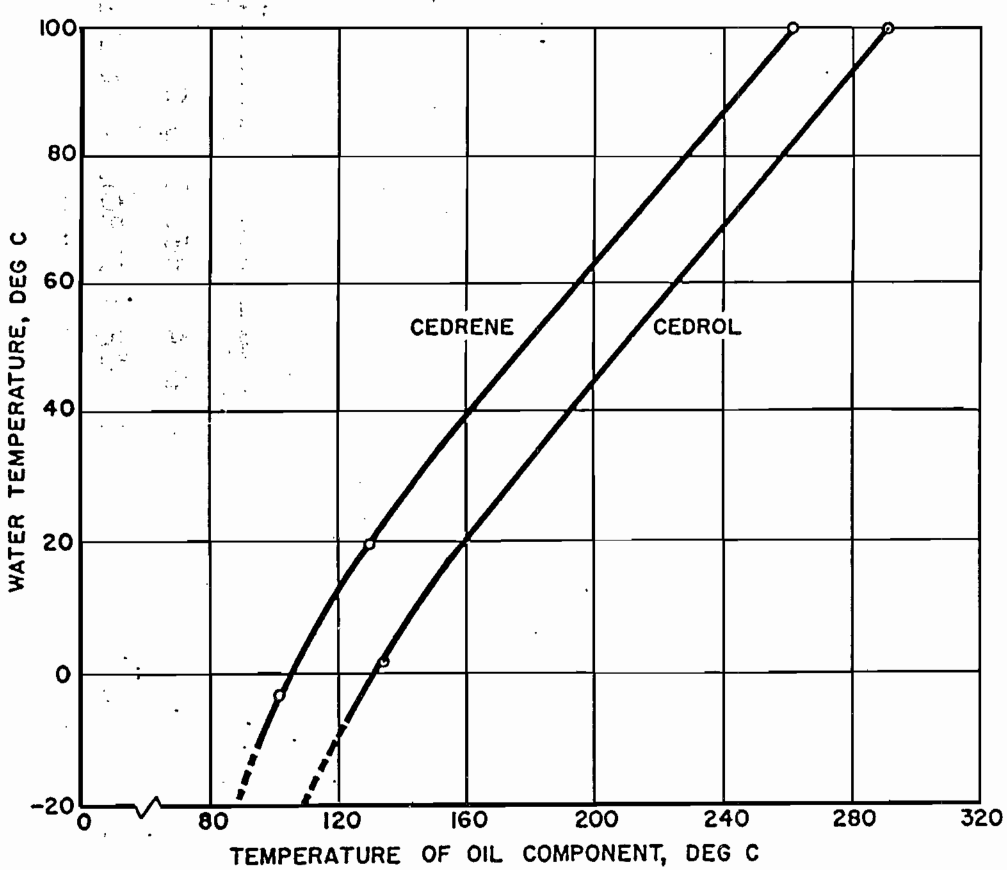


FIGURE 6. DÜHRING VAPOR PRESSURES FOR CEDRENE AND CEDROL.

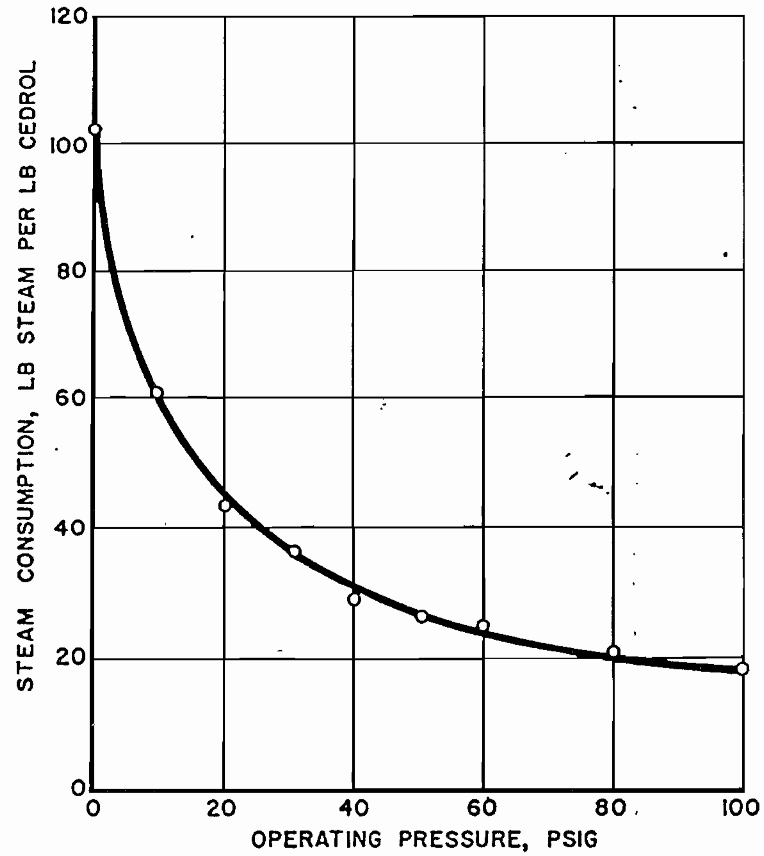


FIGURE 7. THEORETICAL STEAM CONSUMPTION FOR STEAM DISTILLATION OF CEDROL.

# Structure and Productivity of Juniperus occidentalis in Central Oregon [1]

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ABSTRACT: Western juniper ecosystems are well-adapted to the arid environments of central Oregon. In the stand examined, trees rarely exceeded 8 m in height and were uniformly spaced. Although foliage biomass averaged 4315.0 kg $ha^{-1}$, total stand leaf area was only 2.0 ha $ha^{-1}$. Total aboveground biomass averaged 21,161.4 kg $ha^{-1}$. Aboveground net primary production of the juniper was estimated at 1097 kg $ha^{-1}y^{-1}$. Juniper forests have a higher proportion of bark and a much lower stem water-storage capacity than other coniferous forests in the Pacific Northwest. The individual trees examined had leaf areas per unit of stem water-conducting tissue that were less than for fir species on more mesic sites but similar to those for two western pine species. Double sampling provided reliable estimates of means and confidence intervals for juniper biomass and leaf area.

## INTRODUCTION

Western juniper (*Juniperus occidentalis* Hook. subsp. *occidentalis*) occupies the driest of all coniferous forest sites in the Pacific Northwest. In the dry forest zone of central Oregon and Washington (Franklin and Dyrness, 1973), stands of juniper merge with ponderosa pine (*Pinus ponderosa*) forests on the moister sites and border plains of big sagebrush (*Artemisia tridentata*) throughout the region. In the past, fire has controlled the spread of juniper into the adjacent shrub/steppe (Burkhardt and Tisdale, 1976), and the practice of suppressing range fires—widespread during this century—has apparently allowed juniper to invade these recently nonjuniper communities.

Because juniper can compete successfully with more palatable forbs and grasses, range managers generally regard the species as a pest. Furthermore, many of its apparent physical adaptations to this harsh environment, such as a stubby growth form with severe taper, make juniper undesirable for large-scale commercial exploitation by the forest products industry.

As a result of this management status, methods to eradicate juniper, usually to release grazable grasses and forbs (Bedell and Bunch, 1978), have been extensively researched (Martin, 1978). However, little work has focused on juniper's commercial prospects, even as a fuel, and only preliminary work has examined its role as habitat for small mammals, birds and other game animals (Maser and Gashwiler, 1978). Nothing is known of its place in the hydrology, nutrient cycles or production relations of the dry forest zone.

