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SPECIAL FEATURES ISSUE

Versatile CAMELINA

The future of biofuel and much more

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Camelina is the future of fuel. This is a bold statement, yet with its increasing demand as a here-and-now source of biofuel feedstock, this statement is more firmly based in reality now than ever before. Camelina-derived vegetable oil is a key emerging biofuel feedstock for next-generation biofuels and for traditional renewable biodiesel markets. The estimated demand for fuel for the aviation sector in the United States alone is 17 billion gallons (64 billion liters) per year, while the worldwide renewable diesel market (biodiesel and traditional diesel derived from vegetable oils) is more than 2 billion gallons per year. To meet these demands, it is critical to have a vegetable oil that can be produced economically on a large scale without any government subsidy. Camelina-derived oil meets this litmus test.

The increased demand for camelina oil as a feedstock for biofuels is in many ways largely based on the food or fuel debate. Unlike traditional food crops that have crossed over to fuel production, such as corn, soybeans, and canola, camelina is not a food crop in the United States. In addition, its ability to produce high yields while growing on marginal lands with minimal chemical inputs means that it will not displace food crops from fertile land. An added bonus is that the resulting high-protein, high-energy meal can be used in livestock rations, thereby reducing the need for traditional food crops in these rations. In short, camelina is a sustainable crop for biofuels production.

Camelina: What is it?

Camelina sativa is a member of the family Brassicaceae, which includes mustards, rape, cabbage, kale, brussels sprouts, and cauliflower. Similar to these plants, camelina contains glucosinolates, but these glucosinolates are unique to camelina and, like all glucosinolates, offer a natural protection against consumption by insects, thereby reducing insect pressure. It is a high-yield oilseed crop that produces seeds containing approximately 40% oil, yet its meal has a high protein content of nearly 38%. Thus, it is an excellent oil producer for biofuels and provides a residual high-protein meal for use in livestock rations.

Camelina has been cultivated for more than 3,000 years in Europe, and the Romans used its oil as a lamp oil and its meal for livestock feed. The bulk of modern camelina production has been limited to Eastern Europe and Finland, where the oil is niche-marketed as a healthful edible oil. Camelina is a robust seed producer with an oil profile that contains nearly 40% α-linolenic acid (ALA, 18:3n-3) and 20% linoleic acid (LNA, 18:2n-6). (See Table 1.)

Unlike flax oil, camelina oil is stable owing to a high level of natural antioxidants and contains a more balanced amount of n-3 and n-6 fatty acids. The oil also contains fatty acids of up to 24-carbon chain length that

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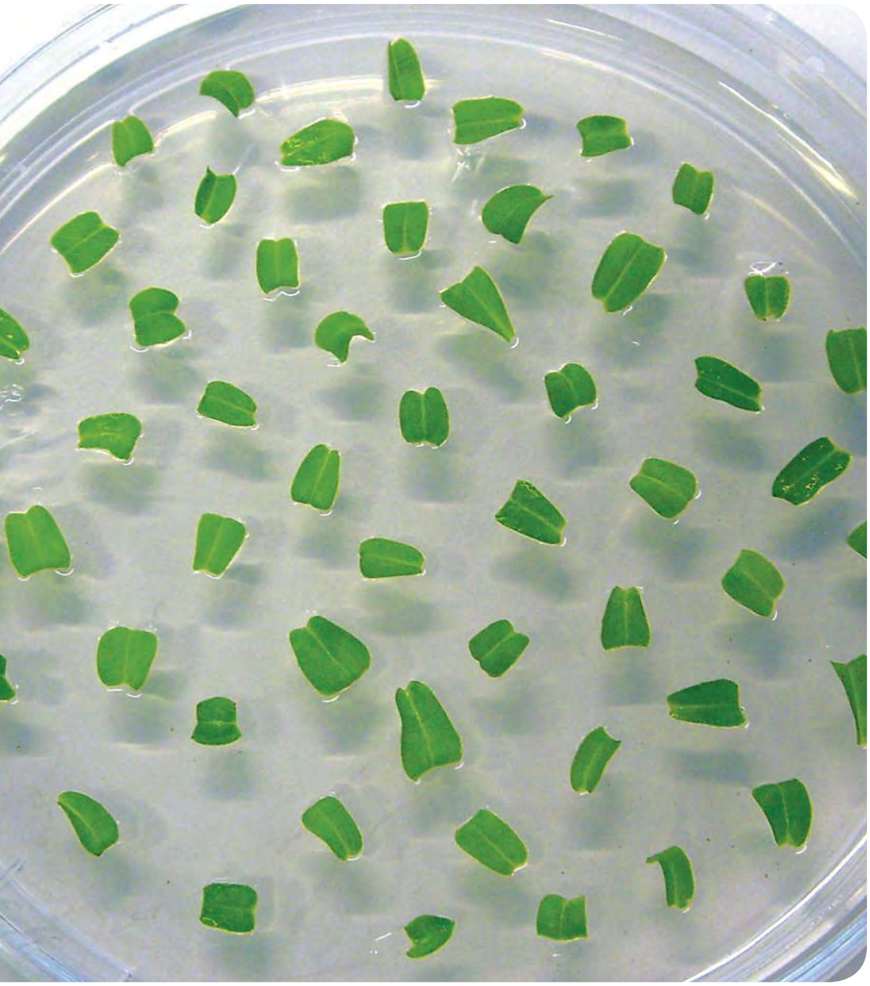

