Agriculture and Agri-Food Canada — Agriculture et Agroalimentaire Canada

Brassica carinata and *Camelina sativa*

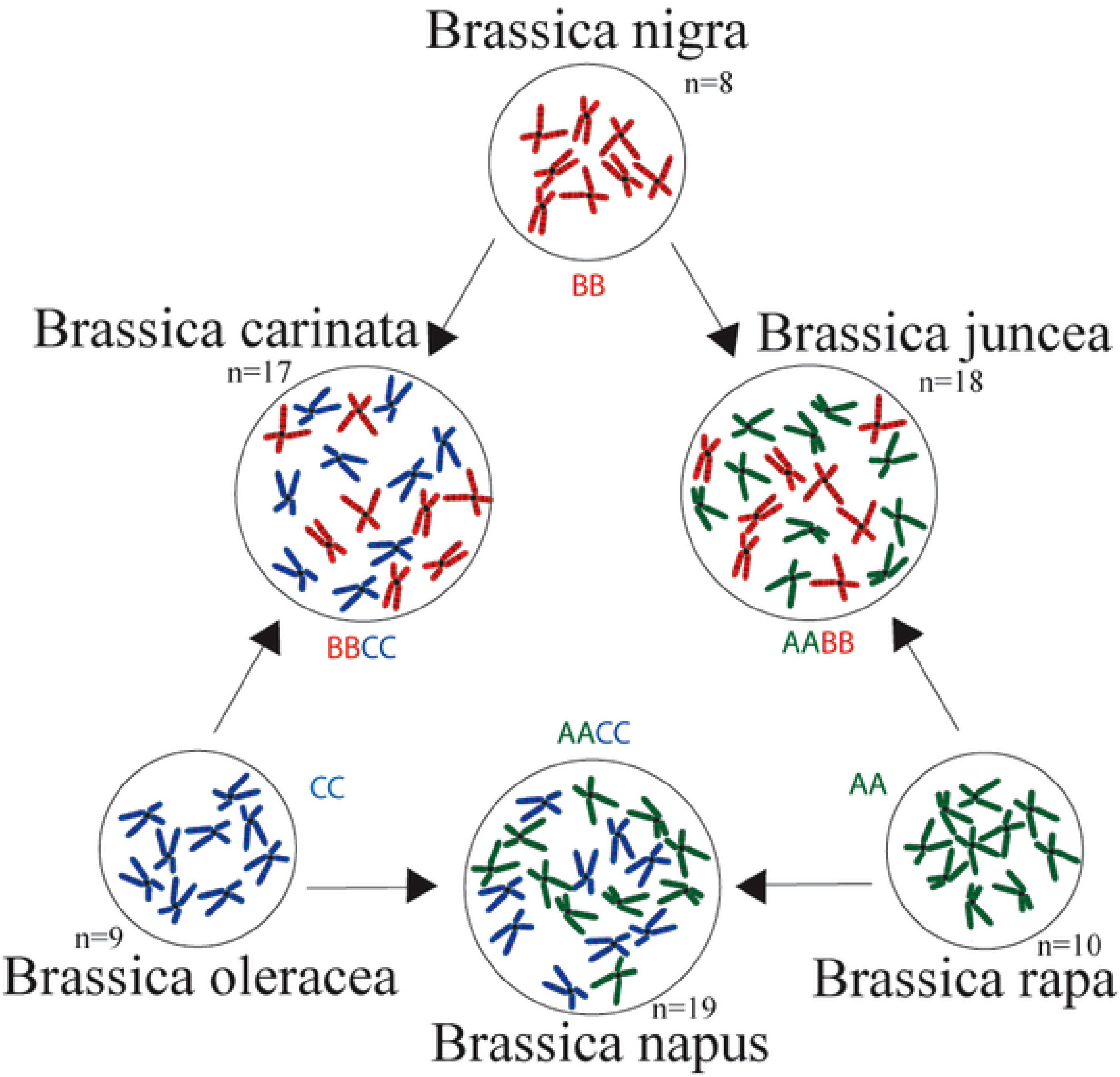
Eric Johnson[1], Kevin Falk[1] and Christina Eynck[2]

[1]AAFC; [2]Linnaeus Plant Sciences Inc.

Canada

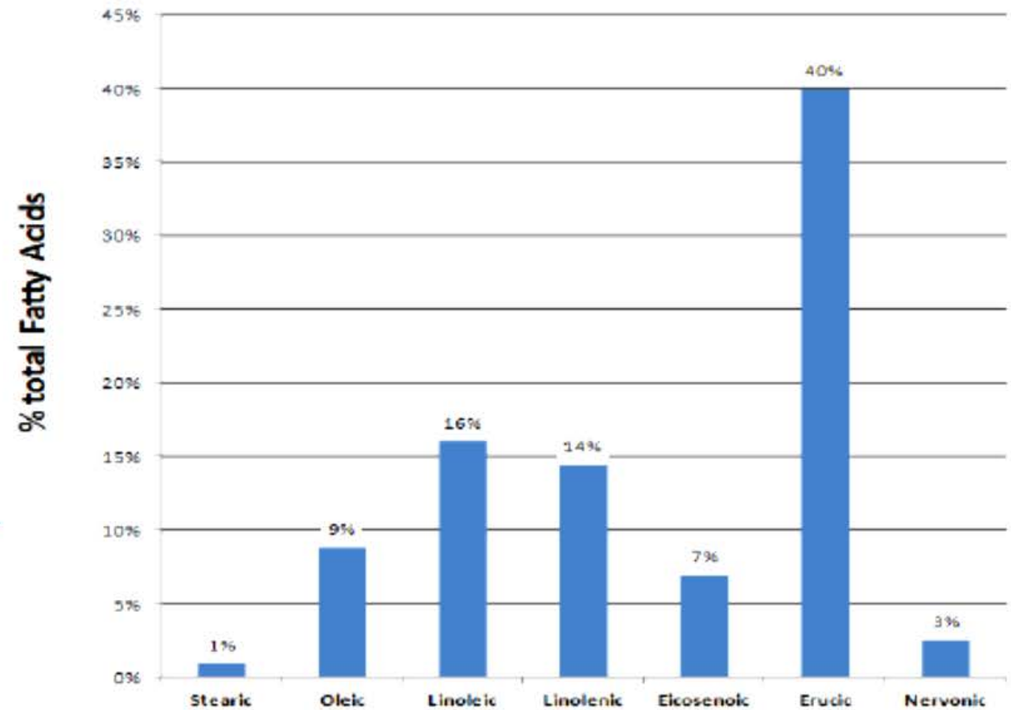
Ethiopian Mustard (*Brassica carinata)*

- Being developed as an industrial oil / bio-platform
- Biodiesel, biopesticide, plastics, polymers, pharmaceutical, and neutraceutical oils.
- Adapted to hotter, drier, longer growing season


Brassica nigra
n=8
BB
Brassica carinata
n=17
BBCC
Brassica juncea
n=18
AABB
AACC
CC
AA
n=9
Brassica oleracea
n=19
Brassica napus
n=10
Brassica rapa

Biodiesel feedstock, high protein meal

- High quality biodiesel, equal to or better than canola
- Low cloud point, oxidative stability
- Non-food oil, low ILUC feedstock



- High protein, low fibre meal
- 20% more crude protein than canola meal, approaching soybean levels

Why Biojet?

