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Plants as a direct source of fuel*

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Summary. Euphorbia lathyris, a plant which has been proposed as an 'energy farm' candidate yields a total of 35% of its dry weight as simple organic extractables. Chemical analyses of the extracts show that 5% of the dry weight is a mixture of reduced terpenoids, in the form of triterpenoids, and 20% of the dry weight is simple sugars in the form of hexoses. The terpenoids can be converted to a gasoline-like substance and the sugars can be fermented to alcohol. Based on a biomass yield of about 25 dry tons ha^{-1} $year^{-1}$, the total energy that can be obtained from this plant in the form of liquid fuels is 48 MJ ha^{-1} $year^{-1}$, 26 MJ in the form of hydrocarbons and 22 MJ in the form of ethanol. A conceptual process study for the large scale recovery of *Euphorbia lathyris* products indicates that this crop is a net energy producer. Several lines of investigation have been started to increase the hydrocarbon yield of this plant. Tissue cultures of *E. lathyris* have been established and will be used for selection, with the aim of regenerating a superior plant. Biochemical studies have been initiated to elucidate regulation of terpenoid metabolism. Future plans include eventual genetic engineering to select the most desirable plant for hydrocarbon production.

Introduction

We have undertaken the investigation of how green plants, such as certain *Euphorbia* species, serve as renewable resources for hydrocarbon production. All higher plants fix CO_2 into carbohydrates. However, there are a number of plant species which can reduce CO_2 further to hydrocarbon-like compounds. A well known plant is *Hevea,* the rubber tree, which belongs to the family Euphorbiaceae. In this family of plants, the genus *Euphorbia* consists of approximately 2000 species, ranging from small herbs and succulents to large trees, the large majority of which produce a milky latex which is often rich in reduced isoprenoids. In general, this genus grows well in semiarid climates. Most of the Euphorbias are native to various parts of Africa but have been found elsewhere throughout the world. At least 20 species are found in South Africa, another half dozen or so in Morocco and Ethiopia and several other candidates for oil producers are indigenous to the Canary Islands. 3 of the wild species which grow in the area of Tenerife, Canary Islands, are shown in figure 1 *(E. regis jubae, E. balsamifera* and *E. canariensis).*

We have chosen *Euphorbia lathyris,* a biennial shrub that grows wild in California as a potential 'energy farm' candidate (fig. 2). *E. lathyris,* like many other

Figure 1. *Euphorbia regis jubae, Euphorbia balsamifera* and *Euphorbia canariensis* from Tenerife, Canary Islands.

latex-bearing plants, has never been cultivated, nor has its chemical content and the biochemistry of its abundant secondary metabolites been studied in detail. Therefore, in order to assess this plant as a hydrocarbon producer, various kinds of basic information need to be obtained: a) Crop yield and cultivation conditions; b) optimal methods for extracting the useful components and chemical characterization of the plant extracts; c) suitable methods for modifying the plant extracts into liquid fuel form; and d) various methods of increasing the 'hydrocarbon' content via plant selection, hormone treatment or by tissue culture techniques.

Crop yields and agronomic inputs

The first effort to cultivate *Euphorbia lathyris* began in 1977-1978, when test plots from wild seeds were established at the South Coast Field Station of the University of California in Santa Ana. After a 9-month growing season, the plants attained a typical dry weight of about 500 g and grew to a height of approximately 1 m. Planting density was 30 cm, centers and the plots were irrigated, receiving a total of 50 cm of water, 25 cm of which was natural rainfall. Preliminary results from these experiments indicate that a biomass yield of 25 dry tons per ha and year may be achieved with *E. lathyris*[1].

More recently, 2 important agronomic inputs were tested by Professor R. Sachs at the University of California, Davis[2]. A plot of 0.1 ha was established to determine the yield of *E. lathyris* as a function of nitrogen. The residual nitrogen in the soil ($85\ kg \cdot ha^{-1}$) was sufficient for this crop; there was no significant response to any added nitrogen. A larger plot (0.25 ha) was established to determine yield as a function of irrigation. The amount of applied water varied from zero at the edge of the plot to 60 cm at the center. The corresponding biomass yields after a 7-month growing season were about 10 dry tons per ha in the region of 0-12 cm of applied water, increasing to a maximum of 20 dry tons per ha at the center of the plot where 60 cm of water was applied. However, the amount of heptane extractables per unit dry weight was not a function of irrigation.

Plant extraction

Euphorbia lathyris exudes a milky latex when cut. However, this plant is not amenable to continuous tapping like some other *Euphorbia* species. In order to obtain the reduced photosynthetic material, the entire plant is extracted after drying at 70 °C to 4% moisture content. The reduced organic material is not uniformly distributed throughout the plant; the leaves contain twice as much as the stems per unit weight. Therefore,

Figure 2.

