Bio-based Polyols
Challenges and Opportunities

Pacific Rim BIO
Linnaeus Plant Sciences
October 11th, 2012

Jack Grushcow
www.linnaeus.net
Linnaeus Mission:
We want to do chemistry in Oil Seeds

- Light and water to make valuable feed stocks
- Increase use of oilseeds as petroleum substitutes
- Increase oilseed values for farmers…beyond fuel
- Reduce environmental impacts of petroleum
- Sustainable crops and production methods
Plant Biotechnology Overview
Potential of Plant Oil Diversity

- The Tropics contain great bio-diversity
- Tropical plants have long been a source of our most valued medicines – *why not industrial feedstocks*?
- These plants produce THOUSANDS of valuable industrial oils BUT they lack agronomics
- Linnaeus is developing germplasm for large scale production
• TAG is synthesized by the sequential addition of fatty acids to a glycerol “backbone”.

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{CH}_2\text{O-R} \\
\text{CH-OH} & \quad \text{CH-OH} \\
\text{CH}_2\text{PO}_4 & \quad \text{CH}_2\text{PO}_4
\end{align*}
\]

TAG assembly

*Modern Philosopher’s Stone*
Relevance to Industry

- New genes will be cloned and engineered
- Patents will control new materials
- Competitive advantages will be conferred
- Developing a feedstock “religion”
- Regulations evolving
- Plants can produce large volumes at low cost
Crop Platform Development
Camelina - Introduction

• *C. sativa* grown in Europe for centuries

• *frost tolerant at seedling stage, heat and drought tolerant at later stages*

• *short growing period (85-100 days)*

• *few insect problems, resistant to flea beetle, blackleg, and alternaria black spot*

• *excellent yield potential in most areas, particularly in short season environments and on poor lands*
The Camelina Opportunity

• Large acreages possible
• Complements Grower’s crop rotation
• No outcrossing
• Uses conventional farm machinery
• Significant breeding potential
• How much agronomy is there?
• Camelina can be THE PLATFORM industrial oilseed
Linnaeus Camelina Program
Breeding is Key

• Access to one of the largest collections of Camelina germplasm

• Largest Field trials ongoing in North America
  – Canadian Food Inspection Agency (CFIA) approval for camelina
  – Confined field trials of HFA camelina underway
  – Agronomic trials to develop best management practices
  – Oil yield increased from 38 to 42+

• M2 population, targets include:
  – Specific oil profiles
  – Herbicide tolerant camelina
  – Disease resistant varieties
Camelina field evaluations 2011 - program in 5th year

performance evaluation of elite lines

- seeded at 15 locations across Canada
- randomized complete block design, 4 replicates
What makes a crop sustainable?
Camelina – Environmental Issues

- Well adapted to all soil zones on the prairies.
- Advantage over HO canola in certain soil zones

### Early maturity
- Alberta
- Saskatchewan
- Manitoba

### Drought, heat tolerance
- Gray
- Dark Gray
- Black
- Dark Brown
- Brown
Displacing summerfallow acreage

- In brown soil zone, summerfallow (chemically or tilling the soil to keep free of vegetation for a growing season) is still widely practiced.

- Summerfallow is used to store moisture and reduce production risk (inefficient).

- About 2.5 M ha (6.5 M acres) of summerfallow annually. Most of it in brown soil zone.
Camelina and Summerfallow

• Camelina, being more drought and heat tolerant than canola, can be grown successfully in the area where summerfallow is prevalent.

• It can displace some of the environmentally harmful summerfallow acreage. It is displacing area where no crop is currently being grown (not interfering with food production).
Nitrogen application required for canola to reach maximum yield is 135 kg/ha; recent studies indicate that maximum yield of camelina is reached at 100 kg/ha, a reduction of 35 kg/ha.

Camelina does not appear to respond to sulphur fertilization indicating a reduction of 20 kg/ha.

Phosphate requirements yet to be determined.

Potential to further improve Nitrogen Use Efficiency of camelina through breeding and genetic transformation.
Pesticide Use

• Requires no fungicide / insecticide seed treatment
• Resistant to most canola insects – Late season insecticide treatment likely not required
• Pod Shatter resistant – can be straight combined
• Doesn’t need to be windrowed – saving fuel
Key Achievements

- Camelina Biology Document
- Weediness
- Outcrossing
- Persistence
- Significant reduction in GM field trial costs
- Elite conventional line ready for 2013
Industry Connections
Linnaeus Plant Sciences entered a licensing agreement with DuPont to use oil gene intellectual property, advanced gene technologies and biotechnology expertise developed by DuPont to accelerate development and commercialization of value-added Camelina oil.