- Global GHG Reduction Goals for Aviation:
  - Carbon neutral growth from 2020
  - 50% emissions reduction by 2050

- Global Airline Agreements:
  - Industry-wide sign on to goals
  - IATA, CAAFI, ICAO driving efforts

- Proactive Efforts to Develop Global Value Chains
  - Airlines, OEMS, agencies all involved

AGRISOMA
Sustainable Energy Founded in Agriculture

- **Higher yields of jet fuel**
  - Higher probability of optimal cracking
  - Two jet fuel molecules (C8-C14) from one C22 acid
  - C18 oils can only produce one jet molecule

ReadiJet™

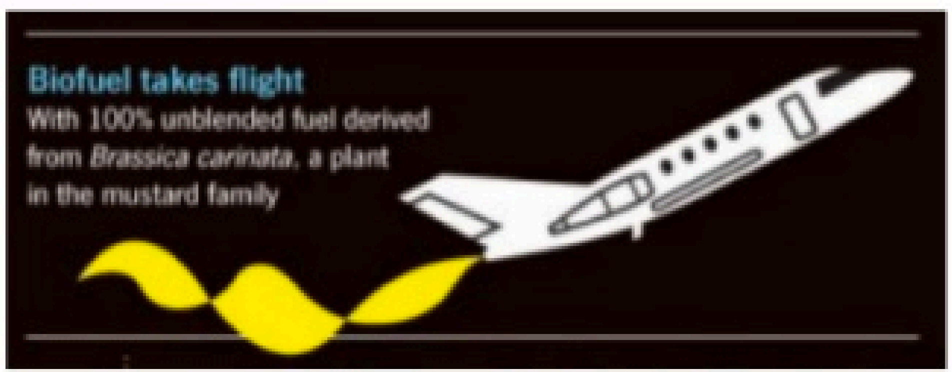
ReadiDiesel™

ARA

December 2012: *Popular Science*

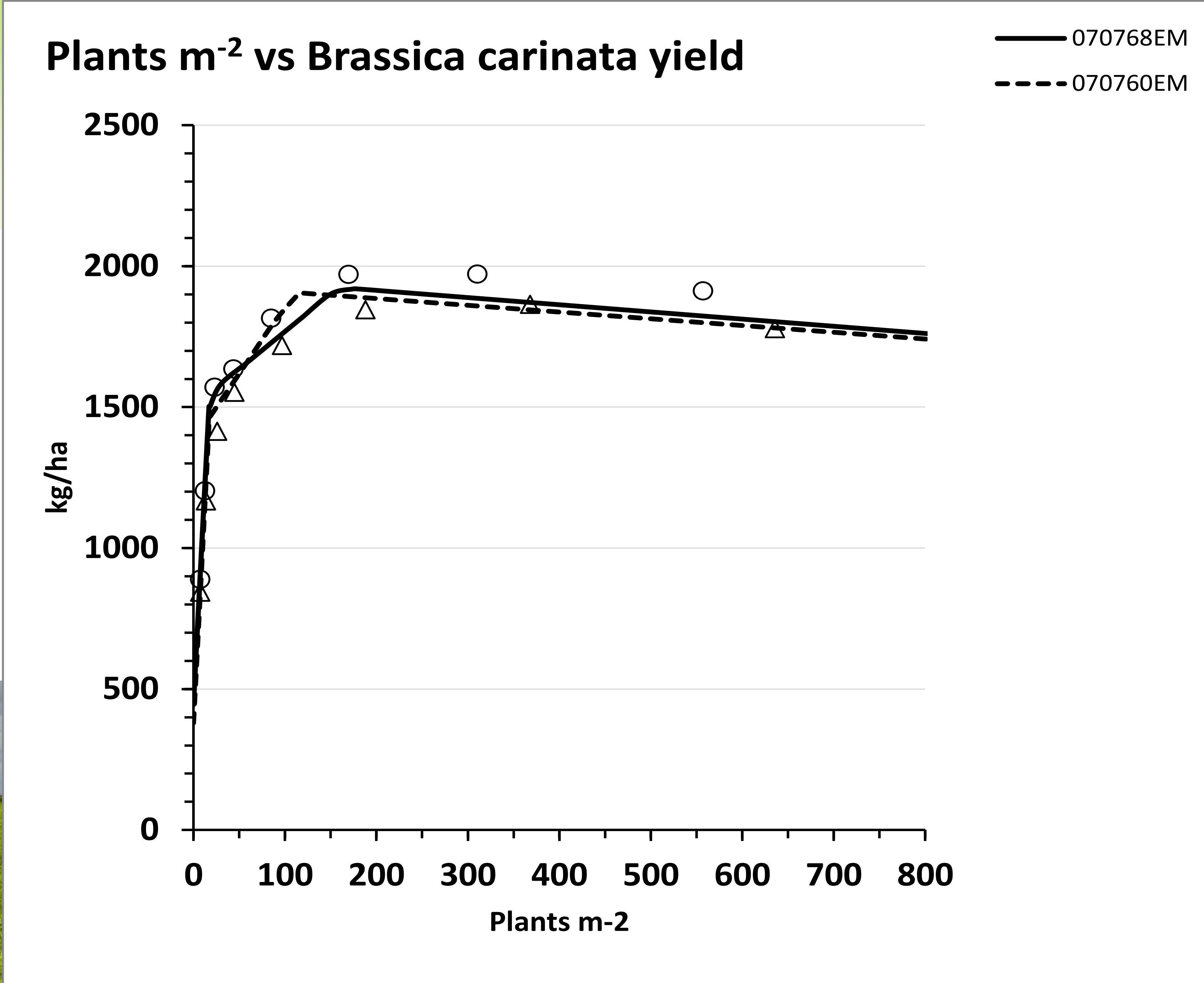
Popular Science Magazine declared the 100% biofuel flight as

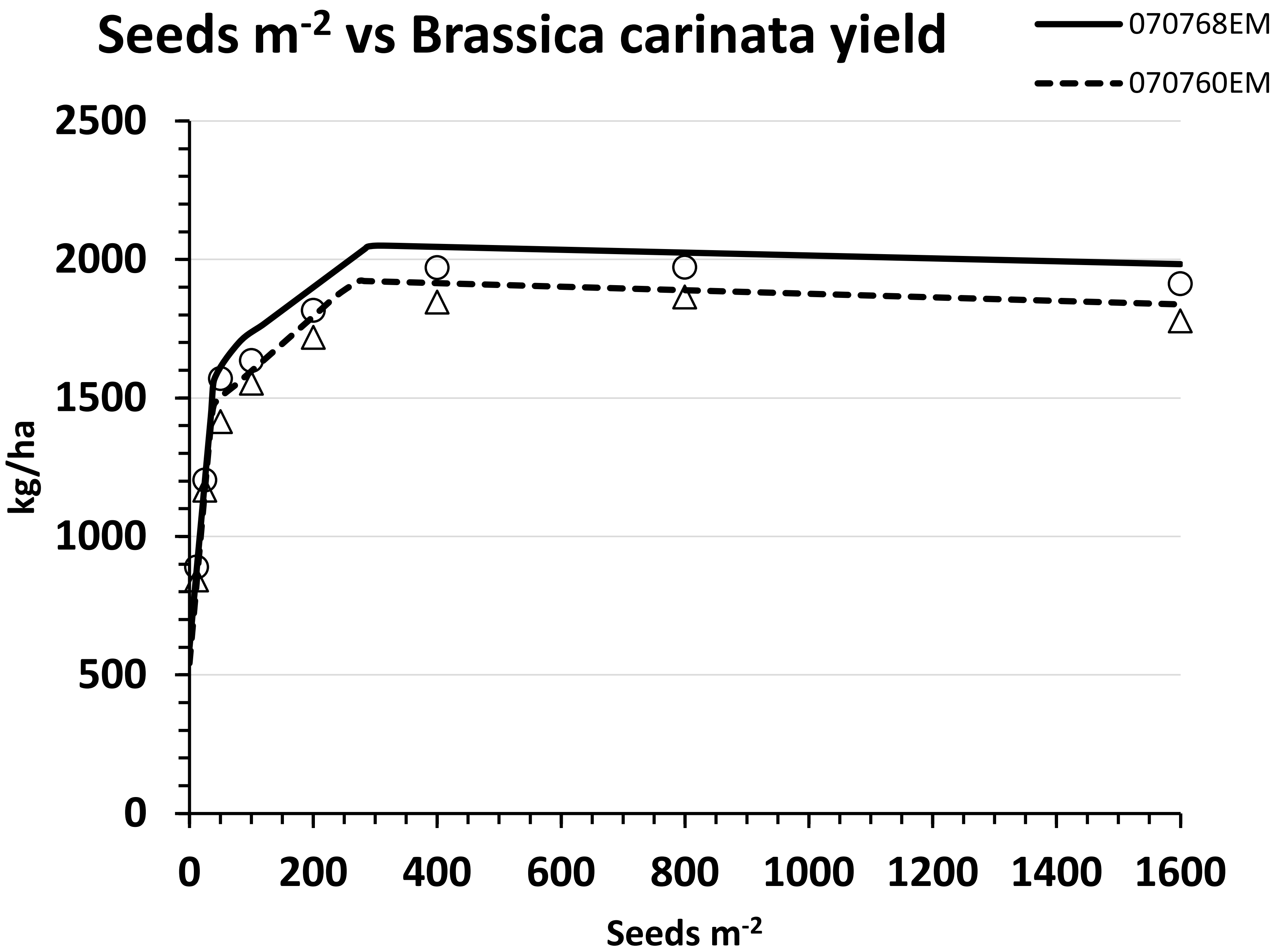
"one of the year's 25 most important scientific events"