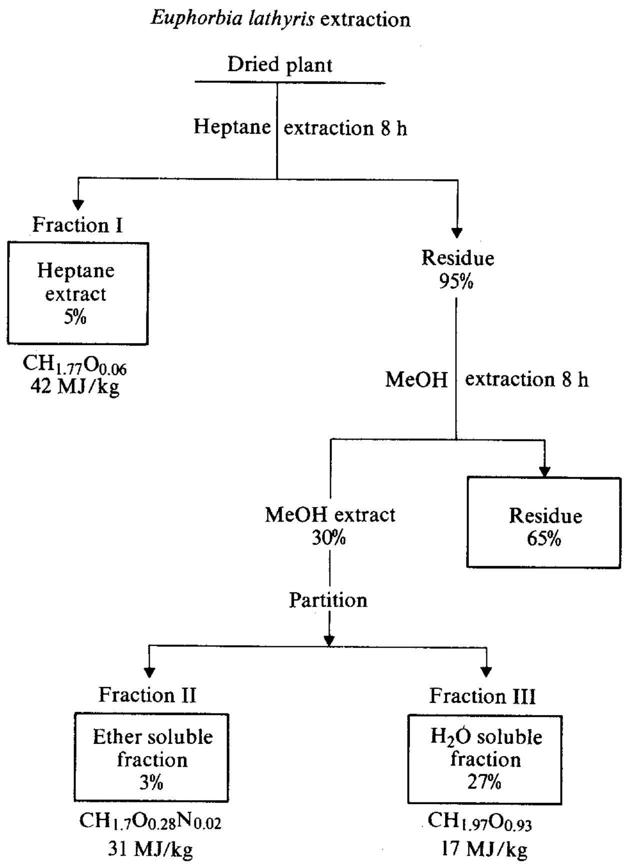

Figure 3.

for uniform sampling, the dried plant is ground in a Wiley Mill to a 2-mm particle size and subsequently thoroughly mixed. A portion of the plant material is then extracted in a Soxhlet apparatus with a boiling solvent for 8 h. Different solvents can be used to extract various plant constituents. One scheme which yields cleanly separated fractions and reproducible results is shown in figure 3.

Acetone can be used as the initial solvent instead of heptane. However, acetone brings down a variable amount of carbohydrates, which precipitate out of solution. This can be filtered off, leaving behind a pure acetone-soluble portion which is 8% of the dry weight of the plant. This is equivalent to the sum of fractions I and II of the extraction scheme (fig. 3) and has a heat value of 38 MJ/kg which corresponds to the weighted average of fractions I and II.

Chemical characterization of the plant extracts

The 2 fractions we have analyzed in detail are the heptane extract (fraction I) and the water soluble portion of the methanol extract (fraction III)[3]. The low oxygen content and high heat value of the heptane extract indicates a potential for use as fuel or chemical feedstock material; because the amount of methanol extract is substantial we investigated its chemical composition as well.

The terpenoids (heptane extract): The heptane extract is a complex mixture which can be separated into crude fractions by adsorption chromatography on silica gel. We have examined the column fractions further by gas chromatography and have obtained structural information on the major components by combined gas chromatography-mass spectroscopy (GC-MS). Molecular formulae were obtained by high resolution mass spectroscopy. The data from the GC-MS analyses indicate that over 100 individual components comprise the heptane extract. About 50 of these are major, and we have either identified or classified them. The major part of the heptane extract consists of various tetra- and pentacyclic triterpenoids functionalized as alcohols, ketones or fatty acid esters. The only non-triterpenoid components of the heptane extract are 2 long chain hydrocarbons which comprise column fraction I and a small quantity of fatty alcohols isolated from column fraction III. The 2 hydrocarbons are straight chain waxes: n-$C_{31}H_{64}$ and n-$C_{33}H_{68}$; the 3 fatty alcohols are $C_{27}H_{53}OH$, $C_{28}H_{57}OH$ and $C_{29}H_{57}OH$. These compounds, however, represent only $\sim$8% of the total heptane extract, 7% of the sample is chlorophyll, so 85% of the extract is composed of only 1 class of natural products, triterpenoids[4].

The major terpenoid components of the latex itself have been identified as 5 triterpenoids. All of the latex components with the exception of euphol (a minor one) could also be detected in the whole plant extract. The plant extract, however, yields a much greater variety of triterpenoids than the latex, suggesting that terpenoid synthesis may take place in other parts of the plant as well.