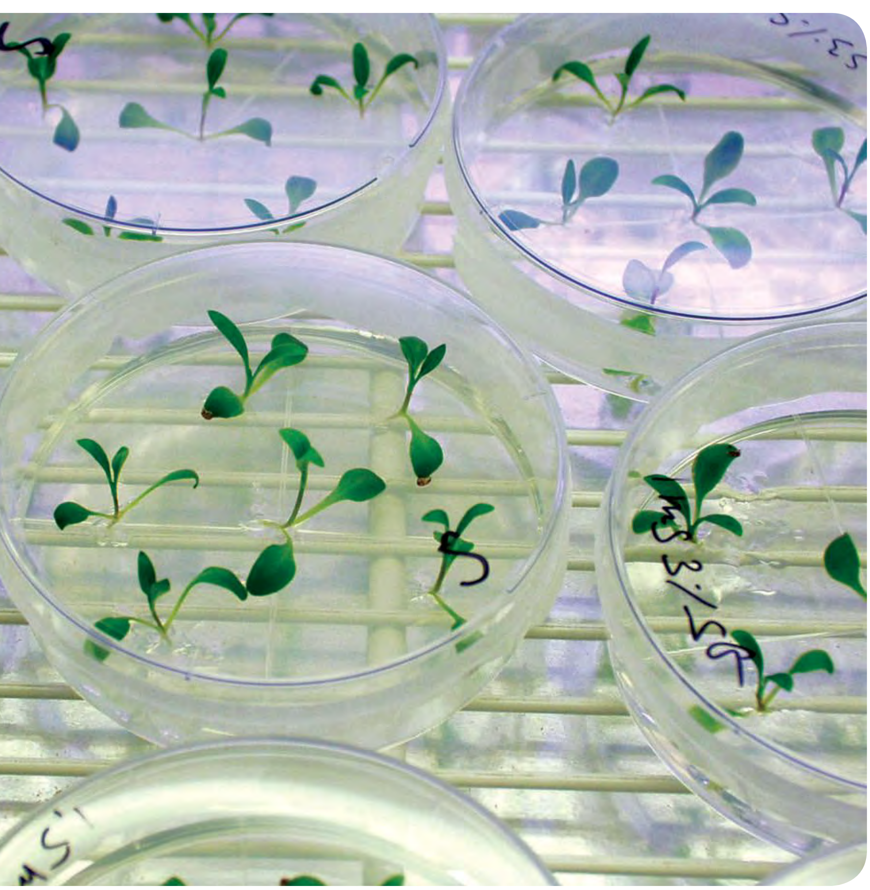

Transgenic camelina is produced by transforming leaflet explants (upper left) into sprouts, which are grown in agar (lower left) and later transplanted to soil (above) for greenhouse growth. This allows many plants with a particular genetic modification to be produced from just a small amount of material. Photos courtesy of Unicrop/Agragen.