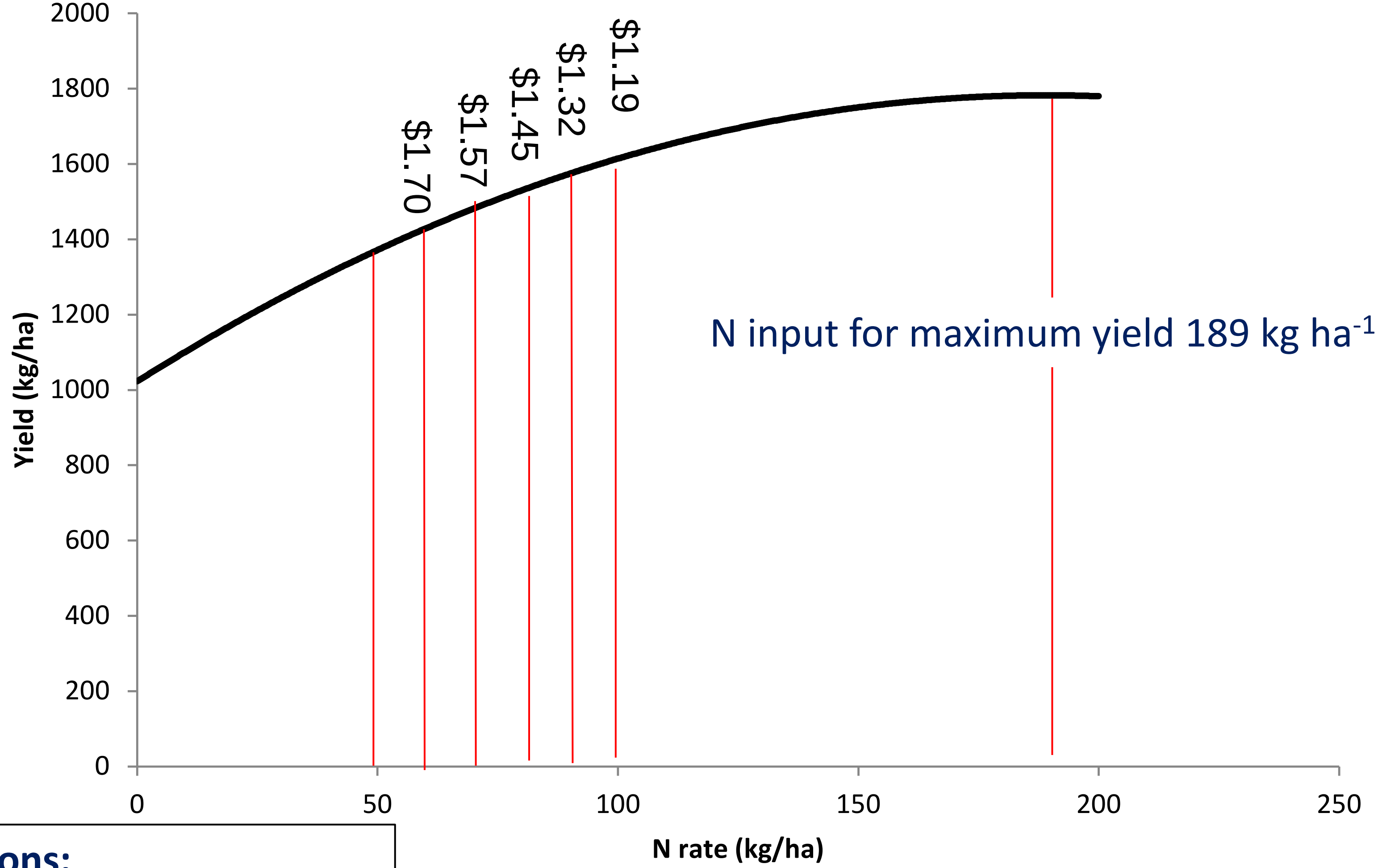
Brassica carinata – Positive Agronomics

- Drought and heat tolerant
- Resistant to blackleg, tolerant to Alternaria
- Large seed size
- Shattering resistant
- Genetically diverse


Plants m-2 vs Brassica carinata yield
070768EM
070760EM
2500
2000
1500
1000
500
0
kg/ha
0
100
200
300
400
500
600
700
800
Plants m-2


Seeds m-2 vs Brassica carinata yield
070768EM
070760EM
2500
2000
1500
1000
500
0
kg/ha
0
200
400
600
800
1000
1200
1400
1600
Seeds m-2

Brassica carinata N response (Adapted from Johnson et al. 2014. CJPS and unpublished data)



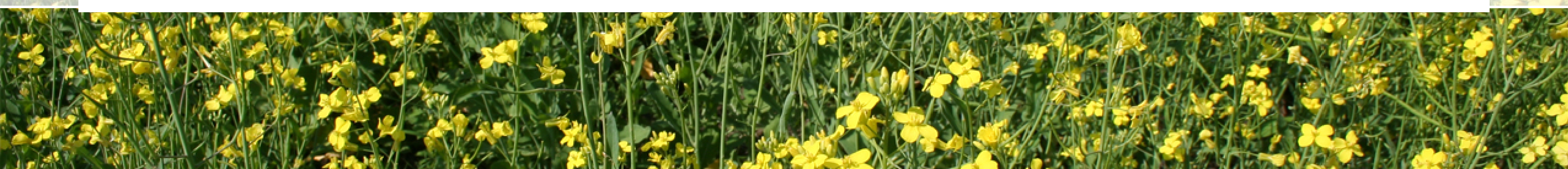
Assumptions:
N fertilizer cost = 65 cents/lb
Carinata Price = $ 9.50 / bushel

Brassica carinata – Agronomic challenges

- Weed control
- Late maturity
  - Maturity has been reduced by 5 to 7 days; however, lines are still 5 to 7 days later than *B. napus.*
  - Target area is southern Prairies, North and South Dakota, Montana.
- Susceptible to aster yellows

Brassica carinata Weed Control

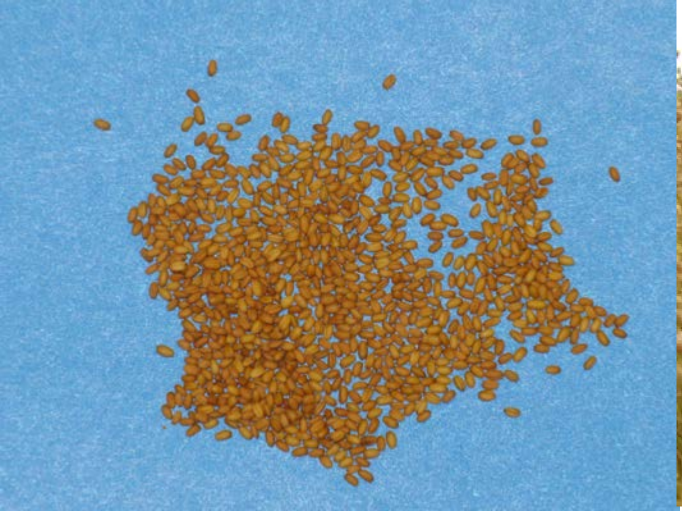
- Major limitation
  - Grass weed control is not a problem – Quizalofop registered
  - Broadleaf weed control
    - Ethametsulfuron (Muster) registered – narrow broadleaf weed spectrum
    - Carinata has tolerance to dinotroaniline herbicides (soil applied). Trifluralin registered; Edge not registered.
    - Sulfentrazone (ppo inhibitor) – seeking minor use registration



Brassica carinata Weed Control

- Major limitation
  - Grass weed control is not a problem – Quizalofop registered
  - Broadleaf weed control
    - Ethametsulfuron (Muster) registered – narrow broadleaf weed spectrum
    - Carinata has tolerance to dinotroaniline herbicides (soil applied). Trifluralin registered; Edge not registered.
    - Sulfentrazone (ppo inhibitor) – seeking minor use registration

