arpa·e
CHANGING WHAT'S POSSIBLE

PETRO Program Overview

B. PROGRAM OVERVIEW

ARPA-E seeks to fund the development of innovative new photosynthetic organisms and related approaches that are capable of producing liquid transportation fuels directly from agricultural practices. Interdisciplinary teams focused on the development of new, dedicated biofuel "crops" that are easily processed to liquid fuels would represent a departure from the sugar-, starch-, or lignocellulose-conversion approaches funded by others. As with the Agency's other programs, ARPA-E seeks to fund the efforts that not only present high technical risk, but also offer the potential to have a transformational impact. In this instance, ARPA-E seeks to fund the creation of a highly productive crop plant engineered specifically to produce liquid transportation fuel.

As the final deliverable of the program, performers will have created a genetically optimized multicellular photosynthetic organism that produces a liquid transportation fuel directly. Such a source must be sufficiently robust that it can be deployed using agricultural practices or the equivalent. The source is envisioned to produce a final product directly, ideally, a high-energy liquid hydrocarbon fuel that can be extracted easily. Specifically, the source should produce fuels with the highest possible energy density, at least that of isobutanol (26.5 MJ/L lower heating value), in a complete system that is significantly more efficient and cost-effective than ethanol derived from fermentation. Because photosynthesis is an energetically inefficient process that has evolved to benefit plants rather than humans, higher energy yields, in the form of liquid fuels, should be accessible without violating fundamental physical laws.

1. Background

Despite significant investments in renewable energy, petroleum and other non-renewable fuels still comprise nearly 90% of total U.S. energy supply.[1] Petroleum is particularly problematic; domestic supplies have dwindled while world demand continues to rise, due to the use of gasoline and other liquid fuels for transportation. Transportation represents 72% of all liquid fuel consumption in the U.S.,[4] and, while domestic demand is relatively stable, worldwide demand is predicted to exceed conventional supplies within the next 20 years.[2] This is clearly an unsustainable situation. Recent interest and significant advances in electric vehicle technologies may mitigate some demand for liquid fuels, but this comes at a price: the transportation support infrastructure in the U.S. has been developed around the use of liquid fuels, and widespread electric vehicle use will require a substantial overhaul of numerous subsystems. For these reasons, liquid fuels will likely remain the dominant form of energy used in the transportation sector, especially for applications where electrification is impractical, such as long-haul trucking and air transportation.

Today's vision of the future of domestically produced biofuels is that they can be derived from fermentable sugars or biomass, or perhaps from dedicated oil crops. This vision has both inherent limitations and inefficiencies that are addressable using innovative technologies.

1. Crops, intended for biofuel production, require large tracts of arable land that inevitably compete with the production of food crops at scale, driving up the cost of both food and fuel. This level of resource utilization is

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[1] U.S. Energy Information Administration (EIA), *Annual Energy Outlook 2010* (2010), reference case
[2] International Energy Agency (IEA), *World Energy Outlook 2010* (2010), summary downloaded from http://www.iea.org/Textbase/npsum/weo2010sum.pdf

intrinsic to modern agriculture, and is related to the light energy absorption and conversion inefficiencies of photosynthesis.[3]

2. Biomass is not easily digested and presently requires an expensive or inefficient process to break down lignocellulose into its components. This difficulty derives from the biological role of lignocellulose, a robust indigestible building material that serves as the major structural component of wood. Further, sugars derived from starch and cellulose require fermentation to produce ethanol, a process that releases one-third of the fixed carbon, and produces a liquid fuel with only 70% of the volumetric energy content of current transportation fuels.
3. Finally, today's oil-rich crops do not provide enough of a step up (based on energy yield per unit area) to be a cost effective alternative for production of transportation fuel at scale.[4] As a result, using currently available technologies, liquid biofuels are projected to comprise only 12% of all liquid fuels by 2035[4]. Several approaches to improve on these technologies are being pursued, ranging from biofuel crop breeding to more efficient lignocellulose digestion, but even the most optimistic projections fall short of forecasting parity with petroleum, either in terms of cost or in terms of scale. Consequently, economically competitive production of biofuels will require a technological paradigm shift that leads to a more efficient process with a concomitant reduction in the use of natural resources.