This study provides data from one western juniper habitat for: (1) the most important aboveground structural features of the juniper stand and the reliability with which they can be assessed; (2) the aboveground net primary production of the tree strata, and (3) the specific structural adaptations to the arid environment of the study area.

## STUDY AREA

Located in central Oregon at 1356-m elevation along a NE-facing slope at the summit of Horse Ridge, the study site lies in the rain shadow of the Cascade Moun-

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tains (44°N lat, 120°W long). May to October rainfall measured 160 mm during 1976 and 80 mm during 1977. Annual snowfall averages 900 mm (Franklin *et al.*, 1972). The evaporative demand from May to October 1977, as measured with evaporimeters (Waring and Hermann, 1966), averaged 40% greater than at several sites W of the Cascades summit.

The site, adjacent to the Horse Ridge Research Natural Area described by Franklin *et al.* (1972), has been little grazed because of the absence of springs and wells along the ridge. In recent years, motorized recreational use has increased in the area, although no evidence was found for disturbances on the study site. Franklin *et al.* (1972) classified the vegetation on the upper part of Horse Ridge as the *Juniperus/Artemisia/Carex filifolia* community type. Because lack of disturbance is necessary to this type, it is restricted in area and was not described by Driscoll (1964).

Soils, derived from aerially deposited pumice, are generally shallow—65 cm to closely packed, fractured basalt bedrock. Soils on Horse Ridge have not been mapped. Pedons examined on this site were extremly stony representatives of the Torriorthent great group. Comparable soil data for other juniper habitats were given by Driscoll (1964).

The soils have low water-storage capacities. In 1977, gravimetric soil water to a depth of 1 m was never present at less than 0.1 atm tension in the spring and reached 15.0 atm by early August. In contrast, western Cascade Mountain sites had soil water at less than 0.1 atm tension in May and still had water at less than 1.0 atm in lower horizons in August, even after 6 weeks of drought. Predawn xylem water potentials averaged -30 atm in mid-August.

The vegetation on the site was sparse. Live juniper trees, averaging 246 per ha, were interspersed with big sagebrush bushes averaging 12% cover and other minor species averaging 3% cover. The juniper was uneven-aged, ranging from less than 30 years to over 350 years.

## METHODS FOR DETERMINING BIOMASS AND LEAF AREA

Double sampling with regression (Cochran, 1963) of the juniper trees in the stand combines two methods of estimating plant components: (1) the complete harvest, separation and measurement of selected (n) plants, and (2) the nondestructive measurements of all plants (n′), including the plants that were destructively analyzed.

All live trees (n′) in seven 20-m-radius circular plots randomly located within a 1-ha area were nondestructively measured and recorded. Ten (n) junipers, randomly selected from three size classes (0-75, 75-150 and > 150 cm in basal circumference) within the circular plots, were destructively analyzed between June and August; four were from the small size class and three each from medium and large size classes. Based on destructive analysis from western Oregon, this sample size was considered maximum for the 8 weeks available for fieldwork. Trees judged to have less than half of a live crown were excepted from sampling with consequences that will be discussed.

Selected plants were cut off at the litter surface. Then the bole was cut into 1- or 2-m sections that were covered with a Plexiglas sheet and tissue paper so that the outlines of the heartwood, sapwood and bark could be traced. Later the tracings were cut up, and the areas were measured with a Lambda Instruments LiCor Portable Surface Area Meter (Model No. LI3000) to estimate stem volumes from extremely irregular patterns of wood and bark development (Fig. 1). All live and dead branches were cut away from the bole into 1-m lengths and grouped according to mean diameters, large (≥ 10 cm) or small (< 10 cm). All small live branches supporting foliage were clipped below the foliage clump to create a third group of small, live, foliage-bearing twigs. Live and dead branches and foliage-bearing twigs were weighed fresh in the field to the nearest 0.5 kg.

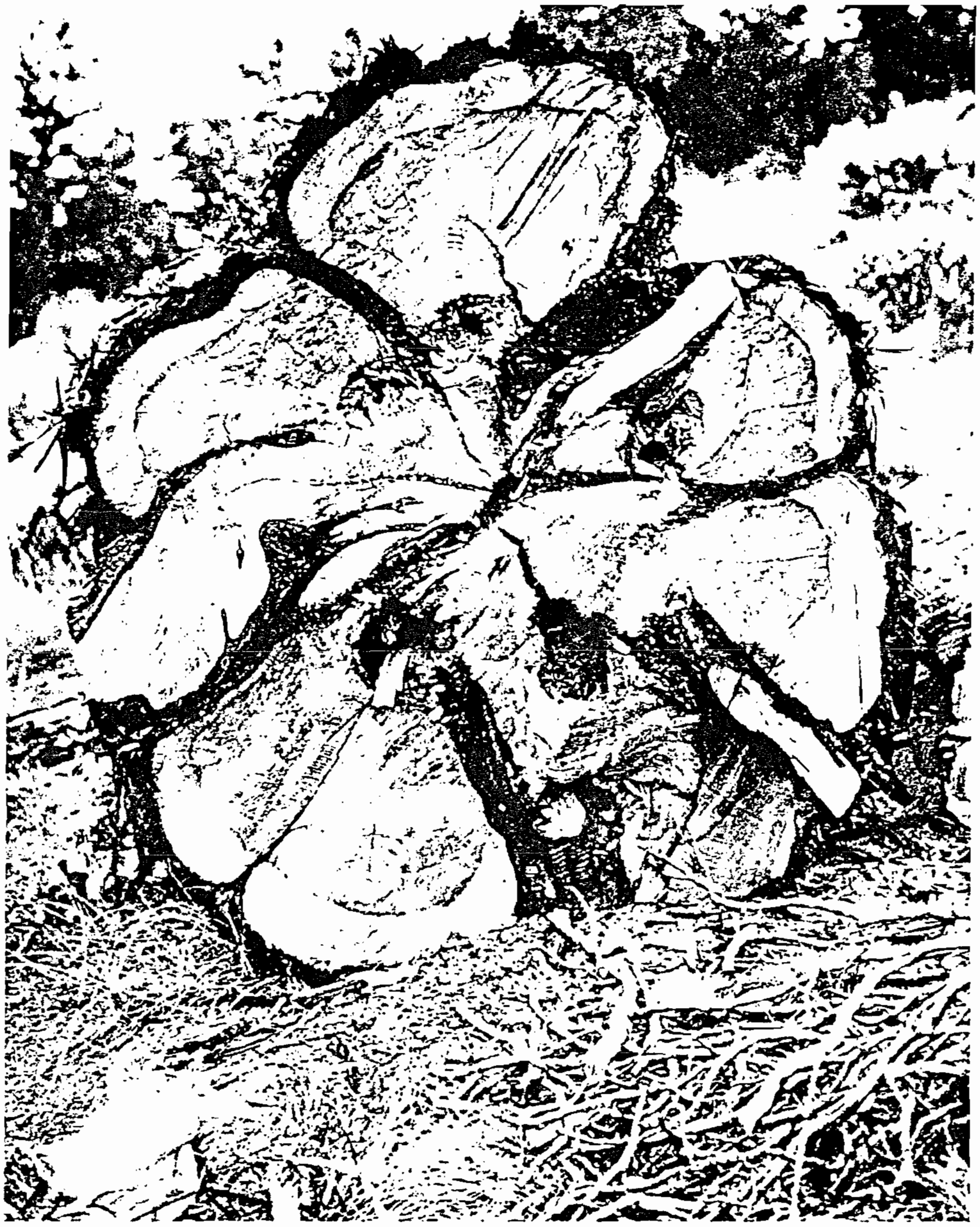

Fig. 1.—Base of the largest juniper harvested for this study (273 cm basal circumference, 8.5 m tall). Sapwood forms a lighter discontinuous band around the outer edge, and bark inclusions commonly penetrate near the center of the stem. At the base of this tree are four isolated pockets of rot

Subsamples of small and large live and dead branches and foliage-bearing twigs were randomly selected and weighed fresh to the nearest 0.5 g in the field, then frozen for laboratory analyses. Finally, stem sections taken 1 m aboveground were cut to determine the specific gravities of the stem and bark. If rot was found, sections were taken in sound wood as near to the 1-m cut as possible. No effort was made to assess belowground biomass.