Herbicide Screening on *Brassica carinata* germplasm – Scott / Saskatoon 2009



Camelina sativa

Seed quality and oil composition– a comparison

Trait	Canola	Flax	Camelina
Seed oil content (%)	45	41.0	41.6
Protein content (%)	26.1	20.0	29.1
Fatty acid profile (%)			
C18:1 (oleic)	60.3	20.0	14.0
C18:2 (linoleic)	18.0	16	18.0
C18:3 (linolenic)	11.6	53	36.0
C20:1 (gondoic)	< 2	-	14.0
C22:1 (erucic)	-	-	2.5

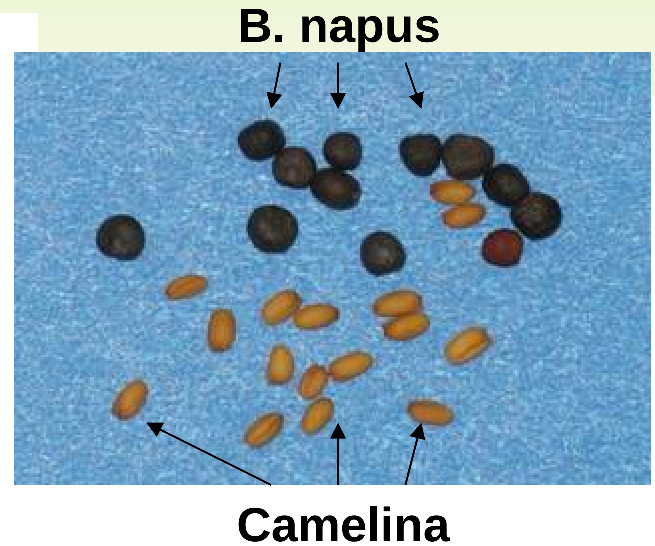
Camelina sativa

- Desirable agronomic traits
  - Early maturity 80 to 100 days
  - Some drought, heat and frost tolerance
  - Resistant to flea beetles, blackleg, alternaria blackspot

Photo courtesy: Venkata Vakulabharanam
Saskatchewan Ministry of Agriculture

Camelina – Agronomic challenges

- Small seed – TKW ~ 1 gram (about 40% establishment rate)
- Broadleaf weed control
  - Tolerant to DNA's; susceptible to pretty well every other broadleaf herbicide
- Susceptible to aster yellows, downy mildew, and sclerotinia



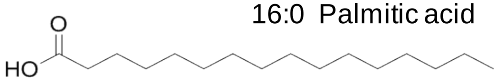
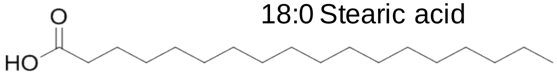
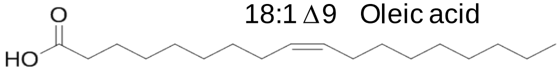
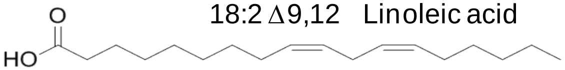
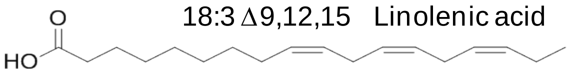
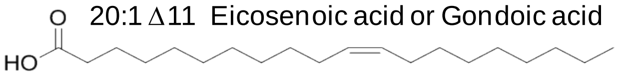
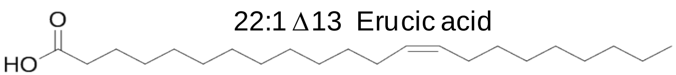
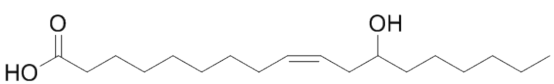
Outlook - Camelina as an industrial crop

Plants oils as petroleum substitutes

- 10% of fossil oil is used in chemical industry
- Products made from petrochemicals have the same value as the remaining 90% of fossil oil consumed as fuel
- Plant oils can easily substitute for petrochemicals. Applications: soaps, paints, plastics, lubricants, hydraulic fluids, cosmetics, etc.
- Added value can also be captured by plant oils!
- Plant oils are renewable

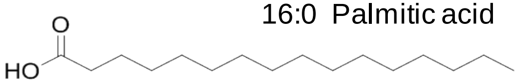
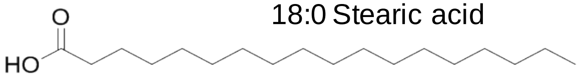
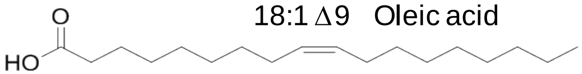
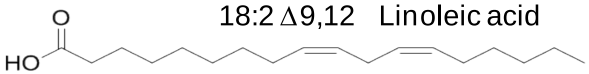
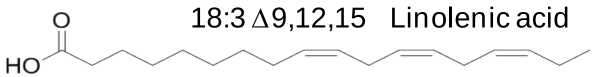
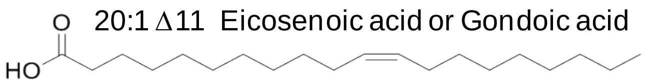
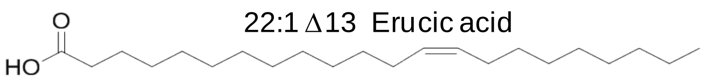
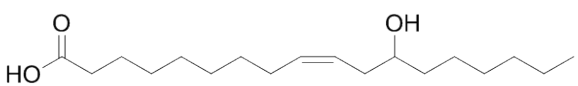
The challenge: oil profile improvement for industrial applications

Fatty acid composition of camelina oil not optimal for oleochemical industry

Fatty acid	Camelina oil
16:0 Palmitic acid 	5.5%
18:0 Stearic acid 	2.5%
18:1 Δ9 Oleic acid 	**14%**
18:2 Δ9,12 Linoleic acid 	**18%**
18:3 Δ9,12,15 Linolenic acid 	**36%**
20:1 Δ11 Eicosenoic acid or Gondoic acid 	**14%**
22:1 Δ13 Erucic acid 	2.5%
 12-OH 18:1 Δ9 Ricinoleic acid	**Zero**

- % PUFAs too high (C18:2, C18:3)
- % MUFAs too low (C18:1, C20:1, C22:1)

Target fatty acid profiles of camelina seed for industrial uses

Fatty acid	Camelina oil	High Oleic	High Gondoic	High Ricinoleic
16:0 Palmitic acid 	5.5%	Low	Low	8-10%
18:0 Stearic acid 	2.5%	Low	Low	Low
18:1 Δ9 Oleic acid 	14%	70-80%	30-40%	55-60%
18:2 Δ9,12 Linoleic acid 	18%	Low	Low	Low
18:3 Δ9,12,15 Linolenic acid 	36%	Low	Low	Low
20:1 Δ11 Eicosenoic acid or Gondoic acid 	14%	Zero	40-50%	Zero
22:1 Δ13 Erucic acid 	2.5%	Zero	Low	Zero
 12-OH 18:1 Δ9 Ricinoleic acid	Zero	Zero	Zero	25-30%

Gondoic acid – jojoha oil
Ricinoleic acid – castor oil

Metabolic Engineering *Camelina sativa* with Fish Oil-Like Levels of DHA

James R. Petrie[1¶], Pushkar Shrestha[1¶], Srinivas Belide[1], Yoko Kennedy[1], Geraldine Lester[1], Qing Liu[1], Uday K. Divi[1], Roger J. Mulder[3], Maged P. Mansour[2], Peter D. Nichols[2], Surinder P. Singh[1*]

1 CSIRO Food Futures National Research Flagship, Canberra, Australian Capital Territory, Australia, 2 CSIRO Food Futures National Research Flagship, Hobart, Tasmania, Australia, 3 CSIRO Materials Science and Engineering, Clayton, Victoria, Australia

Abstract

Background: Omega-3 long-chain (≥C20) polyunsaturated fatty acids (ω3 LC-PUFA) such as eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) are critical for human health and development. Numerous studies have indicated that deficiencies in these fatty acids can increase the risk or severity of cardiovascular, inflammatory and other diseases or disorders. EPA and DHA are predominantly sourced from marine fish although the primary producers are microalgae. Much work has been done to engineer a sustainable land-based source of EPA and DHA to reduce pressure on fish stocks in meeting future demand, with previous studies describing the production of fish oil-like levels of DHA in the model plant species, *Arabidopsis thaliana*.

