# Bio-based Polyols Challenges and Opportunities

Pacific Rim BIO
Linnaeus Plant Sciences
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### 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



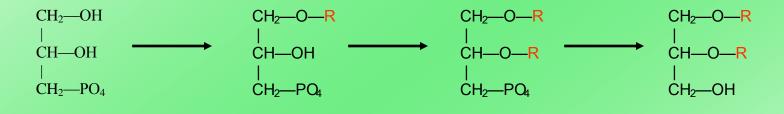
### Potential of Plant Oil Diversity

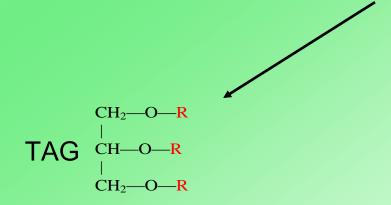
- The Tropics contain great bio-diversity
- Tropical plants have long been a source of our most valued medicines – why not industrial feedstocks?
- These plants produce THOUSANDS of valuable industrial oils BUT they lack agronomics
- Linnaeus is developing germplasm for large scale production



## TAG assembly Modern Philosopher's Stone

• TAG is synthesized by the sequential addition of fatty acids to a glycerol "backbone".







### 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

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# 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 5<sup>th</sup> year

#### performance evaluation of elite lines

- seeded at 15 locations across Canada
- randomized complete block design, 4 replicates



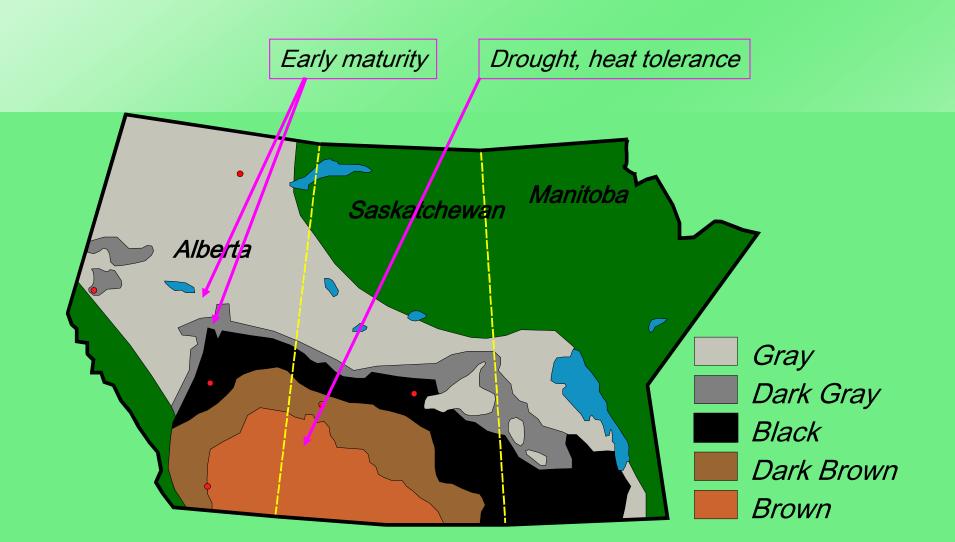


### What makes a crop sustainable?



#### Camelina – Environmental Issues

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



#### 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).



#### Fertilizer Use

- 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



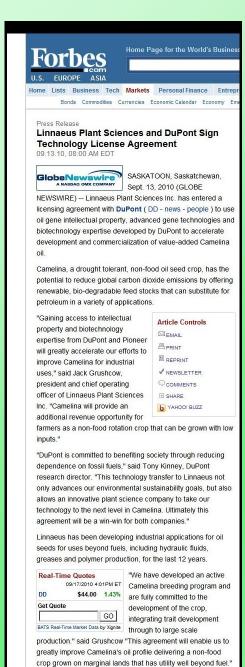
### **Key Achievements**

- Camelina Biology Document
- Weediness
- Outcrossing
- Persistence
- Significant reduction in GM field trial costs
- Elite conventional line ready for 2013



### **Industry Connections**





### **Technology Partnerships**

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 valueadded Camelina oil.