In the laboratory, branch subsamples were dried at 70 C and weighed to the nearest 0.5 g. Foliage was separated from nongreen foliage-bearing twigs, which were then weighed fresh, dried at 70 C and reweighed. Green foliage was again subsampled three times. The projected surface area of each new subsample was estimated with the LiCor meter. Each segment of green "foliage" was assumed cylindrical so that the total leaf area (all sides; Gholz *et al.,* 1976) could be computed as 3.14 times the projected area. To compensate for underestimates from Lambda-meter measurement of very small pieces of foliage, leaf areas were adjusted upward 20%, which was determined by plotting estimated vs. actual areas of paper strips 1 cm long and 1 cm to 0.05 cm wide. Subsamples were then dried at 70 C and weighed to 0.1 mg.

Stem sections were sanded smooth, their areas determined as explained, and their thicknesses measured. Bark was then separated, and wood and bark were dried at 70 C and weighed to 0.5 g.

From the moisture contents, volumes and areas of the subsamples, and from the total fresh weights in the field, 11 whole-plant components were estimated: foliage biomass and surface area, live and dead branch biomass, whole stem volume and biomass, stem bark volume and biomass, stem wood volume and biomass, and sapwood volume.

## Statistical Analyses for Biomass and Leaf Area Determinations

These statistical analyses have been adapted from Uresk *et al.* (1976), after Cochran (1963), and will only be outlined here.

Data from the destructively analyzed n trees were used to compute a set of regression equations relating various plant parts (dependent variables) to basal circumference, chosen as the independent variable because it correlated highest with the most components. Circumference at breast height, crown volume, circumference at half-height, height and diameter squared times height were also evaluated as independent variables. None consistently correlated with the dependent variables as well as did basal circumference (irregular stem development makes diameters difficult to estimate accurately). The equations and the n′ nondestructive measurements were used to compute a mean component size per individual tree ($\overline{Y}_I$) and the variance [Var ($\overline{Y}_I$)].

To obtain a component total per hectare ($\overline{Y}_{tot}$), $\overline{Y}_1$ was multiplied by the mean number of plants per hectare, $\overline{Z}$:

$$\overline{Y}_{tot} = \overline{Y}_I\overline{Z} \quad (1)$$

However, the 10 component data points showed curvilinear relationships with the independent variable, as often occurs with plant biomass estimation, so both axes were log-transformed. The transform resulted in linear correlations and significantly reduced variances; therefore, the statistical analyses were completed entirely in log units, including the evaluation of the regression coefficients, $\overline{Y}_I$, Var ($\overline{Y}_I$), $\overline{Y}_{tot}$ and Var ($\overline{Y}_{tot}$). Accordingly, equation (1) was rewritten:

$$\overline{Y}_{tot} = \overline{Y}_I + \overline{Z} \quad (2)$$

where $\overline{Y}_{tot}$, $\overline{Y}_I$ and $\overline{Z}$ are all in log units. Then the variance of $\overline{Y}_{tot}$ was given by the additive relationship:

$$\text{Var}(\overline{Y}_{tot}) = \text{Var}(\overline{Y}_I) + \text{Var}(\overline{Z}) \quad (3)$$

assuming that $\overline{Y}_I$ and $\overline{Z}$ are independent.

If the analysis is done in log units, a term must be applied before retransformation to arithmetic units (Brownlee, 1967; Baskerville, 1972) to correct the mean value $\overline{Y}_I$ for log bias. This ensures that the arithmetic value of $\overline{Y}_I$ is the mean, the parameter of interest, and not the median. $\overline{Y}_{tot}$ is composed of the already corrected $\overline{Y}_I$ and $\overline{Z}$.

Variances were not corrected. Instead, confidence limits were constructed in log units at a specified probability level, and then these limits were retransformed. The results were mean components per plant and per hectare with respective asymmetric confidence intervals around the mean that estimates the true distribution of the component (assuming that $\overline{Y}_{tot}$ is normally distributed).

If the number of samples is assumed optimal, then a reduction in variance using double sampling—rather than simple random sampling—can be calculated by equations from Cochran (1963, p. 337-339). The optimum ratio of n′ to n can also be estimated as:

$$\frac{n}{n'} = \frac{\sqrt{V_n C_{n'}}}{\sqrt{V_{n'} C_n}} \quad (7)$$

where $V_n$ is estimated by $S^2_{y.x}$; $V_{n'}$ is estimated by $S^2_y$-$S^2_{y.x}$; and $C_n$ and $C_{n'}$ are the relative costs (Uresk *et al.,* 1976).

## Production Estimates

The net aboveground primary production of the juniper in the study area was estimated as the average of annual biomass increment over the last 5 years. To provide average annual increments of stem and live branch biomass (wood and bark) for the 10 destructively analyzed trees, the biomass equations were applied to current basal circumferences and circumferences corrected from stem growth measurements. Linear regressions of the 5-year increment of stem and branch biomass on basal circumference were used with the current basal circumference measured for other trees in the stand to estimate branch and stem increments (plus bark) on an area basis. Foliage production was assumed to be 30% of the total foliage biomass based on *Juniperus osteosperma* data from Utah (Mason and Hutchings, 1968). Production losses to herbivore grazing, nonfoliar litterfall and losses of current tissues to mortality were not estimated, nor was production by nonjuniper species.

## Results

Basal circumference of n plants destructively analyzed averaged 16 cm less than that of n′ plants measured, and the range was less for n plants than n′ plants (Table 1). Crown volumes, heights and sapwood basal areas for the n plants are also included in Table 1; sapwood basal area often is a linear estimator of leaf area

(Grier and Waring, 1974; Waring *et al.*, 1977) and, as such, is a useful variable to document. The study area had 246 ± 20 live juniper trees per ha; aerial photos indicated 194 - 321 live trees per ha within 3 km of the study area. Dead trees were not tallied.

The 12 regression equations used in this analysis represent logarithmic transformations of both variables, with consistently high $r^2$ values (Table 2). The lowest $r^2$ and greatest variance were associated with the dead branch biomass. The linear equations relating leaf surface area to sapwood basal area (Table 2) can be con-

TABLE 1.—Dimensions of live junipers on Horse Ridge: n' = measured and n = the 10 destructively analyzed

| Dimension | $\overline{X} \pm$ SE | Range | Coefficient of variation |
|---|---|---|---|
| Basal circumference (n') | 120.07 ± 7.8 cm | 10.5 - 317.0 cm | 0.61 |
| Basal circumference (n) | 104.10 ± 25.0 cm | 14.5 - 273.0 cm | 0.76 |
| Crown volume (n) | 92.21 ± 44.05 $m^3$ | 0.64 - 303.46 $m^3$ | 1.36 |
| Height (n) | 4.45 ± 0.74 m | 1.00 - 8.50 m | 0.52 |
| Sapwood basal area (n) | 345.56 ± 103.61 $cm^2$ | 12.34 - 1098.06 $cm^2$ | 0.95 |