Principal Findings: In this study we describe the production of fish oil-like levels (>12%) of DHA in the oilseed crop species *Camelina sativa* achieving a high ω3/ω6 ratio. The construct previously transformed in Arabidopsis as well as two modified construct versions designed to increase DHA production were used. DHA was found to be stable to at least the T_5 generation and the EPA and DHA were found to be predominantly at the *sn*-1,3 positions of triacylglycerols. Transgenic and parental lines did not have different germination or seedling establishment rates.

Conclusions: DHA can be produced at fish oil-like levels in industrially-relevant oilseed crop species using multi-gene construct designs which are stable over multiple generations. This study has implications for the future of sustainable EPA and DHA production from land-based sources.

Citation: Petrie JR, Shrestha P, Belide S, Kennedy Y, Lester G, et al. (2014) Metabolic Engineering *Camelina sativa* with Fish Oil-Like Levels of DHA. PLoS ONE 9(1): e85061. doi:10.1371/journal.pone.0085061

Seed News

UK Scientist Seeks GM Camelina Omega-3 Outdoor Trial

Date Posted: January 27, 2014

No GM crops are currently grown commercially in Britain and only two are licensed for cultivation in the European Union (EU).

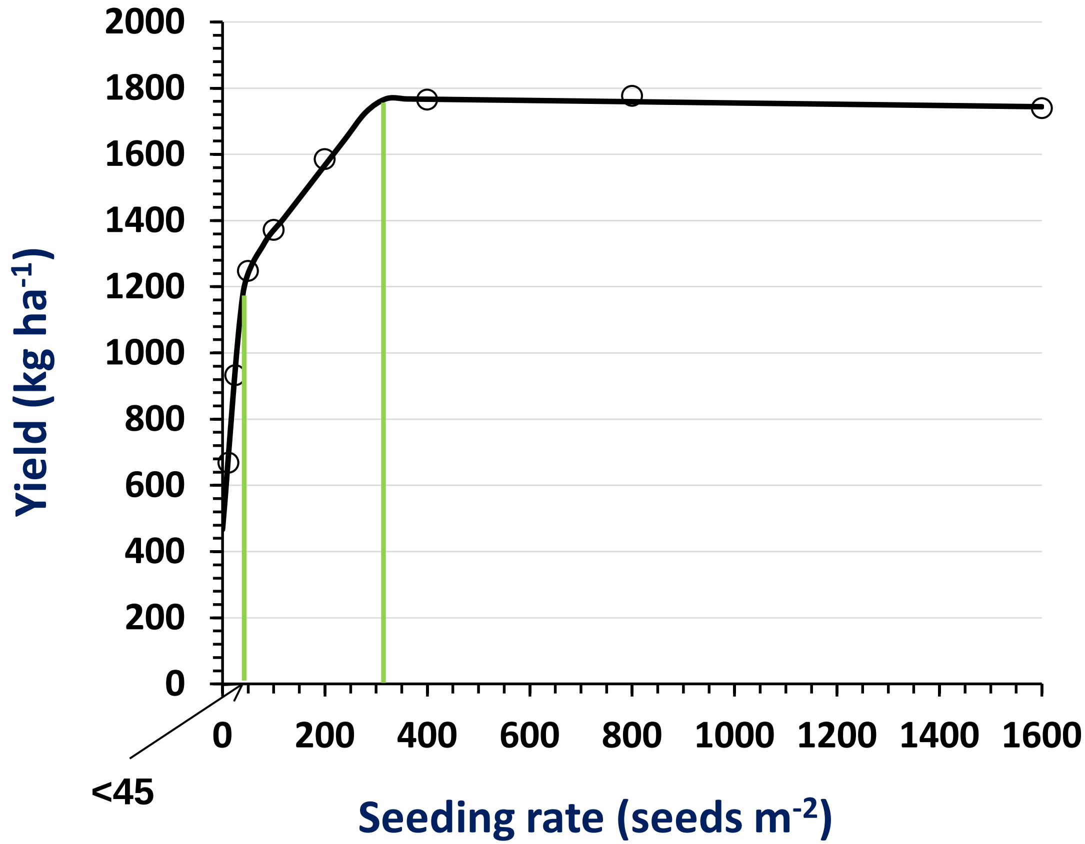
()World Bulletin) -- The proposed trial - likely to generate controversy in a nation where GM foods have little public support - could start as early as May and will use Camelina plants engineered to produce seeds high in Omega-3 long chain fatty acids.

No GM crops are currently grown commercially in Britain and only two - a pest-resistant type of maize and a potato with enhanced starch content - are licensed for cultivation in the European Union (EU).

But scientists at Britain's agricultural lab Rothamsted Research have developed Camelina plants to produce Omega-3 fats that are known to be beneficial to health but normally found only in oils in increasingly limited fish stocks.

The idea, they told journalists at a briefing on their plans, is initially to supply the fish farming industry - which currently consumes around 80 percent of fish oils