To qualify as an ARPA-E project area, it is necessary but not sufficient to identify an unexplored "white space" for technology development. Project areas for current investments must also be poised for progress, combining recent innovation with clarity of purpose. In this context, two questions emerge:

What recent biotechnology innovations are applicable to biofuels? New tools have emerged that reduce both the time and costs associated with complete genomic characterization, and enable the controlled alteration of genotypes through genetic manipulation. Directed genetic manipulations of agricultural crops have become increasingly routine and are currently used to improve yields and reduce costs in agriculture, e.g., by incorporation of pest resistance genes. Despite these advances, systems-level approaches aimed at the shortcomings of photosynthetic production methods, particularly in current biofuel crops, have not been widely attempted. An organism with significantly higher fuel production efficiency through optimized or enhanced metabolism is now within the realm of possibility. If developed, such an organism would represent a paradigm shift in biofuels production with the potential to replace a significant amount of the petroleum currently used for transportation.

What limits and/or inefficiencies should today's technologies focus on? There are several areas of potential interest:

1. *Light Capture*. Chloroplasts originated about 1.5 billion years ago and provide today's biofuel crops with a biochemical light capture mechanism that defines green photosynthetic organisms: Chlorophyll, through Photosystems I & II. While there are many advances being made in understanding the processes and limitations of chloroplast energy transduction, it is difficult to envision a simple path toward improved light utilization that has not been sampled already in the course of plant or cyanobacterial evolution. At the same time, primordial biological pigments that absorb light outside the spectral range of chloroplasts have recently been discovered, and these may be a source of additional photosynthetic energy, if productive uses for the energy can be identified.
2. *Carbon Capture*. Different organisms appear to have developed unique biochemical methods for carbon capture leading to improved evolutionary strategies that depend on specific growth conditions. In particular, C_4 plants grow in direct sunlight, about 50% faster than other plants. In this context, they use about 50% *more* light energy

[3] Zhu, X.-G., Long, S. P, & Ort, D. R. (2010) "Improving Photosynthetic Efficiency for Greater Yield", *Annu. Rev. Plant Biol*. 2010. 61:235–61

[4] Numbers vary, but in both instances, feedstock costs are the single largest cost of production. The per-acre per-year energy yield from corn ethanol is about twice that of biodiesel: Approximately 0.9 hectare is required to produce 370 bushels of corn [based on the average U.S. yield, USDA] and dry mill fermentation to ethanol produces about 2.7 gallons per bushel [USDA Survey, 1988], resulting in 1000 gallons of ethanol from 0.9 hectare. An equivalent volumetric amount of biodiesel from rapeseed oil requires approximately 2.4 hectares [see Scott, S.A. *et al.*, **Current Opinion in Biotechnology** (2010) *21*, 277-286]. The difference in energy density, about 1.4 fold, is insufficient to make up for the intrinsic differences in agricultural productivity.

than C_3 plants, and use the excess energy to concentrate atmospheric CO_2. Further, recent experiments[5] suggest that improving *intracellular* carbon dioxide utilization by transplanting exogenous pathways into photosynthetic organisms can lead to improved growth. This suggests that measuring carbon fluxes and yields, rather than energy losses, will be a useful approach to compare different approaches.

3. *Carbon Flux Redistribution.* This is a well-established approach toward improvement that could benefit from the application of new technologies. Most notably, selective breeding of food crops has resulted in plants that better serve human needs than their own—for example, modern corn devotes about 46% of its above-ground biomass to seed development,[6] much more than is necessary for propagation and survival of the species. Metabolic engineering to redirect photosynthetic energy into fuel molecules or direct precursors is one promising approach to take.