TABLE 2.—Regression equations for estimating component biomass, volume, surface area and biomass increment for western juniper with basal circumference (cm) as the independent variable. The first 12 follow the form ln(Y) = A + B•ln(X) with variances ($S^2_{y.x}$) in logarithmic units. The last four are linear, untransformed equations with variances in arithmetic units

| Dependent variable | A | B | $S^2_{y.x}$ | $r^2$ |
|---|---|---|---|---|
| Stem wood biomass (kg) | −8.5947 | 2.6389 | 0.029 | 0.995 |
| Stem wood volume ($cm^3$) | −0.8568 | 2.6006 | 0.048 | 0.990 |
| Stem bark biomass (kg) | −10.251 | 2.6333 | 0.152 | 0.974 |
| Stem bark volume ($cm^3$) | −2.5414 | 2.6006 | 0.106 | 0.981 |
| Whole stem biomass (kg) | −8.3939 | 2.6344 | 0.029 | 0.995 |
| Whole stem volume ($cm^3$) | −0.6719 | 2.5977 | 0.135 | 0.965 |
| Sapwood volume ($cm^3$) | 0.7232 | 2.1313 | 0.135 | 0.965 |
| Live branch biomass (kg) | −7.3115 | 2.3337 | 0.068 | 0.985 |
| Dead branch biomass (kg) | −11.8460 | 2.8323 | 0.664 | 0.908 |
| Leaf surface area ($m^2$) | −2.5917 | 1.5383 | 0.019 | 0.990 |
| Leaf biomass (kg) | −4.2430 | 1.5606 | 0.024 | 0.988 |
| Height (m) | −1.8616 | 0.7329 | 0.031 | 0.934 |
| Leaf surface area ($m^2$) = 0.559 • (sapwood breast height, $cm^2$) | | | 944.5 | 0.960 |
| Leaf biomass (kg) = 0.140 • (sapwood breast height, $cm^2$) | | | 56.6 | 0.966 |
| 5-year stem biomass increment (wood + bark, kg) = −0.383 + 0.0362 • (basal circumference) | | | 0.930 | 0.910 |
| 5-year live branch biomass increment (wood + bark) = −0.344 + 0.0165 • (basal circumference) | | | 0.356 | 0.840 |

TABLE 3.—Average dimensions of western juniper estimated by double sampling

| Dependent variable | Units per plant ($\overline{Y}_I$) | 90% confidence interval ($\overline{Y}_I$) |
|---|---|---|
| Stem wood biomass | 30.5 kg | 20.3 - 45.8 kg |
| Stem wood volume | 59,700 $cm^3$ | 39,600 - 90,000 $cm^3$ |
| Stem bark biomass | 6.0 kg | 3.9 - 9.4 kg |
| Whole stem biomass | 36.5 kg | 24.5 - 54.5 kg |
| Whole stem volume | 70,812 $cm^3$ | 47,300 - 106,100 $cm^3$ |
| Sapwood volume | 35,600 $cm^3$ | 24,500 - 33,100 $cm^3$ |
| Live branch biomass | 28.0 kg | 19.2 - 40.9 kg |
| Dead branch biomass | 3.9 kg | 2.1 - 7.2 kg |
| Leaf surface area | 82.5 $m^2$ | 64.8 - 105.1 $m^2$ |
| Leaf biomass | 17.5 kg | 13.7 - 22.5 kg |
| Total biomass | 85.9 kg | 59.5 - 125.1 kg |

trasted with cited studies. Estimates of each of the 11 components (Table 3) include the means per plant ($\bar{Y}_I$) and per hectare ($\bar{Y}_{tot}$), plus 90% confidence intervals about each mean. Based on a comparison of percent cover on the study site with one sagebrush community examined nearby for a related study, sagebrush biomass was about 900 kg $ha^{-1}$ and leaf area was 0.30 ha $ha^{-1}$. Biomass of other species (grasses and herbs) was judged negligible in comparison.

Specific gravities varied from tree to tree and throughout the bole. The wood averaged 0.50 g $cm^{-3}$, with a standard deviation of 0.05 and a range of 0.437 - 0.678 (sample size = 20). The bark mean was 0.51 g $cm^{-3}$, with a standard deviation of 0.07 and a range from 0.407 - 0.637 (sample size = 18).

Net production estimates for juniper were 195, 2 and 900 kg $ha^{-1}$ $y^{-1}$, respectively, for stem, branch and foliar biomass increment—a total of 1097 kg $ha^{-1}$ $y^{-1}$. The two regression equations used for determining stem and live branch increments are included at the bottom of Table 2. Confidence intervals for the production estimates were not constructed, but, because the estimates were derived from the biomass equations, the intervals should be comparable to those for the biomass estimates.

## DISCUSSION

*Biomass and leaf area.*—The juniper ecosystem has much less biomass and volume than other mature coniferous forest types (Fujimori *et al.*, 1976; Gholz *et al.*, 1976; Grier and Logan, 1977; Waring *et al.*, 1978). A 2-year-old tropical forest in Colombia (Folster *et al.*, 1976) and a 16-year-old jack pine stand in New Brunswick (MacLean and Wein, 1976) have biomass equal to that of this juniper stand, which has many individuals > 200 years old. Leaf biomass of the juniper ecosystem averaged about one-third and leaf areas averaged about 0.15 of Douglas-fir and western hemlock forests in western Oregon (Gholz *et al.*, 1976; Waring *el al.*, 1978).

The values in Table 3 and biomass values from other studies should be interpreted cautiously. This analysis assumed that the measurement of basal circumference was error-free, which is not strictly true. Sixteen of the 246 live trees per ha were rejected from analysis due to poor canopy vigor (less than half a live crown); this essentially means they were measured incorrectly because their foliage biomass and area were equivalent to a tree with about two-thirds the actual measured basal circumference. Because the analysis did not deduct this difference, foliar characteristics in Table 3 may be somewhat overestimated. If we assume each of the 16 trees had one-half the foliage biomass of other trees of the same basal circumference on the plot, the foliage biomass and leaf area figures in Table 3 would be reduced 9.7% and 9.5%, respectively. Selection of samples according to predeter-

TABLE 3.—(continued)

| | Units per ha ($\bar{Y}_{tot}$) | 90% confidence interval ($\bar{Y}_{tot}$) |
|---|---|---|
| Stem wood biomass | 7,505.7 kg | 4,703.9 - 11,976.5 kg |
| Stem wood volume | 14.7 $m^3$ | 9.2 - 23.5 $m^3$ |
| Stem bark biomass | 1,485.1 kg | 897.1 - 2,458.6 kg |
| Whole stem biomass | 8,989.7 kg | 5,661.0 - 14,275.6 kg |
| Whole stem volume | 17.4 $m^3$ | 10.9 - 27.8 $m^3$ |
| Sapwood volume | 8.8 $m^3$ | 5.6 - 13.7 $m^3$ |
| Live branch biomass | 6,894.9 kg | 4,431.0 - 10,728.7 kg |
| Dead branch biomass | 961.8 kg | 500.4 - 1,848.6 kg |
| Leaf surface area | 2.0 ha | 1.5 - 2.8 ha |
| Leaf biomass | 4,315.0 kg | 3,072.9 - 6,059.4 kg |
| Total biomass | 21,161.4 kg | 13,665.3 - 32,912.3 kg |

mined size classes does not invalidate the sampling technique, but does yield larger confidence intervals while better estimating the means (Uresk *et al.*, 1976). Also, no effort was made to document stem rot, prevalent in large trees, because the regressions were based on the specific gravities of sound wood. Again, this could overestimate stem wood biomass. Volumes, of course, are not so affected.