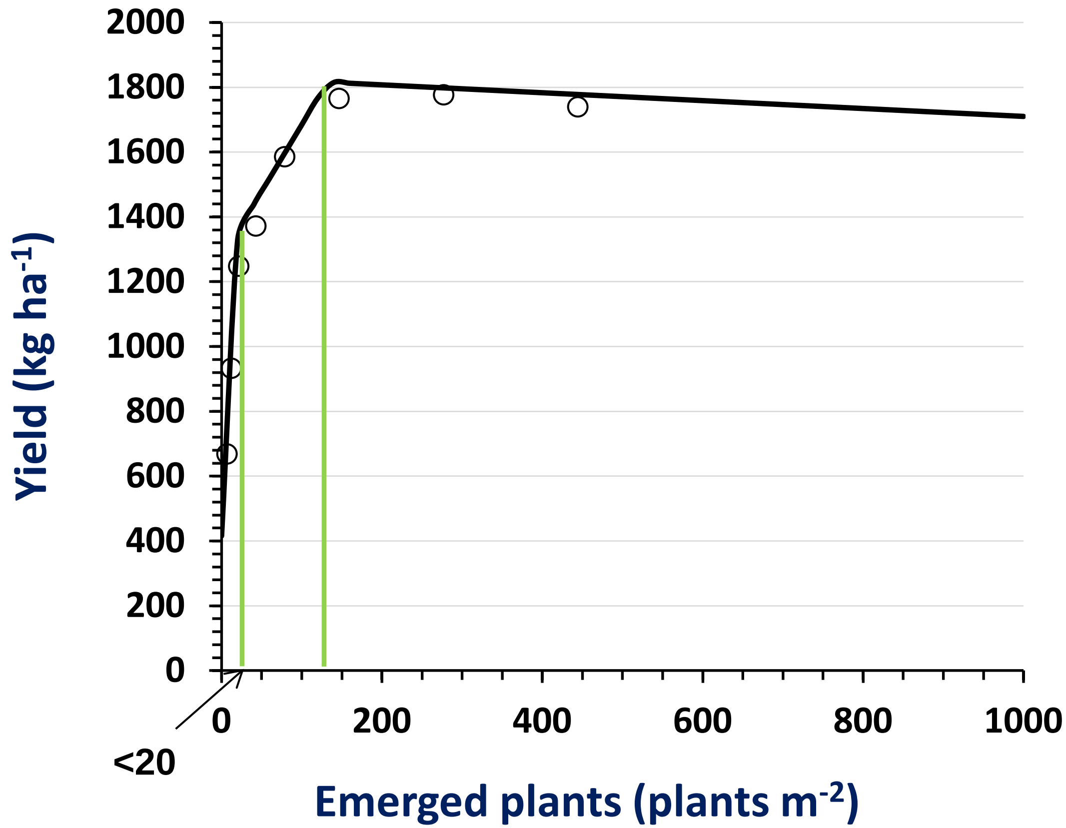
Effect of Seeding Rate on Yield



Minimum seeding rate: **300 seeds/m²**

Eric Johnson et al. unpublished data

Effect of Plant Emergence on Yield



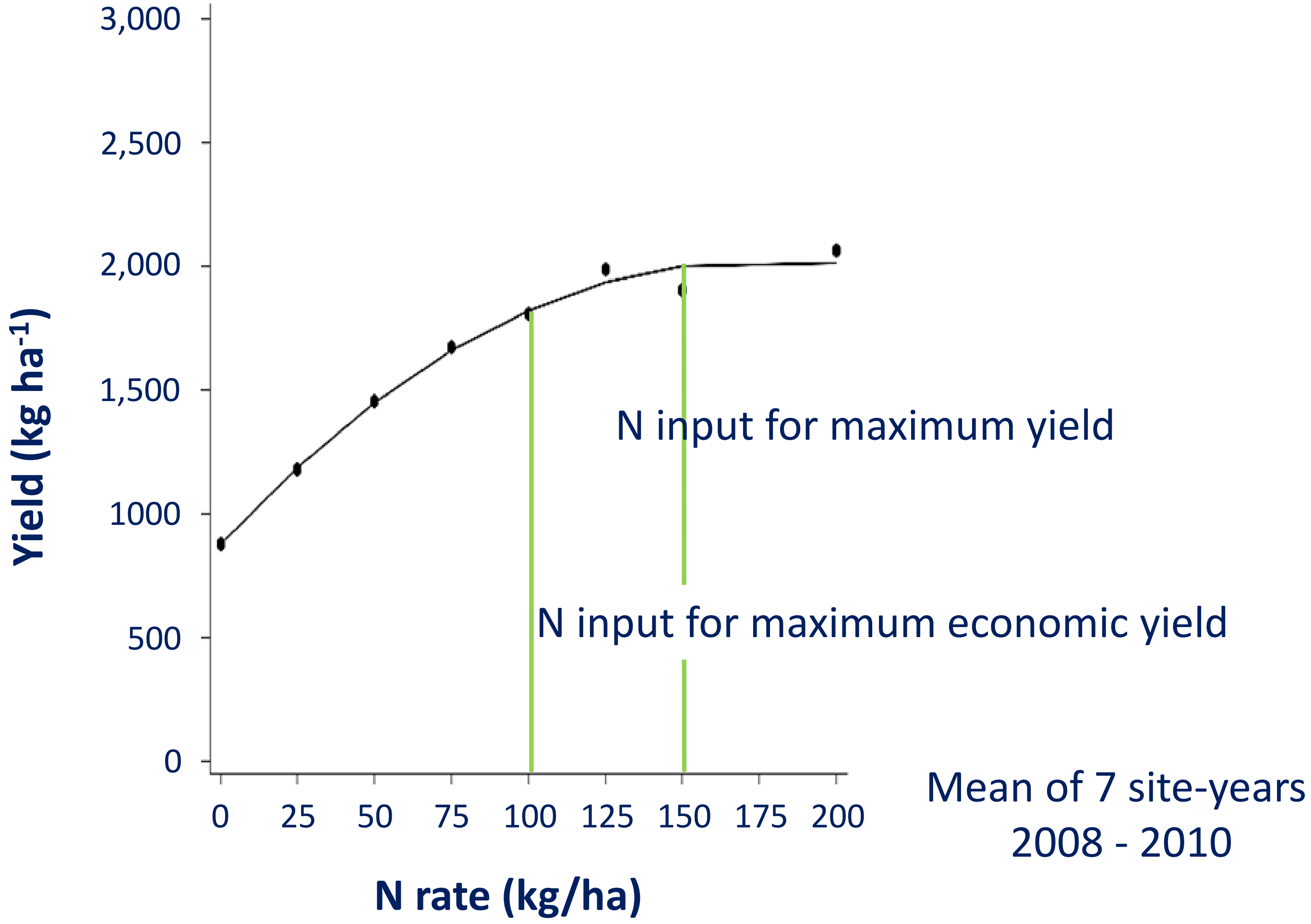
Minimum plant population: **130 plants/m²** (42 % emergence)

Recommended seeding rate: **500 seeds/m² (5 lbs/acre)**

guarantees sufficient plant stands even at reduced emergence

Eric Johnson et al. unpublished data

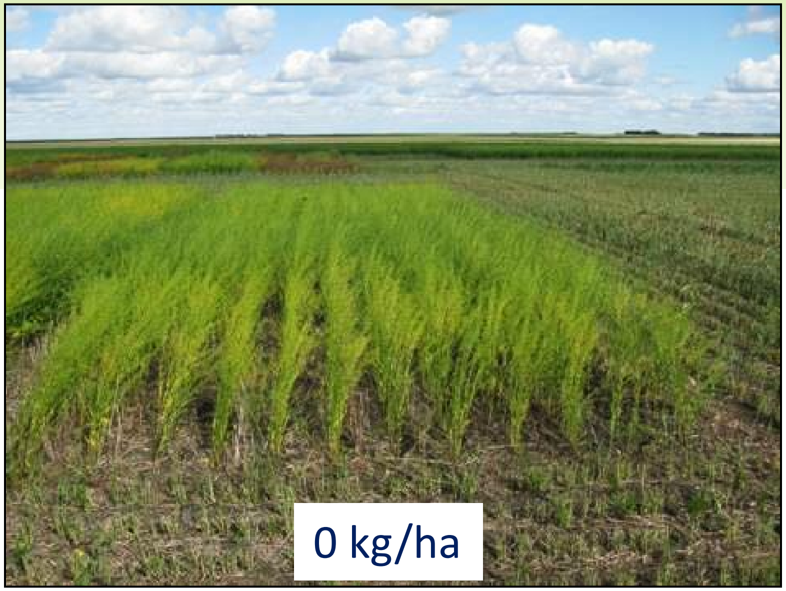
N Response in Camelina

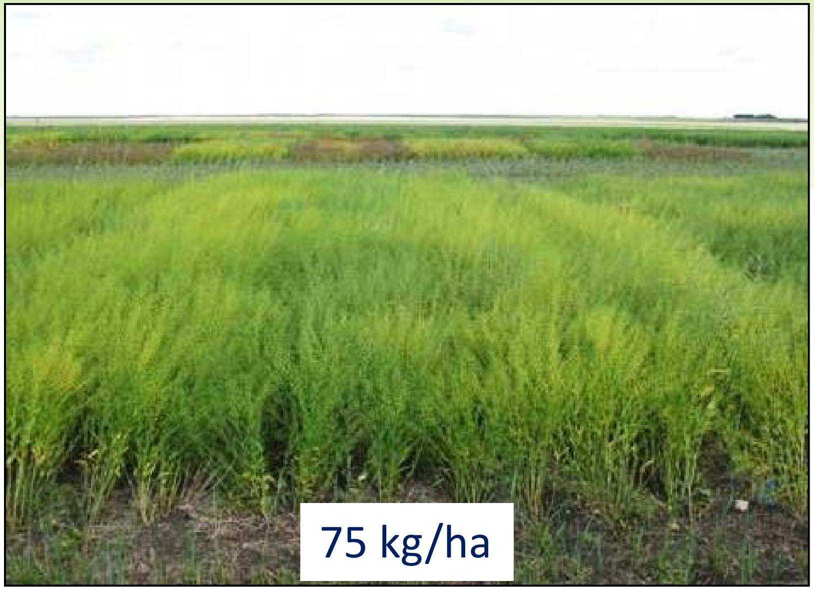


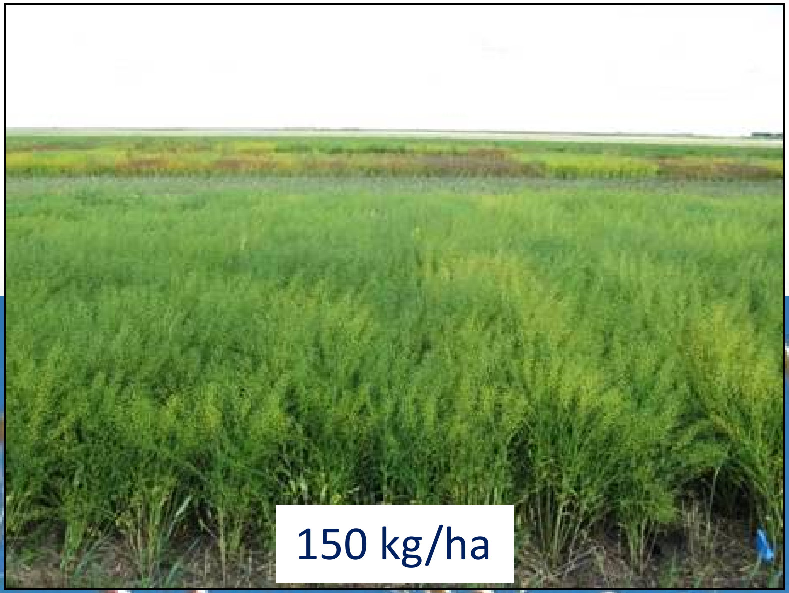
Assumptions:
N fertilizer cost = 45 cents/lb
Camelina Price = 18 cents/lb or $ 9.00 / bushel

