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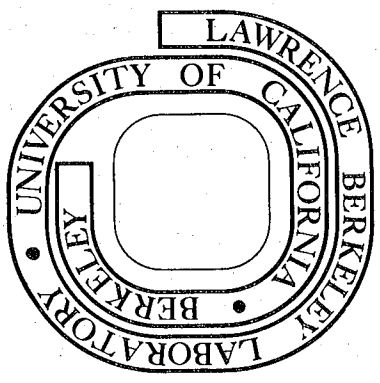

HYDROCARBONS AND ENERGY FROM PLANTS

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Abstract

To explore the feasibility of obtaining fuels and chemical feedstocks by extraction of reduced photosynthetic materials from latex-bearing plants, field studies were undertaken in the cultivation and harvesting of Euphorbia lathyrus, a shrub that grows wild in the California climate. Preliminary results with wild seed and without the benefit of optimization of fertilizer and irrigation conditions gave an annual crop yield of about 12 dry tons per acre. Continuing agronomic studies are suggested for improving this yield. Reduced photosynthate can be extracted with various solvents from the plant material to the extent of 8.7% of dry plant weight. The extract is a complex mixture, averaging between 400 and 500 in molecular weight. It contains some paraffins and carotenoids in addition to the major components, which are apparently pentacyclic triterpenones. A typical extract has a heat of combustion of 17,000 BTU per pound. Results of a very preliminary economic study of a conceptual process, including a biomass operation and a processing plant that extracts the oily material and leaves behind a saleable, cellulosic residue, indicate a cost of $30-$45 per barrel for the oil extract.

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Introduction

It has been suggested[1-4] that certain plants rich in isoprenoids and other hydrocarbon-like materials might be cultivated and grown as renewable sources of highly reduced photosynthetic products. Two distinctly different agricultural methods can be applied. Either we can harvest whole plants as suggested in a biomass plantation or we can tap latex-containing plants as is done in the production of natural rubber. In either case the net product would be a derivative of the total biomass and the process would be unlike many other biomass systems where the whole product is burned for its heat value. It would be more comparable to the production of methane from manure or of ethanol by fermentation. However in the case of hydrocarbon extracts the hope is that the conversion process could be more efficient and less energy demanding because the material is already in a reduced state.

Thus the objective of our program is to explore the feasibility of extracting reduced photosynthetic materials from latex producing plants for use as fuels or for chemical feedstocks. The best example of this is the rubber producing plant Hevea, which belongs to the family Euphorbiaceae. There are some 300 species of latex bearing plants which do not produce rubber, but which might produce lower molecular weight polyisoprenes. To explore this possibility we began in 1976 to develop analytical methods for the separation and identification of latex components and late in that year we surveyed about 2 dozen latex-bearing species, both whole plant and latex, for their content of hydrocarbons, wax, isoprenoids, etc.[5] The major constituents of the latex were identified as tetracyclic triterprenoids and the amount of total extractables was comparable for most of them. Therefore, we selected two species that were available to us for experimental cultivation. One of these, Euphorbia lathyrus, is an annual and can be harvested like a field crop. The other species, E. tirucalli, has a two to three-year growth period to initial harvest so we do not yet have yield data on it nor any extensive chemical analyses. Test plantings of these two species were made with the support of the University of California at its South Coast Field Station near Santa Ana and the Deciduous Fruit Field station in San Jose. All of the

quantitative experimental work was done at Santa Ana, while the plants at San Jose were used mainly for seed production.

The scope of the experimental program was necessarily quite limited and set largely by the availability of plant material during the first year or two of cultivation. Since these and many other latex producing plants have never been cultivated or studied for commercialization of their chemical content, various kinds of basic information were needed:

a) Crop yield and effect of growing conditions: yields of hydrocarbons as well as total caloric yield; effect of intervening winter on perennial crops; effects of irrigation and fertilizer on growth rate.

b) Cultivation and harvesting techniques: optimum harvesting methods; dependence of hydrocarbon yield on harvesting frequency.

c) Chemical composition of products: analysis by compound type as well as molecular weight distribution.

d) Processing methods: optimum extraction procedures; exploratory process chemistry for modification of the product.