The mean ratio of hours in the field and laboratory for the destructive work ($C_n$) to the hours for the nondestructive work ($C_{n'}$) was estimated to be about 500:1. With the high ratio and the very high correlations between component size and basal circumference (Table 2), the double sampling effectively reduced the variances over simple random sampling. For example, $Var(\overline{Y}_I)$ for foliage biomass was decreased 25% over simple random sampling. The optimal ratio of n′ to n under these conditions for juniper was 21:1.

Few studies of biomass express variances associated with estimates. Comparisons with one study from western Oregon (Grier and Logan, 1977) show that the confidence intervals from double-sampling juniper are narrower than those from estimating biomass in Douglas-fir forests by standard regression techniques, even when the latter does not estimate the variation in the number of trees per hectare. Confidence intervals for juniper are larger than those calculated as 1.67 (t value at $p = 0.1$, 60 df) times the standard errors reported for a 16-year-old jack pine stand, but they are smaller than those calculated for other ages of the same vegetation (MacLean and Wein, 1976).

Specific leaf areas ($cm^2 \, g^{-1}$), used to convert foliar biomass to surface area, generally are highly variable as they are extremely sensitive to light and other environmental variables (Gholz, *et al.*, 1976; Gholz, 1978). However, this was one source of variation in juniper that was unusually small. The mean specific area for n trees was 44.0 $cm^2 \, g^{-1}$, with a standard deviation of only 2.0 (range from 40.0 - 46.0, sample size = 27). The low specific leaf areas reflect xeromorphic adaptations (Esau, 1960), as the juniper leaf has a thick-walled sclerenchyma-like hypodermis, a packed epidermis and several palisade layers.

Furthermore, biomass equations generally show wider scatter when a single nonfunctional parameter—such as diameter at breast height (1.3 m) or basal circumference—is used as an independent variable to estimate foliage mass. The tightness of fit of the data points in the regression analysis and the small range in specific leaf areas indicate a uniform environment for the plants on Horse Ridge and perhaps an absence of interference among trees. This, in turn, implies a system in steady state or one at least long undisturbed. Driscoll (1964) noted that the other juniper stands with N aspects and soils similar to those on this study plot had the same evenly spaced, open savannah appearance.

On a per-hectare basis, the very low leaf areas for juniper can be explained as an adaptation to a restricted water environment (Grier and Running, 1977). Contributing factors include lower precipitation, higher evaporative demand and more limited soil water availability than in western Oregon and other areas supporting higher leaf areas.

Recent studies emphasize sapwood as a water storage compartment, 350 $m^3 \, ha^{-1}$ mainly in the stems of 450-year-old Douglas-fir forests (Waring and Running, 1976, 1978), which serves as a buffer during short intervals of water stress. Sapwood in the juniper forest is 2.5% of stem volume, or about 9 $m^3 \, ha^{-1}$, indicating that water storage within the stems is not a major adaptive feature of juniper.

Although Douglas-fir from western Oregon can support almost twice the leaf area per unit area of stem sapwood, the ratios of leaf area to sapwood for juniper (0.56) and pine (0.51) do not differ significantly (Fig. 2). However, the total stand leaf areas for juniper are about one-third those of pine stands, juniper are much shorter with canopies nearer the ground, and maximum sapwood areas per tree are much more restricted in the juniper (700 $cm^2$) than in either the pine or fir (> 2000 $cm^2$).

Bark biomass in the juniper stand was large in relation to whole-stem biomass, averaging almost 17%. In coastal stands of western hemlock and Sitka spruce (*Picea sitchensis*), bark biomass was only 7.7% (Fujimori *et al.,* 1976). In the western Cascades, ca. 10 - 11% of the stem biomass is bark (Fujimori *et al.,* 1976; Grier and Logan, 1977). The highest value for western Oregon forests is 14% in a subalpine stand of noble fir (*Abies procera*) (Fujimori *et al.,* 1976).

*Production.*—Without accurate estimates of foliar production, herbivore grazing losses, nonfoliar litterfall, losses of current tissue to mortality and nonjuniper production, the "net production" values in this paper are tentative (Kira and Shidei, 1967). However, they are included as a basis for initial comparisons with other forest types.

Aboveground net production of 1097 kg $ha^{-1}$ $y^{-1}$ ranks this western juniper community among the least productive of the mature evergreen tree communities in the world (Art and Marks, 1971). In the United States, it is intermediate between the 650 kg $ha^{-1}$ $y^{-1}$ of pygmy conifer-oak scrub in the Santa Catalina Mountains of northern Arizona (Whittaker and Niering, 1975) and the 2100 kg $ha^{-1}$ $y^{-1}$ of *Pinus pungens* heath in the Great Smoky Mountains of Tennessee (Whittaker, 1966). The adjacent ponderosa pine communities of central Oregon annually produce twice as much dry matter as the juniper community. Western Oregon forests of

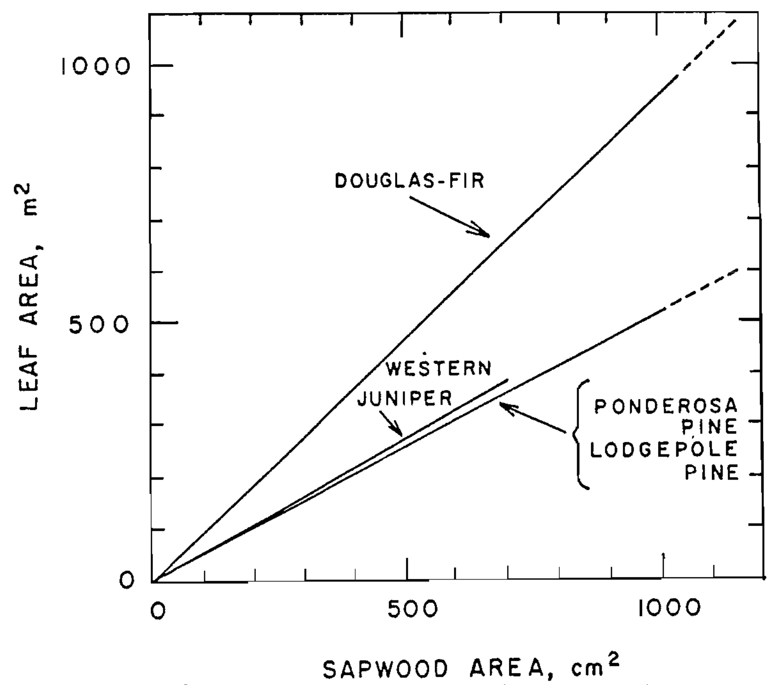


Fig. 2.—Total leaf surface area vs. sapwood cross-sectional area at breast height (1.3 m) for four western evergreens. The Douglas-fir and ponderosa pine (Grier and Waring, 1974) were converted to leaf area using constant specific leaf areas of 130 $cm^2$ $g^{-1}$ and 100 $cm^2$ $g^{-1}$, respectively. Lodgepole pine data are for the Rocky Mountains (S. W. Running, pers. comm. Dep. For. Wood Sci., Colo. State Univ., Fort Collins).

Douglas-fir produce 8000 to 12,000 kg $ha^{-1}$ $y^{-1}$ (Fujimori *et al.*, 1976; Grier and Logan, 1977).

For the juniper, the biomass:net production ratio (biomass accumulation) is 20, the production:foliage biomass ratio is 0.24, and the production:leaf area ratio is 50—all low when compared to other forest types. However, when leaf areas are expressed on an all-side basis, the last ratio is comparable to those of the pygmy conifer-oak scrub in Arizona and a *Tsuga canadensis-Rhododendron* community from the Great Smoky Mountains (Westman and Whittaker, 1975).