Malhi, Johnson et al. 2014. Canadian Journal of Soil Science

N Response in Camelina – Indian Head 2009







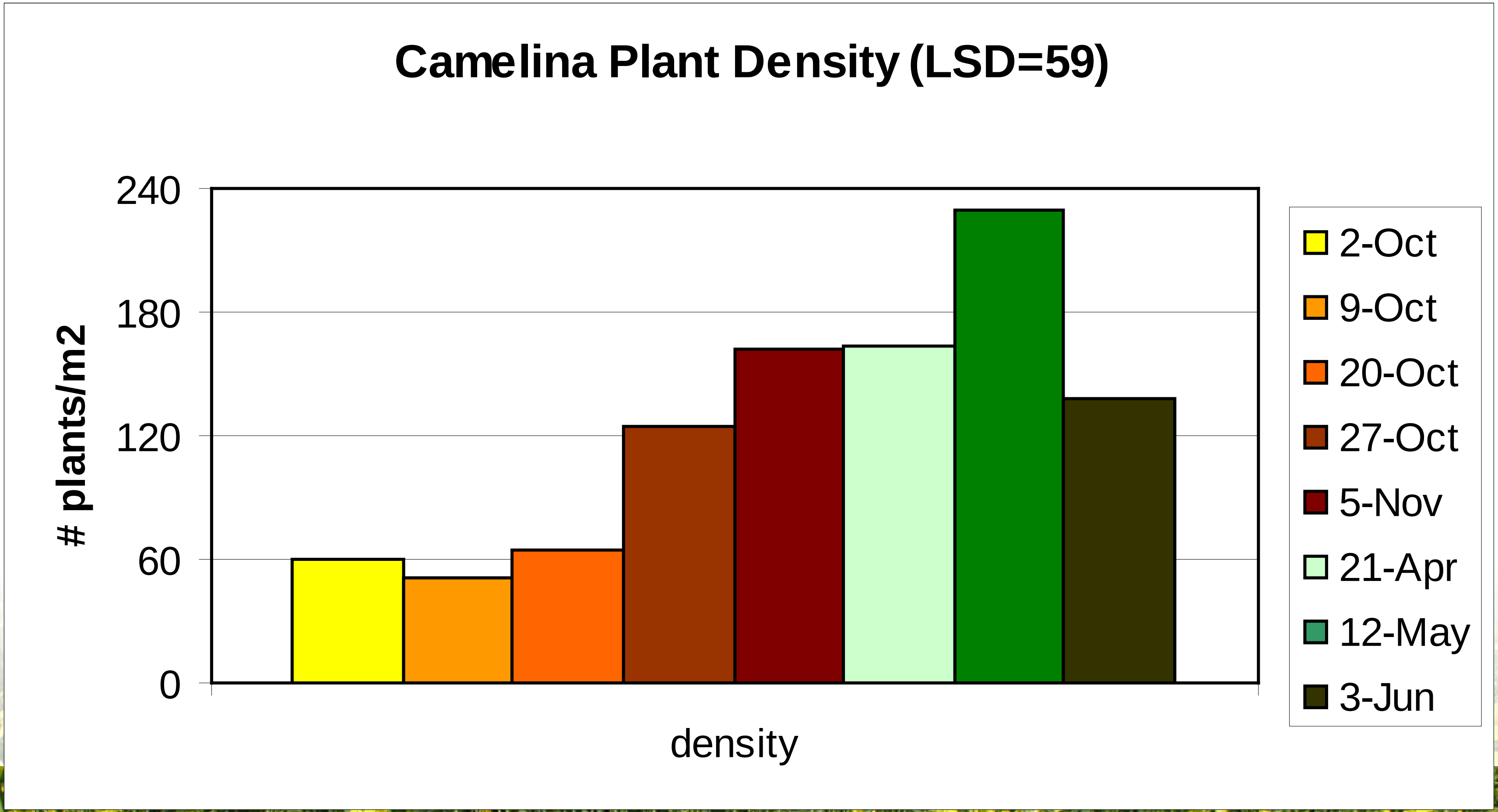
Camelina Seeding Dates

- Why looking at different seeding dates?
  - Small seed
  - Not very competitive early on
- Early maturity
- Success with fall seeding in US and other places – will form rosette in fall if planted early enough

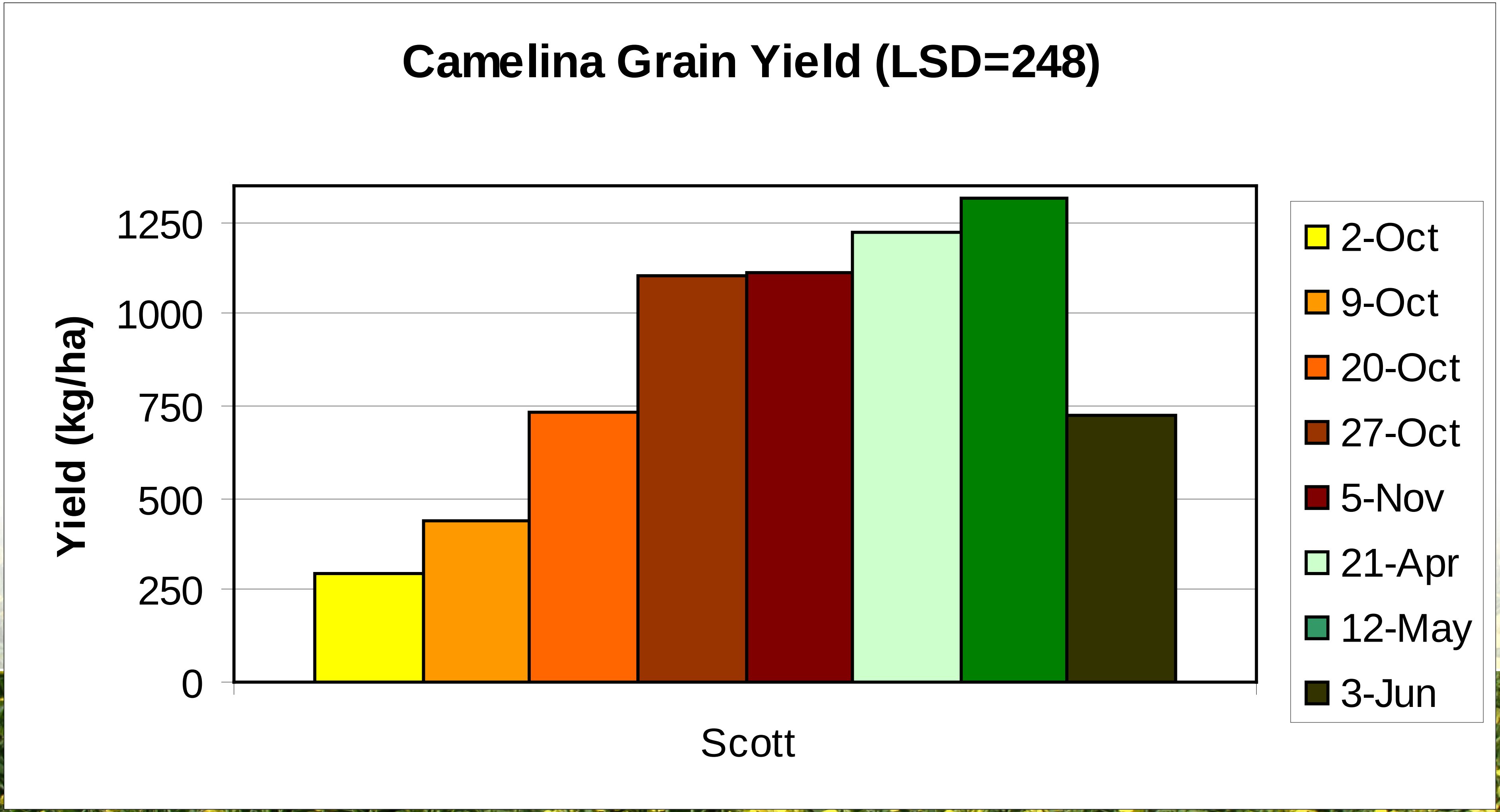
Camelina Seeding Date

- 5 fall and 3 spring seeding dates
- Scott, Swift Current, Indian Head

Scott 2010 Plant Density



Scott 2010



Camelina Grain Yield (LSD=248)
Yield (kg/ha)
1250
1000
750
500
250
0
Scott
2-Oct
9-Oct
20-Oct
27-Oct
5-Nov
21-Apr
12-May
3-Jun