3) Economic evaluation

This paper deals with results to date on the above topics except for b), which we have not yet begun. Other important and related agronomic topics involving seed production, seed propagation, experiments with dry land farming, etc., are largely still in the planning stage, as is the longer term research on genetic development and studies of the plant biochemistry leading to reduced photosynthetic products.

Field Studies

In 1977 seed of E. lathyrus from a northern California source were available in sufficient number to provide plants for one yield plot - i.e., one planting density, one irrigation schedule, and one fertilizer rate. Although we had hoped to test the influence of density, irrigation, and fertilizer on yield in the 1978 trials insufficient seed were available at the optimal time for field planting. Consequently, the 1978 yield trial was with plants grown for another purpose, from a southern California seed source, and at a very low planting density. The data presented are properly termed preliminary. This winter (78-79) seed supplies are adequate for yield trials on density, irrigation, fertilizer response, and planting date. Thus the 1979 field trials should answer most of the agronomic questions raised and provide a sound base for genetic improvement studies as well as for planning other agronomic strategies.

Cultural Conditions and Sampling

1977

Planting. Seed from Healdsburg, CA sown December 22, 1976 in vermiculite until germination (4 Jan. 77), and then transplanted to peat pots in sand/peat soil mix. On February 17 they were planted in the field on 1 ft centers - 43,580 plants/acre. Plots were approximately 16 m^2.

Fertilization was with $(NH_4)_2SO_4$ applied on March 16 [100 lbs N/acre] and again on May 23 [50 lbs/acre].

Irrigation was every 2 weeks commencing at field planting; at each irrigation plants received about 1.2". Total water applied through the October 17 harvest was 19"; rainfall contributed 5.6" water.

Harvest. Single plant samples were harvested and dried. Prior to the October 17 harvest no attempt was made to estimate plant-to-plant variability of the potentially greater growth of border compared to interior plants. Yield calculations were made by multiplying individual plant dry weights by 43,580 (plant/acre). Table 1 shows the progress of growth during 1977 season based on border plant samples, as well as a comparison between an average of 8 interior plants harvested in November and the attained weight of border

Table 1

Euphorbia lathyrus, South Coast Field Station

Single Plant dry Weights and Total Extractables (Acetone, Benzene)

1977 Planting (1 ft centers)

Sampling Date	Location B = Border I = Interior	Plant dry Weight, gm.[a]	No. of Samples	Percent Extractables, Basis Dry Weight
4-77	B	4	1	
5-77	B	23	1	
6-77	B	54	1	8.0
7-77	B	140	1	6.4
8-77	B	234	1	8.2
9-77	B	361 ± 51	10	10.6[b]
10-77	B	321	1	11.4
11-77	I	178 ± 32	8	
4-78	B	626	1	
4-78	I	244 ± 36	5	8.5 ±1.4
1978 Planting (2 ft centers)				
8-78	B	165 ± 70	16	
8-78	I	180 ± 73	9	

[a]Using the average percent extractables as 8.7%, the acre yield in barrels of oil (sp. gr. = 0.9) is given by

$$\text{Barrels/acre} = 6.2 \times 10^{-7} \text{ (plant dry weight)} \times \text{(plants per acre)}$$

The total dry biomass yield is given by

$$\text{Short tons/acre} = 1.1 \times 10^{-6} \text{ (plant dry weight)} \times \text{(plants per acre)}$$

[b]Single plant.

plants measured in September and October. From these data it is apparent that at such high planting densities (43,580 per acre) the interior plants average about 1/2 the dry weight of the border plants.

After the 1977-78 winter 6 more plants were sampled, roughly confirming the ratio of 1:2. However, the dry weight of both the interior and border plants increased considerably (40-60%) between fall and spring.