The carbohydrates (methanol extract): As the data in figure 3 indicate, a substantial amount (30%) of the dried plant weight can be extracted with methanol. The empirical formula of the water soluble portion of this extract is indicative of carbohydrates. Since simple hexoses can be directly fermented to ethanol, a useful liquid fuel, we have determined the carbohydrate content of *Euphorbia lathyris* and identified the specific sugars[4]. The results of gel-permeation chromatography of fraction III (Biogel-P-2) indicated that

Comparison of liquid fuel yield for different crops

Process	Process feed, tons/ton dry biomass	Process efficiency	Tons of liquid fuels/ton dry biomass	Fuel value of liquid (GJ/t)	Energy in liquid fuel (GJ/t biomass)	Dry biomass yield (t/ha·year)	Water requirement (cm rainfall)	Energy in liquid fuel (GJ/ha·year·cm rainfall) related to area and water requirement	Energy in cellulose residue (10^3 GJ/ha·year)	Energy in cellulose residue related to area and water requirement (GJ/ha·year·cm rainfall)
Corn to ethanol	0.32	0.4	0.128	26.8	3.4	12.3	62.5	0.67	115 (3.4 tons)	1.84
Sugar cane to ethanol	0.2	0.4	0.08	26.8	2.15	74.1	195	0.82	813 (24 tons)	4.17
Energy cane to ethanol	0.08	0.4	0.032	26.8	0.85	112	120	1.40	2493 (73.6 tons)	20.78
Euphorbia lathyris to hydrocarbon	0.08	0.86	0.069	36.9	2.5			0.84		
						21.0	62.5		207 (6.12 tons)	3.3
Ethanol	0.2	0.4	0.08	26.8	2.15			0.72 1.56		

there are no poly- or even oligosaccharides present in this fraction. The carbohydrate-containing fractions were identified by the Molish test and were further characterized by 2-dimensional paper chromatography and high pressure liquid chromatography (HPLC). In both these systems only 4 simple sugars were detected: sucrose, glucose, galactose and fructose in a ratio of 7.4:0.5:0.5:1.0, respectively. These 4 sugars comprise 20% of the plant dry weight. The water soluble fraction (fraction III of fig.3) also contains 3 amino acids: alanine, valine and leucine. The amino acid fraction comprises 3–4% of the dry plant weight.

This high carbohydrate content enhances the liquid fuel yield of *E. lathyris* significantly. Even though the heat value of alcohol is about two-thirds that of hydrocarbons, the great abundance of sugars in this plant doubles the energy obtained in the form of liquid fuels. In addition, no pretreatment such as acid hydrolysis or enzymatic digestion, necessary for starch producing plants, is needed to process the *E. lathyris* extract to alcohol.

Isolation and testing of the biologically active components: The latexes of several *Euphorbia* species contain chemicals which are strong skin and eye irritants, some of which exhibit tumor promoting activity. The concentration of these compounds is very low ($\sim$0.01%) compared to the triterpenoids which represent $\sim$50% of the dry latex weight. However, nanogram levels of these compounds exhibit promoter and irritant activity. From the latex of *E. lathyris* we have isolated a fraction which is composed of several ingenol esters. Biological tests showed that this latex-derived extract contains potent compounds which act like the best known, chemically-related tumor promoter 12-tetradecanoylphorbol-13-acetate (TPA). In contrast, the heptane and methanol extracts of the dried plant show little or no activity, indicating that the drying and hot solvent extraction degrades the active components. This finding has significant bearing on future harvesting and larger scale processing plans. Our preliminary results indicate that after mechanical harvesting and drying there should be no toxicological dangers.

Conversion of plant extracts to liquid fuels

Conversion to gasoline. Euphorbia lathyris yields 5–8% of its dry weight as reduced photosynthetic material; however, if this terpenoid extract is to be used as conventional fuel, then further processing of this material is necessary. The conversion of biomass derived hydrocarbon-like materials to high grade transportation fuels has recently been demonstrated by Mobil Research Corp.[5]. Various biomaterials such as triglycerides, polyisoprenes and waxes can be upgraded to gasoline mixtures on Mobil's shape selective Zeolite catalyst. The terpenoid extract of *E. lathyris* was processed under similar conditions with this catalyst[6].

Fermentation to alcohol: The entire crude carbohydrate extract (fraction III of fig.3) as well as the solid residue prior to methanol extraction is fermentable to ethanol. Several different fermentation conditions were tried, using various yeasts. An 80% fermentation efficiency was achieved on the crude extract; the dry residue can also be fermented directly, but with lower efficiency.