The structure and production of juniper on this site reflect the relatively harsh growing conditions found at high elevations in arid regions, and juniper has many characteristics necessary for surviving drought and temperature extremes (Levitt, 1972). In fact, late summer xylem water potentials were similar to values for Douglas-fir on the drier sites in western Oregon (Zobel *et al.*, 1976), indicating that individuals here were under no more water stress than other western conifers. Individuals store little water in the stems, and thick bark covers each juniper stem. Stand leaf areas are low, foliage exhibits xeromorphic adaptations, and water-conducting tissue in the stems is minimal. Production of wood and foliage is very restricted compared with other forest types.

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

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## 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



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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 |

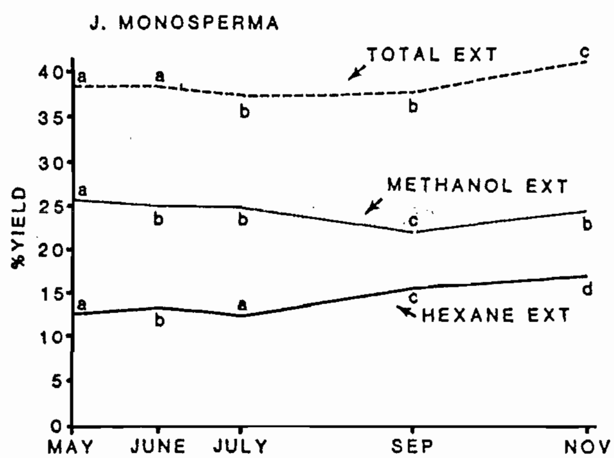


**Fig. 1.** Seasonal changes in the hexane, methanol and total extractables yields from *Juniperus monosperma* (Hansford Co., Texas, USA). Any data points on a line that have the same letter are *not* significantly different by Student–Newman–Keuls multiple range test at $P = 0{\cdot}05$.

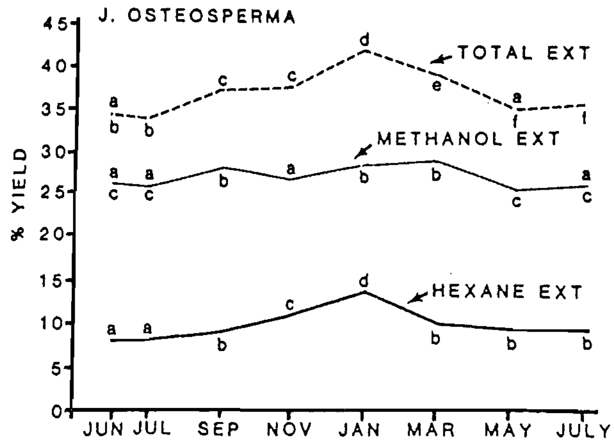


Fig. 2. Seasonal variation in the hexane, methanol and total extractables yields from *Juniperus osteosperma* (Salt Lake Co., Utah, USA). Any data points on a line that have the same letter are *not* significantly different by Student–Newman–Keuls multiple range test at $P = 0{\cdot}05$. Note the large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

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

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**Key Word Index**—*Juniperus*; cupressaceae; termiticides; insecticides.

**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

[Juni]per wood is the domestic source of cedarw[oo]d 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.

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## Results and Discussion

The first test conducted to determine the bioassay for termiticidal activity used fresh 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 se[illegible] [illegible]ferences 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* var. *occidentalis* | Heartwood | 0 (1.5) | 0 (1.5) |
| | Bark/sapwood | 0 (3.0) | 0 (3.0) |
| *J. occidentalis* var. *australis* | Heartwood | 0 (0.5) | 0 (0.5) |
| | 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 O[illegible] [illegible]PER 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 week[illegible]xane and methanol extracts were placed on filter pap[illegible] 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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Extra

Obtained from Vor Karchesy, OSU 1/95

# Antimicrobial Properties of Heartwood, Bark/Sapwood and Leaves of *Juniperus* Species

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**Hexane and methanol extracts of heartwood, bark/sapwood and leaves of twelve taxa of *Juniperus* from the United States were assayed for antifungal and antibacterial activities. The hexane extract of the heartwood of several junipers appeared comparable in antibacterial activity to streptomycin. Antibacterial activity of the hexane extracts from the bark/sapwood of *J. monosperma* and *J. californica* were comparable to streptomycin. No appreciable antibacterial activities were found in the leaf extracts from any species examined. No antifungal activities comparable to amphotericin B were found in either hexane or methanol extracts of the heartwood nor from the bark/sapwood. Antifungal activity against *Cryptococcus neoformans* comparable to amphotericin B was found in the hexane extract of the leaves of *J. occidentalis* var. *australis*. The methanol extracts from the leaves of *J. osteosperma* and *J. californica* had antifungal activities comparable to amphotericin B against *Trichophyton mentagrophytes*.**

*Keywords*: *Juniperus*; antifungal; antibacterial; *Cryptococcus neoformans*; *Trichophyton mentagrophytes*

## 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 (Guenther, 1952). In fact, the preferred status of juniper wood (cedar) for use as fence posts comes from a long history of its use in wet lands. The control of wood rot and termites is a perennial problem in most parts of the United States and the world. Many of the methods for wood preservation have used arsenic and/or chlorinated hydrocarbons which are environmentally hazardous. Carter (1976) 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.

Adams (1987) has recently reported on the yields of the heartwood volatile oils from 12 taxa of *Juniperus*, and noted that in addition to the two species currently utilized (*J. ashei* Buch. and *J. virginiana* L.), two additional species of juniper of the United States might be commercially harvested: *J. erythrocarpa* Cory and *J. scopulorum* Sarg. These species were also examined for their potential as sources of phytochemicals (Adams, 1987). Because plant materials were collected for these analyses an opportunity became available to examine the antibacterial and antifungal activities of the heartwood, bark/sapwood and leaves of these juniper taxa. Previous examination of the hexane and methanol soluble extracts of the leaves of *J. monosperma* revealed considerable bioactivity (McChesney and Adams, 1985).

The purposes of this study were to determine the antibacterial and antifungal activities of the heartwood, bark/sapwood and leaves of the principal *Juniperus* species of the United States.

## MATERIALS AND METHODS

Samples of wood and herbarium vouchers were collected from *J. ashei* (*Adams 5007–5009*, 9 km W of Ozona, Crockett Co., TX; *Adams 5010–5016*, 2 km E of Junction, Kimble Co., TX); *J. californica* 'A' (*Adams 5067–5071*, 13 km NE of I-40, Granite Mtns., San Bernardino Co., CA) and *J. californica* 'B' (*Adams 5072–5076*, 30 km SE of Yucca, Yuma Co., AZ) ('A' and 'B' refer to the two chemical races discovered by Vasek and Scora (1967) and reconfirmed by Adams, von Rudloff, and Hogge (1983) using leaf volatile oils); *J. erythrocarpa* (*Adams 4987–4996*, 32 km N of Alpine, Jeff Davis Co., TX); *J. deppeana* (*Adams 4974–4983*, 32 km NW of Ft. Davis, Jeff Davis Co., TX); *J. monosperma* (*Adams 5027–5036*, 2 km W of Santa Rosa, Guadalupe Co., NM); *J. occidentalis* (*Adams 5077–5086*, 8 km W of Juntura, Malheur Co., OR); *J. occidentalis* var. *australis* (*Adams 5057–5066*, 2 km W of Sonora Jct., Mono Co., CA); *J. osteosperma* (*Adams 5047–5056*, 25 km E of Monticello, San Juan Co., UT); *J. pinchotii* (*Adams 4997–5001*, 28 km E of Ft.