1978

Planting. Seed from Southern California (Santa Ana) were sown 27 February directly in the field; very low germination was recorded by 13 March and, hence, plots were completed with seedlings germinated under field and greenhouse conditions. Plants were placed on 2 ft centers (10,875 plants/acre).

Fertilization was with a slow release fertilizer (Osmocote$^{(R)}$) at approximately 104 lbs N/a, 20 lbs P/a, and 30 lbs K/a on 27 February. On 1 May approximately 100 lbs N/acre was applied.

Irrigation. Owing to very heavy rainfall (10.8") through 30 April irrigation began 1 May. Through harvest 29 August, when plants began to flower, approximately 15" irrigation water was applied (in addition, of course, the plants received 10.8" rainfall).

Harvest. Interior and border plants were harvested individually so that yield comparisons between interior and border plants could be made. The 25 plant square plot consisted of 16 border and 9 interior plants. Average dry weights were 165 ± 70 and 180 ± 73, respectively, showing that at the 2' spacing there is no competition and therefore no border effect.

Comments and Conclusions

1) There is great variability in germination among different seed lots suggesting that some of the seed has not been "after-ripened" or may be of poor quality.

2) At the high planting densities of 1977, with the climatic conditions for that year the irrigation and fertilization schedule may have been close to optimal.

3) Climate, particularly temperature, plays an important role in determining the performance of E. lathyrus. In the greenhouse E. lathyrus is a rapidly growing plant but under field conditions at Santa Ana, particularly during periods of low temperature (such as persisted from February through April, 1978) it is a very slow growing plant with perhaps a poor root system, that may ultimately give low yields during the subsequent summer months.

We believe that the key to success with E. lathyrus is to get good field germination in the cooler winter to spring months so that it develops an extensive root system. It should then be able to exploit the higher temperatures, longer days, and higher light intensities of late spring and summer and continue growth through harvest.

4) The 1978 trials revealed, too, that at low plant densities acre yields may be quite low - even though individual plant size is not very different from that of plants at higher densities. The reason is that at the 6-month harvest period the plants may not have grown sufficiently to "fill" the surface. That is, they never attain the size where interplant competition becomes the yield-limiting factor.

5) Perhaps the most significant finding for determining harvest is the data point showing a 40% gain in yield between November and April. This suggests that E. lathyrus continues to photosynthesize and store dry weight in its shoot system even though there is no apparent increase in plant height. The preliminary laboratory data show no decrease in percent extractables. Therefore, the increased growth probably includes its share of the desired hydrocarbons.

We conclude, therefore, that highest yields for E. lathyrus may be with a 12-month seeding to harvest cycle. A summary of the acre yield figures for the dense plantings, using only interior plants when replicates were available, gives:

	Short tons biomass	Barrels oil
9 month harvest	8.5	4.7
14 month harvest	11.7	6.5

Chemical Studies

In February, 1977 a test plot of Euphorbia lathyrus, a fast growing plant, was started at the South Coast Field Station. We have, therefore, directed almost all our work toward studying this one plant. The research is in two categories:

I. Methods of extraction of the dried plants.
II. Chemical characterization of the extract.

I. Methods of Extraction.

Several different methods of isolating the hydrocarbon-like material from E. lathyrus have been investigated. In addition to determining the best method of extraction, we also need one method which allows convenient and fast comparison of different plant samples on a laboratory scale. To this end we have been using hot solvent extraction of air-dried plants which have been finely ground for uniform sampling. Drying, however, is energy intensive; therefore we have started to investigate different ways of extracting the fresh plant. Our quantitative results to date, however, have only been obtained from dried plants and are discussed below.

Extraction of dried plants

The acetone-benzene system is the traditional method of extraction for rubber-producing plants.[6,7] The initial acetone extraction removes all the lipids, and the subsequent benzene extraction removes the polyisoprenes as well as some nonpolar waxes. This simple method can therefore be used to estimate roughly the polyisoprene content of a plant. Since it was of interest to determine whether E. lathyrus produces any polyisoprenes, several plant samples were extracted by this method (Method A).