TABLE 1. Oil profile of field-grown and greenhouse-grown camelina[a]

Fatty acid	Greenhouse-grown		Field-grown
Lauric 12:0	BLD	BLD	BLD
Myristic 14:0	0.1	0.1	BLD
Palmitic 16:0	6.5	6.6	5.3
Stearic 18:0	3.1	3.0	3.0
Oleic 18:1n-9	17.9	19.2	18.7
Linoleic 18:2n-6	17.4	17.4	16.0
Linolenic 18:3n-3	27.8	25.6	38.1
Arachidic 20:0	2.2	2.1	1.4
Eicosenoic 20:1n-9	14.6	16.4	11.6
Eicosadienoic 20:2n-6	1.8	1.9	1.8
Eicosatrienoic 20:3n-3	1.5	1.3	1.3
Behenic 22:0	0.5	0.4	<1
Erucic 22:1n-9	3.6	3.7	2.5
Lignoseric 24:0	0.2	0.1	<1
Nervonic 24:1n-9	2.9	1.4	<1
Camelina parent line	Blaine Creek	GP68	Celina

[a]BLD, below limit of detection; <1% indicates trace fatty acids; all values represent mole%. Note: Other field-grown camelina varieties have 18:3n-3 content in the range of 34–41%.

are derived from elongation of mainly 18:1n-9, but also of 18:2n-6 and 18:3n-3, indicating a relatively robust level of elongase activity.

More than fuel: camelina meal in livestock rations

Production of large quantities of oil for biofuels results in a substantial amount of meal as a by-product. Camelina meal is an excellent source of protein and energy owing to the residual fatty acid content. Meal produced by Great Plains Oil and Exploration (GPOE; Cincinnati, Ohio, USA), has a residual oil content of about 8% (by wt), and this oil is also rich in n-3 fatty acids. (Great Plains Oil and Exploration is a sister company to Agragen LLC, a Cincinnati-based biotechnology company for which I am chief scientific officer and executive vice president for research and development.) In laying hens, this level of residual n-3 fatty acids increases the egg n-3 fatty acid content, including heart-healthy eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). GPOE's proprietary production process produces a meal in which myrosinase, the enzyme that breaks down glucosinolates to isothiocynates and other antinutritive compounds, is inactivated, thus limiting the level of these antinutritive compounds in the meal. The net result is a more healthful meal for livestock, which was demonstrated in laying hens in which a ration containing 20% camelina meal had no adverse impact on hen health as determined by weight gain or egg-laying days.

However, to be consistent with an inclusion rate of not more than 10% camelina meal in rations for broiler chickens and cattle set by regulatory agencies, we requested from the US Food and Drug Administration (FDA) the same inclusion rate for camelina meal in laying hen diets and received from the FDA a letter of no objection for laying hen rations to contain up to 10% camelina meal. The ultimate large-scale production of camelina for biofuels provides a high-quality meal as a by-product that will provide options for livestock producers to reduce the amount of other grains also used for human consumption in livestock diets, such as barley or corn in cattle or soy meal in poultry.

Lessons from camelina: trials and tribulations of introducing a new crop

While Agragen, LLC and Unicrop, Oy (Helsinki, Finland) have more than 14 years of experience in using biotechnology approaches to introduce agronomic improvements to camelina via our patented technology platform, GPOE has worked diligently to introduce a new crop to US and Canadian farmers, while also expanding operations around the globe. Growing more than 100,000 acres (40,000 hectares) of camelina in 12 states in the United States and four Canadian provinces, GPOE has been at the forefront of introducing camelina for large-scale agriculture. As the world's largest producer of camelina seed stock and producer of camelina-derived oil and meal, GPOE has experienced the pitfalls of introducing a crop that has had minimal development of best farming practices. It is estimated that in the United States alone, there are 20 million acres of marginal land that could be used to grow camelina. Because of its 90-day growth cycle from emergence to harvest, camelina offers farmers who grow cotton and soybeans a double-crop

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In the greenhouse, transgenic camelina goes on to flower (left) and produce seed pods (above) of its own. Photos courtesy of Unicrop/Agragen.

option in certain regions of the country such as Texas, Oklahoma, and Arkansas. This dramatically increases the number of available acres for camelina in the United States alone.

Camelina grows well on marginal lands and requires minimal inputs in terms of fertilizer or herbicide applications. Combined with no need for spraying and single-pass harvesting, the planting and harvesting of camelina requires minimal field work, resulting in a low carbon footprint for its growth. Because it grows well in areas with 6–8 inches (15–20 cm) of precipitation and on marginal lands, camelina can be grown in regions with poorer soils not suitable for corn or soybeans.