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Stockton, Pecos Co., TX); (*Adams 5002–5006*, 10 km W of Sheffield, Pecos Co., TX); *J. scopulorum* (*Adams 5037–5046*, 5 km E of Clines Corner, Torrance Co., NM); and *J. virginiana* (*Adams 5017–5025*, 7 km W of Bastrop, Bastrop Co., TX). Voucher specimens are deposited at Baylor University.

The samples consisted of wood (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 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 °C) to determine the percent moisture. Extracts were obtained from fresh heartwood, bark/sapwood and leaves by Soxhlet extraction of each set of materials for 6 h (Adams and McChesney, 1983). 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 °C) after the hexane extraction to remove the hexane before extraction with methanol (see Adams, Balandrin and Martineau, 1984, for detailed notes on the extraction protocol).

Qualitative antimicrobial screening was carried out using the agar-well diffusion assay (Clark *et al.*, 1981) against the following organisms: *Bacillus subtilis* 6633, *Staphylococcus aureus* 6538, *Escherichia coli* 10536, *Pseudomonas aeruginosa* 15442, *Mycobacterium smegmatis* 607, *Cryptococcus neoformans* 32264, *Saccharomyces cerevisiae* 9763, *Pycnoporus sanguineus* 14622, *Aspergillus flavus* 9170, *Aspergillus fumigatus* 26934, *Trichophyton mentagrophytes* 9972.

All test organisms were obtained from the American Type Culture Collection (Rockville, MD USA). Crude extracts and fractions were tested at a concentration of 20 mg/mL in ethanolic or aqueous ethanolic solution. Results of the qualitative screen were recorded as the average radius of the zone of inhibition surrounding the well containing the test solution (after 48 h incubation for bacteria and 72 [h] incubation for fungi) and are reported according [to] the following code: − = no activity; ± = questionable activity; + = 1–3 mm zone radius; ++ = 4–7 mm zone radius; +++ = 8–12 mm zone radius; ++++ = ≥13 mm zone radius. Streptomycin sulfate (1 mg/mL) and amphotericin B (1 mg/mL) were included [as] positive controls for antibacterial and antifungal activity, respectively.

## RESULTS AND DISCUSSION

Antibacterial activity was assayed for the heartwood, bark/sapwood and leaf extracts. Essentially no activity was found against *E. coli* from the hexane extracts of the heartwood (Table 1). Nearly all species showed activity against *S. aureus*, particularly in the hexane extracts of the heartwood (Table 1). Little or no activity was observed against *P. aeruginosa* (Table 1). Almost all the species had antibacterial activity against *B. subtilis* and *M. smegmatis* (Table 1). The results of this screen indicate that follow-up research (bio-guided fractionation) will be needed.

Antibacterial activity of the bark/sapwood extracts was very similar to that of the heartwood extracts (cf. Tables 1 and 2). In general, more activity was found in the non-polar extracts than the polar extracts (Table 2) and activity was observed against the Gram-positive bacteria, *S. aureus* and *B. subtilis*, and the acid-fast bacterium *M. smegmatis*. Activities comparable to streptomycin were found in the hexane extract from *J. californica* and *J. monosperma* (Table 2).

The leaf extracts exhibited less antibacterial activity, in general, than the wood extracts (cf. Tables 1, 2 and 3). However, these extracts were from unground leaves and some of the active components may be sequestered in glands. Small antibacterial activities were found in both the non-polar and polar

Table 1. Antibacterial activity of juniper heartwood extracts after 48 hours

| Species | *E. coli* | | *S. aureus* | | *P. aeruginosa* | | *B. subtilis* | | *M. smegmatis* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Hex | MeOH | Hex | MeOH | Hex | MeOH | Hex | MeOH | Hex | MeOH |
| *J. ashei* | ± | − | ++ | + | − | − | ++ | NT | +++ | + |
| *J. californica* 'A' | + | − | + | + | − | − | + | + | ++ | + |
| *J. californica* 'B' | − | − | + | + | − | − | + | NT | + | + |
| *J. deppeana* | − | − | ++ | + | − | − | ++ | + | +++ | ++ |
| *J. erythrocarpa* | − | − | ++ | + | − | − | NT | NT | +++ | − |
| *J. monosperma* | + | − | + | + | − | − | + | NT | ++ | + |
| *J. occidentalis* var. *australis* | − | − | ++ | + | − | − | ++ | + | ++ | + |
| *J. occidentalis* var. *occidentalis* | + | − | ++ | + | − | − | ++ | + | ++ | + |
| *J. osteosperma* | − | − | ++ | + | − | − | ++ | + | ++ | + |
| *J. pinchotii* | NT | − | NT | + | NT | − | NT | NT | NT | + |
| *J. scopulorum* | − | − | ++ | ++ | − | ± | ++ | + | ++ | ++ |
| *J. virginiana* | − | − | ++ | + | − | − | ++ | + | +++ | + |
| Streptomycin sulfate 1 mg/mL | ++ | | +++ | | ++ | | +++ | | ++++ | |

HEX, hexane extract. MEOH, methanol extract.
Activities are reported as: + = 1–3 mm; ++ = 4–7 mm; +++ = 8–12 mm; ++++ = greater than 12 mm (average radius of the zone of inhibition). (−) = no inhibition.
Extracts were tested at 20 mg/mL, 100 μL applied. NT, not tested.

**Table 2. Antibacterial activity of juniper bark/sapwood extracts after 48 hours**

| Species | *E. coli* Hex | *E. coli* MeOH | *S. aureus* Hex | *S. aureus* MeOH | *P. aeruginosa* Hex | *P. aeruginosa* MeOH | *B. subtilis* Hex | *B. subtilis* MeOH | *M. smegmatis* Hex | *M. smegmatis* MeOH |
|---|---|---|---|---|---|---|---|---|---|---|
| *J. ashei* | − | − | ++ | + | − | − | ++ | + | − | ± |
| *J. californica* 'A' | − | − | ++ | + | − | − | ++ | + | + | + |
| *J. californica* 'B' | − | − | ++ | + | − | − | +++ | + | + | + |
| *J. deppeana* | − | − | ++ | + | − | − | + | + | + | + |
| *J. erythrocarpa* | − | − | ++ | + | − | − | ++ | + | + | + |
| *J. monosperma* | − | − | +++ | + | − | − | ++ | + | + | − |
| *J. occidentalis* var. *australis* | − | − | ++ | + | − | ± | ++ | + | ++ | + |
| *J. occidentalis* var. *occidentalis* | − | − | ++ | + | − | − | ++ | + | + | + |
| *J. osteosperma* | − | − | ++ | + | − | − | ++ | + | + | + |
| *J. pinchotii* | − | − | ++ | + | − | − | ++ | + | ++ | + |
| *J. scopulorum* | − | − | ++ | + | ± | − | ++ | − | ++ | + |
| *J. virginiana* | − | − | + | + | − | − | ++ | + | ++ | − |
| Streptomycin sulfate 1 mg/mL | ++ | | +++ | | ++ | | +++ | | ++++ | |

Abbreviations and symbols as Table 1.