To add more clarity to the "carbon accounting" approach for project evaluation, Table 1 below contains a list of established benchmarks. Descriptions of the levels of carbon accounting in the table are provided in the glossary. Examples of hypothetical energy crops as anticipated responses to this Funding Opportunity Announcement are provided later on in this document, for further clarity.

[5] Kebeish R, et al. "Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in *Arabidopsis thaliana*." **Nat. Biotechnol.** (2007) *25*:593–99

[6] Pordesimo, L.O.; Edens, W.C.; Sokhansanj, S. **Biomass and Bioenergy** (2004) *26*, 337-343

Table 1: Carbon flux from atmospheric CO_2 for current biofuel crops

[NOTE: Only carbon is counted as part of weight.]

	Maximum Photosynthetic Rate A_n 50 $t_C \cdot ha^{-1} \cdot y^{-1}$ [7] [based on carbon, mw=12]					
	Maize (*Midwest*) (8, 9, 10, 11, 12)		Soybean (*Midwest*) (11, 13, 14, 15, 16, 17, 18)		Sugarcane (*LA, TX, FL*) (19, 20, 21, 22, 23)	
	$t_C \cdot ha^{-1} \cdot y^{-1}$	Yield	$t_C \cdot ha^{-1} \cdot y^{-1}$	Yield	$t_C \cdot ha^{-1} \cdot y^{-1}$	Yield
Captured	7.7	15%	3.1	6.3%	24.	48.%
Harvested	3.9	7.8%	1.3	2.5%	16.	32.%
Purified	2.7	5.4%	0.38	0.77%	7.7	15.%
Processed	1.5	3.0%	0.34	0.69%	4.0	8.0%
Final Energy Content ($GJ \cdot t_C^{-1}$)	52 (Ethanol)		50 (FAME)		52 (Ethanol)	
Overall Fuel Yield ($GJ \cdot ha^{-1} \cdot y^{-1}$)	79		17		207	

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[7] Following Collatz, GJ et al. "Physiological and environmental regulation of stomatal conductance, photosynthesis and transpiration: A model that includes a laminar boundary layer", *Agric. & Forest Meteorology* **1991**, *54*, 107-136, using a daily average of 30 umol•m^{-2}•s^{-1} based on an average of 12 hours of sunlight per day over an entire year. Maximum theoretical photosynthetic carbon fixation rates vary depending on many environmental and seasonal conditions—the given number serves as guidance in tracking the carbon utilization from CO_2 capture through produced liquid fuel. For a similar approach involving algal biomass, see Sukenik et al., "Optimizing algal biomass production in an outdoor pond: A simulation model", J. App. Phycology **1991,** *3*, 191-201.

[8] Per hectare biomass yields were derived from the measured net ecosystem exchange level of carbon for maize and soybean during the growing season, while harvested grain yields were concurrently measured in the same fields. Hollinger et al., "Carbon budget of mature no-till ecosystem in the North Central Region of the United States", *Ag & Forest Res.* **2005,** *130*, 59-69. Per acre grain yields are derived from USDA, National Agricultural Statistics Service, **2009**.

[9] Loomis, R.S.; Lafitte, H.R. "The carbon economy of a maize crop exposed to elevated CO_2 concentrations and water stress, as determined from elemental analyses". *Field Crops Res.* **1987**, *17*, 63-74.

[10] Latshaw, W.L.; Miller, E.C. "Elemental composition of the corn plant", *J. Agric. Res.* **1924**, *27*, 845-861.

[11] Bunge Milling "Typical Composition of Yellow Dent Corn," downloaded on 1/5/10 from http://www.bungenorthamerica.com/news/pubs/03_Bunge_Milling_Process_Diagram.pdf

[12] Bothast, R.J.; Schlicher, M.A. "Biotechnololgical processes for conversion of corn into ethanol", *Appl. Microbiol. Biotechnol.* **2005**, *67*, 19-25.