As a comparison an alternate solvent system was also tried: heptane followed by acetone (Method B). A non-polar solvent like heptane could be expected to bring down most of the hydrocarbon-like compounds, and the more polar constituents should be washed out by the acetone.

Continuous extractions were done in a soxhlet apparatus for eight hours with each solvent. Approximately 10g of dried plant material was

extracted with 300cc of solvent. Longer extraction times (up to twenty-four hours) did not increase the yield. Tables 2 and 3 show the percentage of extractables, elemental analyses, and heat values of several samples. All these plants were taken from Plot No. 1 F. 28 of the South Coast Field Station; they were planted on February 17, 1977 and harvested on March 17 and 20, 1973. Samples 1 through 4 were taken from the inside of the plot. Sample 3 is a flowering plant; the seed heads were removed and only the vegetative part was extracted. Sample 4, the vegetative plant, was a neighbor of Sample 3 and is meant for comparison. Plant Sample 5 was taken from an outside row; the leaves were separated from the stems and the two parts were extracted separately.

As can be seen from Table 2, the benzene extractables are always an insignificant portion of the total. The proton nmr spectra of these extracts do show absorptions which can be attributed to a polyisoprene structure, but no further characterization of these trivial quantities was attempted.

By using a different solvent system (Method B) approximately the same amount of total extractables can be obtained, however, the acetone extractables have an extremely high oxygen content. At this time we do not have a satisfactory explanation for this. One possibility is that the initial heptane extraction removes the surface waxes of the plant very efficiently and thereby makes an underlying layer accessible for further acetone extraction. If this were the case, however, then the total amount of extractables should be higher for Method B than for Method A. By substituting pet. ether, a lower-boiling nonpolar solvent for heptane, or by doing the extraction under an inert atmosphere the same results are obtained.

One other solvent was tried for the extraction of the dried plant: methylene chloride, the advantage of this being its nonflammability. From plant Sample 1, 4.5% could be obtained by continuous extraction for eight hours. The extract gave the following elemental analysis: %C: 76.94, H: 10.94, N: 0.27, S: 0.06.

Table 2

Extraction of oven dried E. lathyrus

SAMPLE:	1	2	3 Flowering	4 Vegetative	5 Leaves 211 g	5 Stems 415 g
METHOD A						
% Acetone extractables:	7.6	8.8	8.79	10.02	9.5	4.23
Elemental Analysis:						
%C	77.41	76.18	78.99	78.29	80.48	77.93
H	10.72	10.23	10.72	10.63	11.36	10.91
N	0.22	0.26	0.17	0.25	0.41	0.07
P	<0.01	0.03	<0.01	<0.01	<0.01	<0.01
O content*	11.64	13.3	10.11	10.82	7.74	11.03
10^3 BTU/lb	16.46	15.96	16.78	16.59	17.43	16.67
% Benzene extractable:	0.32	0.244	0.23	0.08	0.49	0.2

*Oxygen content was calculated by difference.

Table 3

Extraction of Oven Dried E. lathyrus

SAMPLE:	1	2	3 Flowering	4 Vegetative	5 Leaves	5 Stems
METHOD B					211 g	415 g
% Heptane extractable:	3.96	4.42	4.23	3.99	7.85	3.51
Elemental Analysis:						
%C	79.95	79.31	79.99	80.24	79.91	78.93
H	11.39	11.24	11.41	11.37	11.38	11.25
N	0.11	0.25	0.22	0.19	.16	.16
P	0.16	0.11	0.09	0.12	.12	.11
O content*	8.39	9.09	8.29	8.08	8.43	9.55
10^3 BTU/lb	17.32	17.12	17.35	17.37	17.31	17.04
% Acetone extractable:	4.93	2.48	9.0	6.76	3.23	4.29
Elemental Analysis:						
%C	50.51	58.16	50.72	58.24	62.11	57.80
H	7.75	8.27	7.72	8.61	8.79	8.31
N	.71	1.09	.56	0.80	1.38	0.98
P	<.01	0.08	0.03	0.04	0.08	0.14
O content*	41.02	32.4	40.97	32.31	27.64	32.77
10^3 BTU/lb	9.44	11.31	9.47	11.48	12.4	11.25