We can therefore obtain not only hydrocarbons from *Euphorbia lathyris* but a substantial quantity of ethanol as well. Per unit dry weight the sugar content of *E. lathyris* equals that of sugar cane. Our present biomass yield of 25 dry tons per ha and year yields approximately 2 tons crude extract which is converted to gasoline and 5 tons of sugars fermentable to alcohol.

Comparison with conventional biomass processes

Since *Euphorbia lathyris* and other hydrocarbon-producing crops are new species from the point of view of cultivation, their agronomic characteristics, requirements and yield potentials are not yet well known[7]. With further agronomic research and plant selection the biomass as well as the terpenoid yield is expected to increase. Nevertheless, it is interesting to compare in terms of energy yield a new crop like *E. lathyris* to other established crops such as corn or sugar cane.

The table shows the basic crop and the derived liquid fuel characteristics needed for this comparison, as well as the liquid fuel yield in terms of a very important agronomic input, the water requirement. The different fertilization requirements for these crops are not taken into account; fermentation efficiency is kept constant at 80% for the purpose of a comparison. In terms of liquid fuel production the crop which is comparable to *E. lathyris* is so-called 'energy cane' under development in Puerto Rico. Energy cane produces less sugar than normal cane (8% vs 20%). However, it yields an extraordinary 100 dry tons of biomass per ha. As the last column in the table indicates its yield of cellulose per unit of water input is significantly better than the other crops; however, its yield of liquid fuel (ethanol) is comparable to *E. lathyris.*

Increasing the biomass yield as well as the terpenoid content is of primary importance in the development *E. lathyris* as a new energy crop. In particular, raising the terpenoid yield per unit dry weight leads to large cost reduction in processing[8].

Plant tissue culture

Actively growing green cultures of *Euphorbia lathyris* have been established. The plant source materials (hypocotyl segments of seedlings and leaves of young plants) and the media and hormone requirements for producing actively growing callus cultures were determined. These cultures have been maintained for over a year. In addition, the cells have been placed in suspension culture for several months, and then brought back to callus culture on a semisolid medium. Roots have been regenerated from newly produced callus, and both roots and shoots have been produced from cultured hypocotyl segments through normal manipulation. Current efforts are directed at regenerating entire plants from callus cultures so that any yield improvements made in vitro can be tested in regenerated plants.

Triterpene biosynthesis in callus cultures: Since tissue cultures quite frequently do not synthesize the secondary metabolites characteristic of the parent plant at all, or do so in minute amounts, it was necessary to establish first whether *E. lathyris* callus tissue produces any terpenoids. By using our previously established techniques for triterpenoid isolation and characterization, we determined that *E. lathyris* callus cultures grown in the dark do produce 0.1–0.2% triterpenoids on a dry weight basis. This yield is comparable to that reported for secondary metabolism in other callus species.

Since *Euphorbia lathyris* tissue cultures do have the capability of triterpenoid biosynthesis, we now have a system which can be effectively exploited for the selection of desirable callus lines as well as for the exploration of secondary metabolic pathways.

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Wood as biomass for energy: results of a problem analysis

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1. *Wood as biomass for energy*

Under the pressure of a world-wide crisis in nutrition, raw materials and energy, a new field of research has evolved which attempts, through interdisciplinary cooperation, to promote the production of economically convertible 'biomass' rapidly and effectively using modern biotechnological methods. Biomass is defined as all organic material produced by living things. Biomass has potential uses as food, as raw material for economically important products and as an energy source.

One of the main aims of this new research is to find suitable organisms producing the greatest quantity of economically convertible biomass under well-adjusted conditions. Trials on the cultivation of fast-growing woody plants in intensively managed plantation-type monocultures and the production – with the shortest possible time lapse between planting and cropping (short-rotation forestry) – of a maximum of woody biomass for energy or chemistry, are to be evaluated from this viewpoint. This cultivation method for fuel wood differs in several ways from the common practice in forestry to date. Therefore it is expedient to examine more closely the widespread suggestion that fuel wood be produced on special farms; we will do this within the framework of a general survey of the topic 'Wood as raw material and energy source'.

2. *Systems analysis approach*

Forest management is a very complex dynamic system, which can be divided into 4 closely interrelated subsystems:

a) the *ecological* sub-system deals with the relationship between woody plants and their animate and inanimate environment;

b) the *forestal* sub-system is founded on all those observations made during the course of man's efforts to guide the development of wood-producing plants towards specific aims;

c) the *timber-economic* sub-system concerns itself with the production, processing, distribution and utilization of wood in the commercial sector, and its restoration to the natural ecosystem;