**Table 3. Antibacterial activity of juniper leaf extracts after 48 hours**

| Species | *E. coli* Hex | *E. coli* MeOH | *S. aureus* Hex | *S. aureus* MeOH | *P. aeruginosa* Hex | *P. aeruginosa* MeOH | *B. subtilis* Hex | *B. subtilis* MeOH | *M. smegmatis* Hex | *M. smegmatis* MeOH |
|---|---|---|---|---|---|---|---|---|---|---|
| *J. ashei* | − | − | ± | − | − | − | + | + | + | + |
| *J. californica* 'A' | ± | − | − | + | − | − | ± | + | − | + |
| *J. californica* 'B' | − | − | − | + | − | − | − | + | − | + |
| *J. deppeana* | − | + | − | − | ± | + | + | ++ | ± | + |
| *J. erythrocarpa* | − | − | − | + | − | + | + | + | − | + |
| *J. monosperma* | − | + | − | − | − | + | + | ++ | + | − |
| *J. occidentalis* var. *australis* | − | − | − | + | − | − | − | + | ++ | + |
| *J. occidentalis* var. *occidentalis* | − | − | + | + | ± | − | NT | + | ± | + |
| *J. osteosperma* | − | − | + | + | − | ± | NT | + | + | ++ |
| *J. pinchotii* | + | + | + | − | − | + | + | ++ | ± | + |
| *J. scopulorum* | − | + | − | + | − | − | − | + | ± | + |
| *J. virginiana* | + | + | − | + | − | ± | + | ++ | + | + |
| Streptomycin sulfate 1 mg/mL | ++ | | +++ | | ++ | | +++ | | ++++ | |

Abbreviations and symbols as Table 1.

leaf extracts (Table 3). A more thorough examination of the leaf extracts from ground material is in progress.

Only minor antifungal activities of the heartwood extracts were observed against any of the fungi (Table 4). However, hexane was removed with heat, so no volatiles were present in the hexane extracts. It should be noted that Oda *et al.* (1977) found high insecticidal activity in the volatile oils of *Juniperus recurva*. A study of the antimicrobial activity of the volatile heartwood oils is in progress. Essentially no antifungal activity was found in the bark/sapwood (Table 5), which is a little surprising because the antibacterial activity of the bark/sapwood extracts roughly paralleled the antibacterial activity of the corresponding heartwood extracts (Tables 1 and 2).

The antifungal activity of the extracts from unground leaves (Table 6) was strong against *C. neoformans* and *T. mentagrophytes* from a number of taxa. *Juniperus osteosperma* and both varieties of *J. occidentalis* were particularly active against *C. neoformans* (hexane extract, Table 6). The hexane extracts of these taxa, which are active against *C. neoformans*, are noticeably ineffective against *T. mentagrophytes*. The methanol (polar) extracts of *J. californica* and *J. osteosperma* showed activity against *T. mentagrophytes* (Table 6). This would seem to imply that a different component(s) is active against these two fungi. Almost no activity was found against the other fungi.

Overall, many positive antifungal activities were found in the heartwood and leaf extracts which will warrant a further examination by bio-guided fractionation.

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**Table 4. Antifungal activity of juniper heartwood extracts**

| Species | *C. neoformans* 32264 | | *S. cerevisiae* 9763 | | *P. sanguineus* 14622 | | *A. flavus* 9170 | | *A. fumigatus* 26934 | | *T. mentagrophytes* 9972 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hex | MeOH | Hex | MeOH | Hex | MeOH | Hex | MeOH | Hex | MeOH | Hex | MeOH |
| *J. ashei* | + | − | + | − | + | − | ± | − | ± | − | ++ | ± |
| *J. californica* 'A' | ± | ± | − | − | − | − | − | − | − | − | ± | + |
| *J. californica* 'B' | − | − | − | − | − | − | − | − | − | − | ± | + |
| *J. deppeana* | + | − | + | − | ++ | − | − | − | ± | − | ++ | − |
| *J. erythrocarpa* | ++ | + | + | ++ | + | − | + | − | ± | − | ++ | + |
| *J. monosperma* | + | − | + | − | + | − | ± | − | ± | − | + | − |
| *J. occidentalis* var. *australis* | + | ± | + | ± | + | − | ± | − | ± | − | + | + |
| *J. occidentalis* var. *occidentalis* | ± | + | ± | + | + | − | − | − | ± | − | + | ± |
| *J. osteosperma* | + | ± | + | − | − | − | − | − | ± | − | + | ± |
| *J. pinchotii* | NT | ++ | NT | ++ | NT | ± | NT | − | NT | − | NT | + |
| *J. scopulorum* | ± | ± | + | ± | + | ± | − | − | − | − | + | + |
| *J. virginiana* | + | ± | + | − | + | − | − | − | ± | − | ++ | + |
| Amphotericin B 1 mg/mL | +++ | | ++ | | NT | | ++ | | ++ | | ++ | |

Abbreviations and symbols as Table 1.

**Table 5. Antifungal activity of juniper bark/sapwood extracts**

| Species | *C. neoformans* 32264 | | *S. cerevisiae* 9763 | | *P. sanguineus* 14622 | | *A. fumigatus* 26934 | | *T. mentagrophytes* 9972 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Hex | MeOH | Hex | MeoH | Hex | MeOH | Hex | MeOH | Hex | MeOH |
| *J. ashei* | ± | − | − | − | − | − | − | − | − | − |
| *J. californica* 'A' | − | − | − | − | − | − | − | − | − | − |
| *J. californica* 'B' | − | − | − | − | − | − | − | − | − | − |
| *J. deppeana* | − | − | − | − | − | − | − | − | − | − |
| *J. erythrocarpa* | − | − | − | − | − | − | − | − | − | − |
| *J. monosperma* | + | − | − | − | − | − | − | − | − | − |
| *J. occidentalis* var. *australis* | − | − | − | − | − | − | − | − | − | − |
| *J. occidentalis* var. *occidentalis* | − | − | − | − | − | − | − | − | − | − |
| *J. osteosperma* | + | − | ± | − | − | − | − | − | − | − |
| *J. pinchotii* | − | − | − | − | − | − | − | − | − | − |
| *J. scopulorum* | − | − | − | − | − | − | − | − | − | − |
| *J. virginiana* | − | − | − | − | − | − | − | − | − | − |
| Amphotericin B 1 mg/mL | +++ | | ++ | | NT | | ++ | | ++ | |

Abbreviations and symbols as Table 1.

**Table 6. Antifungal activity of juniper leaf extracts**

| Species | *C. neoformans* 32264 | | *S. cerevisiae* 9763 | | *P. sanguineus* 14622 | | *A. flavus* 9170 | | *A. fumigatus* 26934 | | *T. mentagrophytes* 9972 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hex | MeOH | Hex | MeOH | Hex | MeOH | Hex | MeOH | Hex | MeOH | Hex | MeOH |
| *J. ashei* | − | + | − | ± | ± | ± | − | − | − | − | ± | ± |
| *J. californica* 'A' | + | + | − | + | − | ± | − | − | − | − | − | +++ |
| *J. californica* 'B' | − | + | − | ± | − | − | − | − | − | − | − | +++ |
| *J. deppeana* | ± | + | − | + | − | ± | − | − | − | − | − | + |
| *J. erythrocarpa* | − | + | − | + | ± | − | − | − | − | − | − | ± |
| *J. monosperma* | − | + | + | + | − | ± | − | − | − | − | ± | +++ |
| *J. occidentalis* var. *australis* | +++ | + | + | + | − | − | − | − | − | − | − | + |
| *J. occidentalis* var. *occidentalis* | ++ | − | ± | − | − | − | − | − | − | − | − | + |
| *J. osteosperma* | ++ | + | + | ± | + | ± | ± | − | − | − | ± | ++ |
| *J. pinchotii* | − | + | − | + | ± | − | − | − | − | − | − | − |
| *J. scopulorum* | ± | + | ± | ± | ± | − | − | + | − | − | − | ++ |
| *J. virginiana* | + | + | ± | + | − | ± | − | − | − | − | − | ++ |
| Amphotericin B 1 mg/mL | +++ | | ++ | | NT | | ++ | | ++ | | ++ | |

Abbreviations and symbols as Table 1.

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