*Oxygen content was calculated by difference

II. Chemical Composition of the Extract

Euphorbia lathyrus contains a very minor amount of polyisoprenes; the acetone and heptane extractables are clearly not just hydrocarbons, but they are sufficiently low in oxygen content for possible use as fuels. In order to determine the most suitable method of processing these extracts some information about their chemical composition is needed. We have therefore started to investigate the composition of the acetone extractable material (Method A).

At first we have attempted to fractionate this extract by gel permeation chromatography; over 90% of the sample coeluted on two different supports, indicating a narrow molecular weight range. Adsorption chromatography, however, can be used successfully to separate the mixture. The extract can be partitioned between heptane and methanol; 68% of it is soluble in heptane. This heptane soluble fraction can be further separated by column chromatography on silica gel, eluting with solvents of increasing polarity. The results are shown below:

Fraction eluted with	Color	% by weight of the hexane fraction
I Heptane	white crystals	7
II Benzene	Yellow	15
III EtOAc	Green	40
IV Acetone	Yellow-green	9
V MeOH	Green	29

These fractions are being analyzed separately and in some detail using IR and UV spectroscopy together with combined gas chromatography and mass spectrometry. Preliminary results indicate that I is composed of hydrocarbons, mainly n-$C_{31}H_{64}$ and n-$C_{33}H_{68}$ while II shows evidence of extensive conjugation and it probably contains carotenoids. Fraction III is a complex mixture, the main components of which are apparently pentacyclic triterpenones.

Economic Evaluation

The economic assessment of a conceptual operation that begins with growing an annual crop such as Euphorbia lathyrus, harvesting it, and supplying it to a processing plant where it is converted to an oil and a cellulosic residue is in progress but has not yet been completed. It will appear in a report by SRI International: "Mission Analysis for the Federal Fuels From Biomass Program."[8] There have been many assumptions made in this analysis because we have no experimental data yet on the optimum extraction and processing techniques. However, for a base case of the processing step, the SRI report takes as its starting point a value of $16. per dry ton of biomass feed to the processing plant ($1. per million BTU), assuming that the biomass production can be achieved at this cost, and takes a credit of $1. per million BTU for the by-product cellulosic residue. The plant is sized to process a million dry tons of harvested plant per year with an oil content of 8.7%. Using financing computations applicable to a regulated producer, the cost of oil would be $45 per barrel for this base case and $30 for a more optimistic set of processing parameters.

Since about half of this cost arises from the capital investment in the processing plant, it is evident that future research should be directed toward increasing the oil yield from the biomass and reducing the capital (power) requirements for extracting the oil. We are presently beginning efforts in both of these directions.

REFERENCES

1. M. Calvin, Science 184, 375 (1974).
2. M. Calvin, Photochem. Photob. 23, 425 (1975).
3. M. Calvin. In "Living Systems as Energy Converters", p. 231, R. Buvet, et al. eds. Elsevier Publishing Co., New York (1977).
4. M. Calvin, Energy Res. 1, 299 (1977).
5. P. Nielsen, H. Nishimura, J. W. Otvos, M. Calvin, Science 190, 942 (1977).
6. Loren G. Polhamus. "Rubber Content of Miscellaneous Plants". Production Report No. 10, Agricultural Research Service, U. S. Dept. of Agriculture, 1957.
7. Annual Report. Rubber Research Institute of Malaya, 1972.
8. (Exact reference to be supplied)

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