[13] Huber, G.W.; Iborra, S.; Corma, A. "Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering", *Chem. Rev.* **2006**, *106*, 4044-4098.

[14] Osborn, T.W. "Elemental composition of soybean meal and interlaboratory performance", *J. Agric. Food Chem.* **1977**, *25*, 229-232.

[15] Domalski, E.S.; Jobe, T.L.; Milne, T.A. National Institute of Standards and Technology, "Thermodynamic Data for Biomass Conversion and Waste Incineration" **1986**

[16] Renewable Biofuels Inc., "Soy Methyl Ester B100" product specification data sheet, downloaded on 1/5/10 from:http://www.rbfuels.com/assets/files/RBF_Soy_Methyl_Ester_B100_Data_Sheet.pdf

[17] Hill, J.; Nelson, E.; Tilman, D.; Polasky, S.; Tiffany, D. "Environmental, economic,and energetic costs and benefits of biodiesel and ethanol biofuels", *PNAS* **2006**, *103*, 11206-11210.

[18] El-Darier, S.; Hemada, M.; Sadek, L. "Dry matter distribution and growth analysis in soybeans under natural agricultural conditions", *Pak. J. Biol. Sci.* **2002**, *5*, 545-549.

[19] Per acre sugar yields are derived from USDA, National Agricultural Statistics Service, **2009**. Note that these values were determined using different methodology than for maize and soybean.

[20] Beeharry, R.P. "Carbon balance of sugarcane bioenergy systems", *Biomass Bioenergy* **2001**, *20*, 361-370.

[21] Da Rosa, A. Fundamentals of Renewable Energy Processes. **2009**, Elsevier Academic Press, Burlington, MA.

[22] Goldemberg, J.; Coelho, S.T.; Nastari, P.M.; Lucon, O. "Ethanol learning curve—the Brazilian experience", *Biomass Bioenergy* **2004**, *26*, 301-304

[23] Walford, S.N. "Composition of cane juice", *Proc. S. Afr. Sug. Technol. Ass.***1996**, *70,* 265-266.

2. Program Objectives

ARPA-E's mission is to fund projects that propose transformational technologies to reduce America's dependence on foreign energy imports, to reduce U.S. energy related emissions (including greenhouse gases), to improve energy efficiency across all sectors of the U.S. economy, and to ensure that the U.S. maintains its leadership in developing and deploying advanced energy technologies.

The major objective of this program is to develop a new, dedicated biofuel "crop" that converts photosynthetic energy to liquid fuels more efficiently than existing sources (see Table 1) and that can be grown, harvested, and processed in an economically viable manner. This new crop would be designed for agricultural implementation in the U.S. with an average carbon yield through to fuel (per unit area per year) at least twice that of corn-based ethanol within that same climate and region. The current state of the art is to use corn as a feedstock for ethanol production via fermentation, with substantial efforts underway to incorporate biomass or dedicated oil crops as additional sources. This program seeks applications for substantially higher efficiency organisms than are currently envisioned, and that could be planted, harvested, and processed to liquid fuels in a fully scalable manner.

The key problems to be addressed by this program are as follows: to capture and retain more light energy more effectively, and to divert the bulk of this energy toward a viable liquid fuel. While there are biological precedents and suggestions that such transformations are feasible, a comprehensive effort to develop a dedicated biofuel crop and process has not been attempted.

In view of ARPA-E's mission, development of such a crop and process would have two impacts: the resulting fuel products would replace or supplement fuels that are currently imported, and the process would recycle carbon dioxide, providing no net increase in this greenhouse gas. Because the crop would be deployable and developed in the U.S., it would help to improve the nation's self-reliance and economic competitiveness.

To more effectively and substantially address the program objectives, applicants are strongly encouraged to form interdisciplinary teams that bring an integrated approach to addressing various aspects of the problem.