In areas such as the Pacific Northwest (PNW) of the United States, where cropping options are limited, camelina offers a good option for farmers as a rotational crop. It is an excellent rotation option for dry-land wheat operations. When used as a rotational crop with wheat, the yield of the wheat crop following camelina increases up to 15%. However, GPOE has experienced crop failures in this region due to residual amounts of class-2 herbicides in the soil. This indicates that camelina is highly susceptible to class-2 herbicides, which are heavily used in wheat operations in this region. This presents a unique problem for camelina in the PNW, one which Agragen worked to solve using a biotechnology approach.

Biotechnology and camelina: lessons learned

When initially presented with the puzzling problem of PNW crop failure, we immediately speculated that this failure was due to residual class-2 herbicides in the soil. This was a problem that scientists at Agragen and Unicrop solved using biotechnology to engineer specific mutations in the gene encoding the enzyme acetolactate synthase (ALS). ALS is the first committed step for branched-chain amino acid biosynthesis and the target enzyme inhibited by class-2 herbicides. By combining specific amino acid changes in the active site, we achieved a herbicide-dependent increase in tolerance between 1,000- and 10,000-fold in two different varieties of camelina.

We have also used a biotechnology approach to introduce specific changes in camelina fatty acid composition and in its oil content. Again, these strategies use multiple gene constructs and our patented high-throughput transformation protocol. We have produced a high lauric acid (12:0) camelina while preserving the ALA (18:3n-3) content. This is important for using the meal as a high-energy, residual n-3 fatty acid source for poultry applications. With an academic third party we have introduced specific genes in the triacylglycerol biosynthesis pathway and have entered into collaboration with another academic partner to enhance the oil content of camelina using a novel strategy.

Agragen's and Unicrop's strong intellectual property portfolio in terms of issued patents and in proprietary technology in camelina plant science offers a number of different partners the capacity to rapidly make agronomic changes in the plant using our nonselection transformation technology. This strategy is important for downstream commercialization efforts to produce a meal without any genes that impart antibiotic resistance. Our rapid, high-throughput transformation technology is highly effective with a very high level of transformation efficiency. This is important because common strategies for transformation of camelina are ineffective, although a number of laboratories and competitors have had success using a floral dip transformation strategy. In our hands this method has not been overly successful in meeting the needs of a commercial high-throughput operation, and its use for commercial applications may be limited by our recently awarded patents for our transformation method in the United States and the European Union. Nonetheless, the floral dip method does offer noncommercial entities interested in camelina options with regard to transformation.

Beyond biofuels: plant-made pharmaceuticals

Agragen, LLC was founded as a plant-made pharmaceutical company with the vision of producing biological pharmaceuticals at a much lower price than the traditional method of producing drugs in mammalian cells. After our acquisition of Unicrop, Oy, we shifted our emphasis to producing cytokine trap molecules for use in a variety of disease states. Why is camelina an ideal platform for producing these biological pharmaceuticals? Simply, camelina is for all essential purposes a self-pollinating plant and a nonfood crop. Hence, the possibility that pharmaceutical-producing camelina might cross with weeds or other plants is extremely limited, and its use as a crop only for biofuels limits the liability seen with other food crops such as corn or rice.

Agragen's lead compound, AGR131, is a cytokine trap designed to reduce the levels of tumor necrosis factor alpha in patients with inflammatory conditions, such as rheumatoid or psoriatic arthritis. It binds the target cytokine with an affinity equal to or greater than Enbrel, a well-known drug in this drug class. Using our patented protein expression system combined with our patented rubisco [ribulose-1,5-bisphosphate carboxylase/oxygenase] promoter technology, we have developed a very efficient sprouting expression system, putting our expression of proteins on par with yeast or slightly better than mammalian cells. Additional proprietary technology limits the addition of plant-derived sugars, overcoming a significant challenge that had limited the growth of the plant-made pharmaceutical industry. Because we harvest the seed and use our sprouting technology, we can efficiently recover the protein from the sprouts with yields approaching 85% and a purity of the final protein product exceeding 99%. Thus, although camelina is the future of fuel, it very well may be the future of biological pharmaceutical production, lowering the cost point of these drugs to enhance their affordability. Who knew that camelina would be so versatile? ■