Camelina, a drought tolerant, non-food oil seed crop, has the potential to reduce global carbon dioxide emissions by offering renewable, bio-degradable feed stocks that can substitute for petroleum in a variety of applications.

"Gaining access to intellectual property and biotechnology expertise from DuPont and Pioneer will greatly accelerate our efforts to improve Camelina for industrial uses," said Jack Grushcow, president and chief operating officer of Linnaeus Plant Sciences Inc. "Camelina will provide an additional revenue opportunity for farmers as a non-food rotation crop that can be grown with low inputs."

"DuPont is committed to benefiting society through reducing dependence on fossil fuels," said Tony Kinney, DuPont research director. "This technology transfer to Linnaeus not only advances our environmental sustainability goals, but also allows an innovative plant science company to take our technology to the next level in Camelina. Ultimately this agreement will be a win-win for both companies."

Linnaeus has been developing industrial applications for oil seeds for uses beyond fuels, including hydraulic fluids, greases and polymer production, for the last 12 years.

"We have developed an active Camelina breeding program and are fully committed to the development of the crop, integrating trait development through to large scale production," said Grushcow. "This agreement will enable us to greatly improve Camelina's oil profile delivering a non-food crop grown on marginal lands that has utility well beyond fuel."
Linnaeus – Arkema Partnership

- World’s 5th largest chemical company
- Largest user of Castor oil
- Desires a sustainable Castor substitute
Linnaeus Industrial Oil Profiles
# Target Fatty Acid profiles of Camelina seed for Industrial uses

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Camelina oil</th>
<th>High Oleic</th>
<th>High Gondoic</th>
<th>High Ricinoleic</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 Palmitic acid</td>
<td></td>
<td>5.5%</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>18:0 Stearic acid</td>
<td></td>
<td>2.5%</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>18:1 Δ9 Oleic acid</td>
<td></td>
<td>14%</td>
<td><strong>70-80%</strong></td>
<td><strong>30-40%</strong></td>
</tr>
<tr>
<td>18:2 Δ9,12 Linoleic acid</td>
<td></td>
<td>18%</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>18:3 Δ9,12,15 Linolenic acid</td>
<td></td>
<td><strong>36%</strong></td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>20:1 Δ11 Eicosenoic acid or Gondoic acid</td>
<td></td>
<td>14%</td>
<td>Zero</td>
<td>40-50%</td>
</tr>
<tr>
<td>22:1 Δ13 Erucic acid</td>
<td></td>
<td>2.5%</td>
<td>Zero</td>
<td>Low</td>
</tr>
<tr>
<td>12-OH 18:1 Δ9 Ricinoleic acid</td>
<td></td>
<td>Zero</td>
<td>Zero</td>
<td>25-30%</td>
</tr>
</tbody>
</table>
T3 homozygous FAD2amiRNA camelina

GM-Camelina lines with 80% Monounsaturated Fatty Acids
Summary of High-Oleic/Ricinoleic Camelina Project

In conventional camelina…
• 80% MUFA
• 10% PUFA
• 10% SFA
by silencing FAD2

In HFA Camelina…
• 12% Ricinoleic Acid
• Zero Densipolic Acid
by silencing FAD3
• Zero Lesquerolic Acid
by silencing FAE1

In progress…
• Greenhouse Seed Increase for Field Seed Increase Summer 2012
• GENE STACKING
Rilsan® Production Chain

Crude Oil -> Naphtha
Butadiene
Lauryl Lactame
Polymerization & Compounding
Rilsan® PA12

Castor oil
Methyl Ricinoleate
Amino 11 Undecanoic Acid
Polymerization & Compounding
Rilsan® PA11
Solvent Fractionation of Methyl Esters

**Conditions**
- FFA or methyl esters
- Based on the polarity
- Solvents: Hexanes/Methanol/Acetone
- Different Oil-to-solvent ratio
- Temperature: 10 to -25°C
- Separate Top and Bottom fractions
- Evaporate solvent to recover the FFA or ME
Fractionation of Gondoic Acid from Camelina Oil: Physical Methods

Chemical Modification and Distillation collaboration with University of Saskatchewan BioProcessing Pilot Plant
Summary

*Camelina sativa* seed oil has successfully been engineered to produce

- Oleic acid 18:1 Δ9 60%
- Ricinoleic acid 18:1-OH 12.5%
- Gondoic Acid 20:1Δ11 23%

In Progress…

- Seed increases of High Oleic and Ricinoleic Camelina lines in field Summer 2012
- Gene-stacking of amiRNA FAD2, FAD2, and FAE1 to continue to optimize above traits
- *Enzymatic and Physical* separation of Gondoic Acid from the camelina oil
Thanks for your support…