3. Areas of Interest

Systems Development. As a general guiding principle, any funded application must result in a liquid transportation fuel that costs no more than alternative fuels at scale, presently $10 per GJ fuel or $50 per barrel of oil equivalent. Such cost estimates must account fully for the life cycle costs of biological conversion, starting with sunlight and ending at liquid fuels, following well-established financial models, e.g., corn agriculture combined with corn-based ethanol production. Applicants must demonstrate facility with such models, and should be careful not to ignore the costs of *all* raw materials, energy, and transportation. For example, systems operating at ambient concentrations of CO_2 are strongly preferred because supplementation adds a significant feedstock cost to fuel production. Approaches that augment these ambient levels may be feasible, but the cost of supplementation at scale must be elucidated completely. Applications that assume either zero or negative-cost "waste" (e.g., CO_2 from coal-fired power plants), or that do not factor in other direct costs of supplementation (e.g. infrastructure additions, transportation, and maintenance), will be rejected as non-responsive. Similarly, applications that achieve cost metrics via non-technological means (e.g., co-product sales or government subsidies) will also be rejected.

Component Development. While applications that address the entire process leading from sunlight to a liquid fuel are both anticipated and strongly encouraged, those that lead to potentially generalizable approaches (i.e., ones that could apply not only to production of biofuels but also to the growth of food crops) are more useful than those that provide specific approaches to specific fuel molecules. To this end, innovative components of applications that make significant advances towards a key aspect of the overall process or system but do not produce an integrated solution that meets all of the "Primary Technical Targets" (and thus does not qualify for a Systems Development award) may be considered for separate funding in the Component Development category.

- **Areas of Particular Interest:** Any combination of technologies capable of meeting or exceeding the "Primary Technical Targets" (see below) will be considered for a Systems Development award under this funding opportunity. A technology that makes significant advances towards a key aspect of the overall system, but does not meet all of the "Primary Technical Targets" will be considered for a Component Development award. Examples of particularly interesting technologies include, but are not limited to, the following:
    - Improvement in light utilization, including the use of alternative capture molecules and/or redistribution/minimization of the light-harvesting antenna to decrease wasteful non-photochemical quenching processes;
    - Production of energy-dense fuel molecules, e.g. terpenes, via the diversion high-energy Calvin cycle intermediates (upstream of glucose) or the use of otherwise wasted energy and/or reducing equivalents;
    - Use of alternatives to the Calvin-Benson-Bassham cycle (via RuBisCO) for carbon fixation; and
    - Approaches that employ metabolic pathways found in widely divergent organisms, such asthose derived from prokaryotes, archaebacteria, or extremophiles.
- **Areas of Supplemental or Secondary Interest:** Any combination of technologies capable of meeting or exceeding the "Primary Technical Targets" (see below) will be considered for a Systems Development award under this funding opportunity. A technology that makes significant advances towards a key aspect of the overall system, but does not meet all of the "Primary Technical Targets" will be considered for a Component Development award. Examples of supplemental technologies that may enhance an application but will *not* be sufficient on their own merits are the following:
    - Recovery of the biochemical energy lost to photorespiration (which has been demonstrated primarily in C_3 organisms);
    - Replacement or transformation of chloroplasts in C_4 plants based on cyanobacterial precedents to improve photosynthesis;
    - Symbiotic combinations of organisms that separate light capture and fuel production; and
    - Development of productive photobiological combinations that absorb light more effectively, e.g., through mismatched action spectra.
- **Areas Specifically Not of Interest:** Many Federal funding agencies currently support programs to advance the development of biomass-based biofuels. Consequently, applications that either use, or enhance, biomass as a feedstock will *not* be considered for this FOA. As a general rule, incremental improvements to, or combinations of, existing products and technologies, wherein no significant transformational advances in understanding or reductions in technical uncertainty are achieved, do not fit ARPA-E's mission, and will also not be considered. Additional excluded categories are:
    - Incremental improvements to, or combinations of, existing products and technologies, wherein no significant advances in understanding or reductions in technical uncertainty are achieved;
    - Incremental improvements in existing liquid fuel processes, or approaches that rely solely upon improved farm practices or selective breeding of existing plant species;