Fall

Test 88 - Biodiesel Potential of Alternative Species

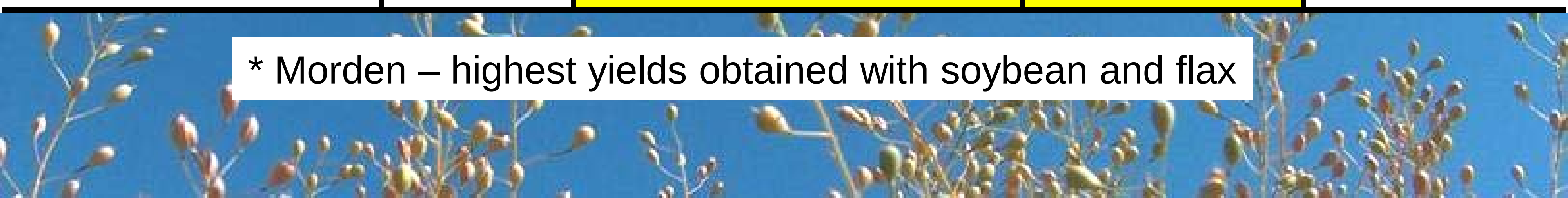
- Objective
  - To evaluate the agronomic performance and the biodiesel quality of ten different oilseed species or cultivars.
- Locations
  - Lethbridge, Swift Current, Scott, Indian Head, Morden over 2 years

Crops / Cultivars Evaluated

1. *Brassica napus* canola *cv.* Invigor 5440
2. *Brassica rapa* canola *cv* ACS-C7
3. *Camelina sativa cv.* Calena
4. *Brassica carinata* ethiopian mustard (Common seed)
5. *Sinapis alba* yellow mustard *cv.* Andante
6. Canola quality *Brassica juncea cv.* Xceed 7784
7. *Brassica juncea* oriental mustard *cv.* Cutlass
8. Flax *cv.* Bethune
9. Soybean *cv.* LS0036RR
10. Soybean *cv.* OAC Prudence

Relative Yields (kg/ha) Camelina vs *Brassica napus*

Location	Year	Brassica napus	Camelina	
Lethbridge	2008	2850	4150	>
	2009	4040	2120	<
Swift Current	2008	1780	1910	=
	2009	520	740	>
Scott	2008	2720	1840	<
	2009	2260	2360	=
Indian Head	2008	3230	1530	<
Morden*	2008	1730	1850	=
	2009	1890	1580	=



* Morden – highest yields obtained with soybean and flax

Weed Control in Camelina

- Options are limited
- Quizalifop registered for grass weed control; glyphosate registered for pre-harvest weed control
- Some growers using Trifluralin or Ethafluralin

Untreated

Imazmethabenz

Fluroxypyr-MCPA

2,4-D amine

MCPA amine

Bentazon

Bromoxynil

2,4-DB

Clopyralid

Glufosinate

Sulfentrazone

Imazamox/
Imazethapyr

Progress in ALS (Group 2) resistance

Wild-type Untreated

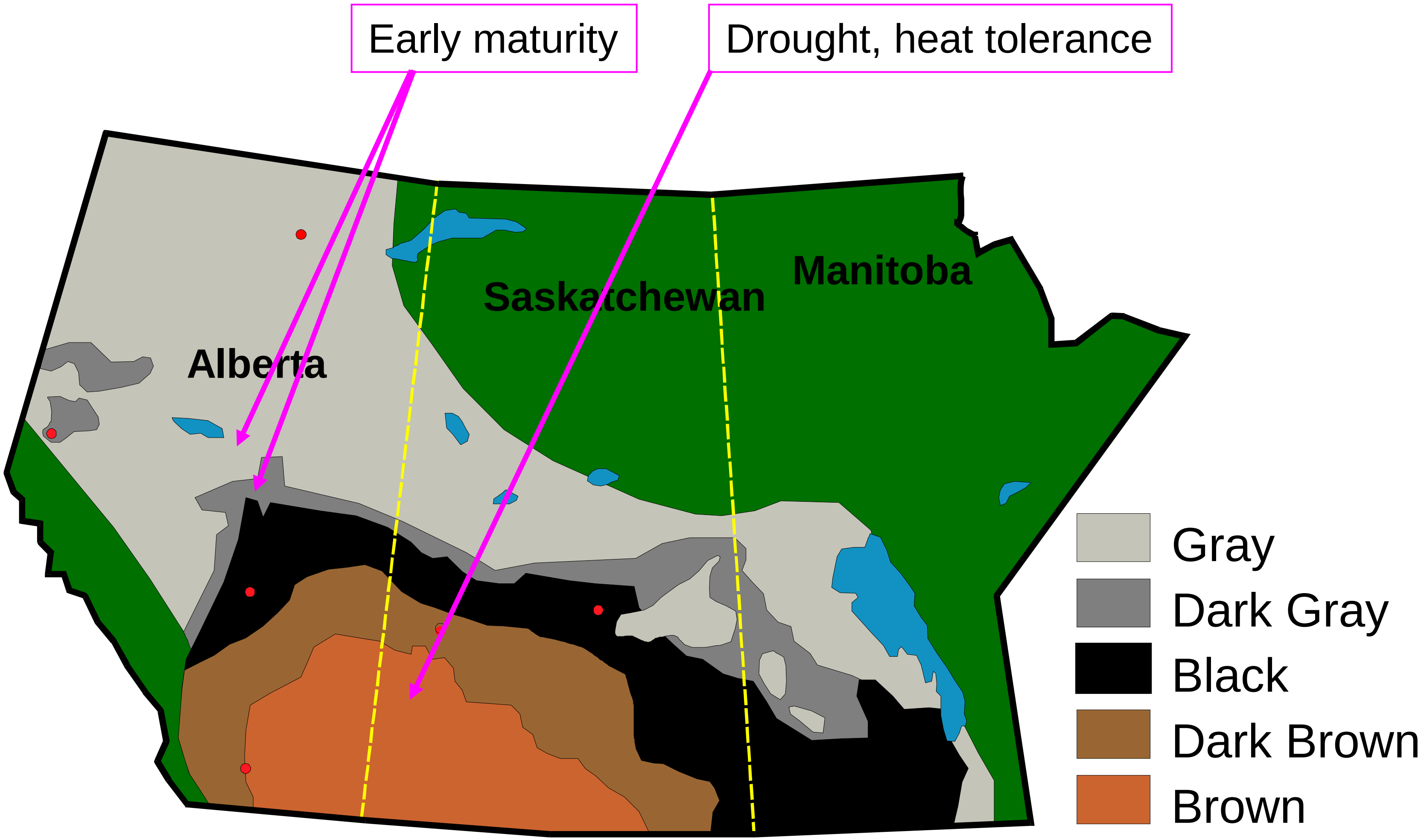
Wild-type Treated with Refine SG 1X

Mutated Line Untreated

Mutated Line Treated with Refine SG 1X

Camelina – Future

- Well adapted to all soil zones on the prairies.
- Should be targeted to:



Early Maturity - Facilitate Seeding of Winter Wheat

Summarize

- New crops
  - The next Cinderella crop? Who knows?
  - Timing of introduction to growers and market is critical
    - Credible agronomy will do nothing but help even it exposes “warts”
  - Weed control challenge for adoption of crops
    - High producer expectations for weed control
  - Strive for long-term sustained growth.

Acknowledgements

- Growing Forward I and II
- Western Grain Research Foundation
- Saskatchewan Agriculture Development Fund
- Mustard 21 Canada Inc.
- Agrisoma Biosciences Inc.
- Saskatchewan Mustard Development Commission
- Dow AgroSciences Canada Inc.
- Linnaeus Plant Sciences