### Linnaeus – Arkema Partnership

- World's 5<sup>th</sup> largest chemical company
- Largest user of Castor oil
- Desires a sustainable
   Castor substitute



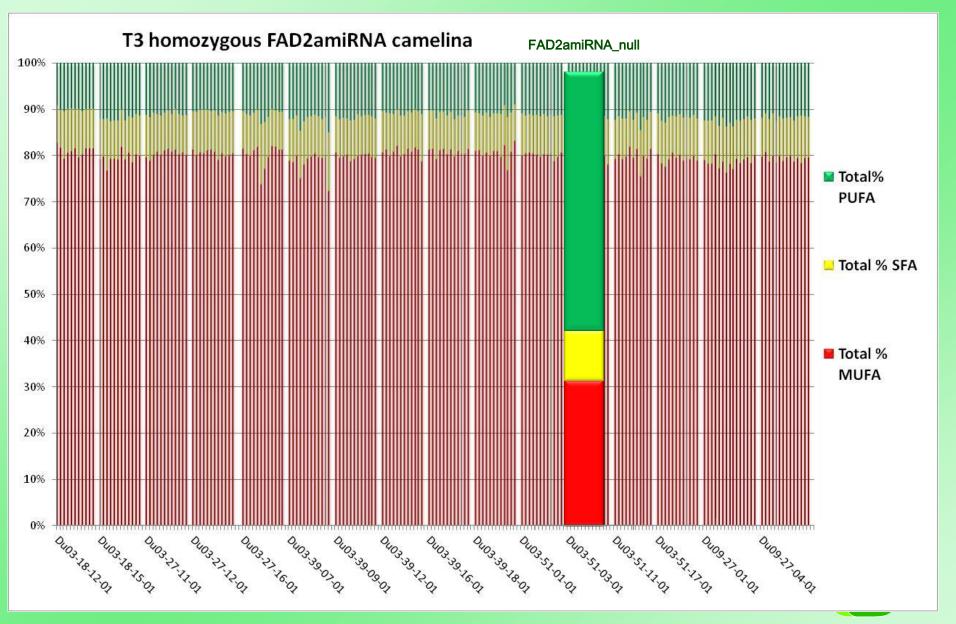


### **Linnaeus Industrial Oil Profiles**



#### Target Fatty Acid profiles of Camelina seed for Industrial uses

Fatty acid	Camelina oil	High Oleic	High Gondoic	High Ricinoleic
O 16:0 Palmitic acid				
но	5.5%	Low	Low	8-10%
O 18:0 Stearic acid	2.5%	Low	Low	Low
O 18:1 Δ9 Oleic acid	14%	70-80%	30-40%	55-60%
18:2 $\triangle$ 9,12 Linoleic acid	18%	Low	Low	Low
18:3 $\triangle$ 9,12,15 Linolenic acid	36%	Low	Low	Low
ο 20:1 Δ11 Eicosenoic acid or Gondoic acid	14%	Zero	40-50%	Zero
22:1 $\Delta$ 13 Erucic acid	<b>2.5%</b>	Zero	Low	Zero
OH HO OH 12-OH 18:1 Δ9 Ricinoleic acid	Zero	Zero	Zero	25-30%
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### 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

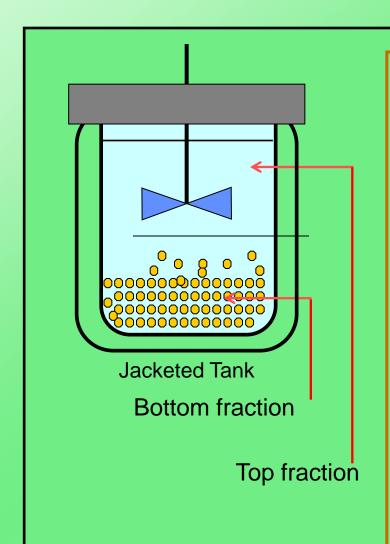


Rilsan® PA11





#### Solvent Fractionation of Methyl Esters



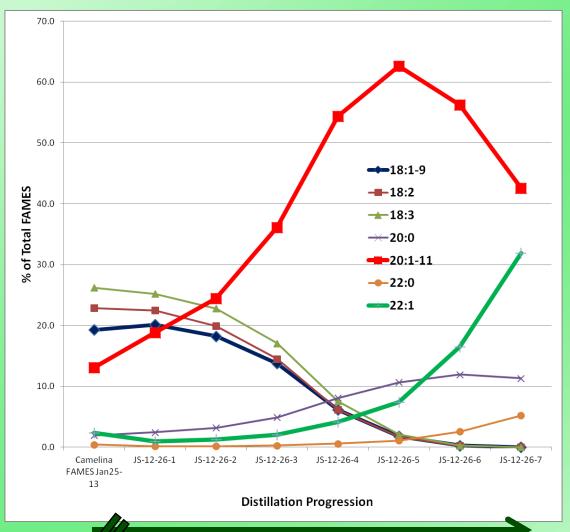
#### **Conditions**

- 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 20:1∆11 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

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• Enzymatic and Physical separation of Gondoic Acid from the camelina oil

### Thanks for your support....













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