- Demonstration projects that do not involve a significant degree of technical risk;
- Artificial (abiotic) or "bio-hybrid" photosynthetic systems that mimic natural photosynthetic systems or incorporate inorganic components that do not result from genetic expression;
- Systems in which the final product organism cannot be deployed as an agricultural crop [NOTE: Unicellular aquatic organisms such as microalgae, cyanobacteria, and diatoms will *not* be considered to be deployable in this manner. However, the use of insights and developments from such systems is valuable and is actively encouraged.]; and
- Purely engineering approaches that rely on existing organisms, e.g., the development of engineered photobioreactors or containment vessels for use with unicellular organisms.

Any Concept Papers or Full Applications that focus on "Areas Specifically Not of Interest" will be rejected as nonresponsive and will not be reviewed or considered.

4. Technical Performance Targets

Applications will not be considered for funding unless they have a well-justified, realistic potential to meet or exceed all of the Primary Technical Targets by the end of the period of performance for the proposed project. This funding opportunity focuses on research and development projects that lead to an innovative organism for the production of liquid transportation fuels. To be deployed successfully, the organism must increase carbon-use efficiency and reduce cost when compared to corn agriculture and corn-based ethanol (see Table 1). Additionally, applicants should quantitatively describe the approach to scale the developed organism (i.e., the process hurdles that stand between lab scale demonstration and the production of 100 million gallons per year in a commercial facility). Applicants are expected to describe crops that can be grown in the United States on a level of acreage similar to corn. The applicant is also encouraged to highlight aspects of the application that would provide an opportunity for U.S. leadership in the production of biofuels.

Applications will receive favorable consideration if they meet or exceed at least one of the Secondary Technical Targets. Preference will be given to applications that have a well-justified, realistic potential to meet or exceed most, if not all, of the Secondary Technical Targets.

Applications should account for the time required to measure the end-of-project target, particularly the length of time to grow the plant to its harvest stage.

The Primary Technical Targets and Secondary Technical Targets for this FOA are stated below.

a. Primary Technical Targets

ID Number	Category	*Basis/Current Perceived Limit*	Value (Units)
1.1	Energy Density of the liquid fuel extracted	iso-Butanol	≥ 26.5 MJ/L (LHV)
1.2	Melting point of the liquid fuel extracted	Gasoline	≤ -40 °C
1.3	Boiling point of the liquid fuel extracted	Isoprene	≥ 35 °C

1.4	Per hectare energy yield at scale	See Table 1	≥ 2 x ethanol yield from corn (≥160 GJ/Ha/year)
1.5	Process cost at scale	OECD/IEA[24]	≤ $10/GJ ($50/BOE)

b. Secondary Technical Targets

ID Number	Category	*Basis/Current Perceived Limit*	Value (Units)
2.1	CO_2 Use	Corn	Atmospheric CO_2
2.2	Water Requirements	Corn	≤ 22 inches/year
2.3	Fertilizer Requirements	Corn	N ≤ 179 lbs/acre P ≤ 62 lbs/acre K≤ 50 lbs/acre

c. End-of-Project Targets

Category	Scale	Approach/Form Factor
Deliverable	Demonstration of yield through direct side-by-side comparison with established standard organism	A plant or other multicellular organism ready for field-testing

During award negotiations, ARPA-E will establish materially significant "go/no-go" metrics to track the progress of each project selected. Further, an independent external partner designated by ARPA-E will be employed to independently validate the performance of all organisms.

<u>EXAMPLES:</u>

<u>Example Biofuel Crop A</u>

The applicant proposes to engineer switchgrass with multiple traits to produce an easily extractable hydrocarbon. One set of modifications significantly increases the synthesis of the cyclic terpene limonene ($C_{10}H_{16}$, mp -74 °C, bp 176 °C), which is combustible for fuel, to achieve limonene levels of 20% dry weight in switchgrass. A second set increases biomass production 50%. Limonene can be directly extracted from the switchgrass biomass and there is no processing step anticipated for biofuel production.

	$t_C \cdot ha^{-1} \cdot y^{-1}$	Yield
Total Captured	17.	35.%
Harvested Biomass	11.	23.%
Purified/Processed	4.1	8.3%
Final Energy Content ($GJ \cdot t_C^{-1}$)	51.1 (Limonene)	
Overall Fuel Yield		

The applicant then calculates the energy content in the fuel molecule as a measure of the carbon flux through engineered switchgrass, based on observed growth yields in the field and the efficiency of extracting limonene.

ARPA-E considers the crop is responsive to the FOA, as the fuel yield from the limonene produced in

techno/essentials.htm, January

Example Biofuel Crop B

The applicant proposes to utilize a natural strain of duckweed (*Spirodela polyrrhiza*) that was selected in the laboratory for high starch production, and based on laboratory studies is estimated to produce yields of 15 tons dry biomass/ha/yr. A novel production system utilizing a tiered aquaculture platform will be developed that allows two crops to be grown simultaneously on a single site, doubling yields of starch for ethanol fermentation. The entire biomass can be processed for starch extraction, utilizing all of the photosynthetic energy captured by the crop.

	$t_C \cdot ha^{-1} \cdot y^{-1}$	Yield
Total Captured	14.	28.%
Harvested Biomass	14.	28.%
Purified	6.3	11%
Processed	3.5	5.6%
Final Energy Content ($GJ \bullet t_C^{-1}$)	52 (Ethanol)	
Overall Fuel Yield ($GJ \bullet ha^{-1} \bullet y^{-1}$)	182	

The applicant then calculates the energy content in the fuel molecule as a measure of the carbon flux through the duckweed following Table 1. These calculations are based on observed growth yields in small ponds and the efficiency of producing ethanol from starch.

ARPA-E considers the crop non-responsive for several reasons. First, at 22.77 MJ/L, ethanol does not have sufficiently high energy density to be considered as a product. Second, light will be limiting during several periods, such that the two tiers are unlikely to work independently. Third, it is not feasible to implement a large-scale aquatic production platform on large areas across the United States. Finally, the strategy focuses on physical engineering approaches instead of biological.

Example Biofuel Crop C

The applicant proposes to engineer sugarcane by altering metabolic pathways so that 60% of the solar energy that normally goes into sucrose production is redirected to produce triglycerides. The triacylglycerol can be easily extracted from the cane and processed into biodiesel in the same manner as soybean oil, while the cane sugar can still be extracted and converted into ethanol if desired.

	$t_C \cdot ha^{-1} \cdot y^{-1}$	Yield
Total Captured	24.	48.%
Harvested Biomass	16.	32.%
Purified	4.5	9.0%
Processed	4.0	8.1%
Final Energy Content ($GJ \bullet t_C^{-1}$)	50 (FAME)	
Overall Fuel Yield ($GJ \bullet ha^{-1} \bullet y^{-1}$)	201	

The applicant then calculates the energy content in the triacylglycerol as a measure of the carbon flux through the engineered sugarcane as presented in Table 1. These calculations are based on observed growth yields in FL, LA, and TX, and the efficiencies of biodiesel from triacylglycerol.

Crop C meets the target for overall fuel yield, but will not be considered responsive because sugarcane is a tropical crop and cannot be grown over a wide range of the United States. However, if cold tolerance is also engineered in this sugarcane, allowing it to survive freezing temperatures and expand its growing range to that of maize, then Crop C would be considered responsive.