Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

# Research at SLU Targeting Industrial Quality Oils in Traditional and New Oil Crops

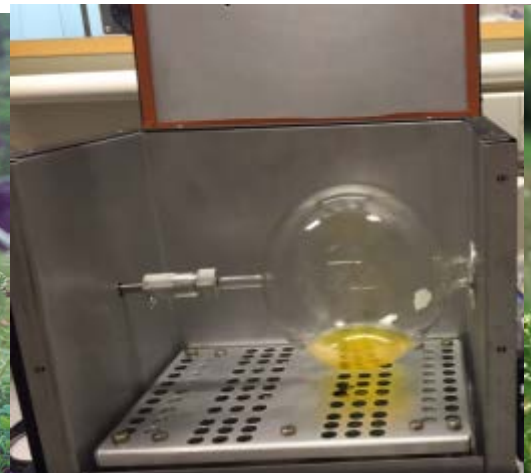
Prof. Anders S. Carlsson/ Head of
Department of Plant Breeding

May 9th 2017

# Overview

- Department of Plant Breeding, SLU
- Crambe abyssinica as a platform for production of tailor made industrial oils
- Lepidium campestre (Field cress) as a new under-sown oil crop
- GM camelina producing an oil that contains sex pheromones for specific insect pests
- Identifying the underlying mechanisms for oil composition in Rapeseed
- Precision breeding in Rapeseed




# Department of Plant Breeding at SLU

Ca. 75 colleagues
(incl. about. 25 PhD students (many on fellowships)
15 technical personnel
30 junior & senior researchers)

Head of Department: Prof. Anders S. Carlsson anders.carlsson@slu.se
Deputy Head: Dr. Mariette Andersson mariette.andersson@slu.se

**Five divisions with their subject area**

- Agricultural Plant breeding
  Lead by Prof. Rodomiro Ortiz rodomiro.ortiz@slu.se

- Horticultural Plant breeding
  Lead by Dr. Kimmo Rumpunen Kimmo.rumpunen@slu.se

- Agricultural plant quality
  Lead by Prof. Eva Johansson Eva.johansson@slu.se

- Horticultural plant quality
  Lead by Prof. Marie Olsson Marie.olsson@slu.se

- Plant biotechnology
  Lead by Assoc. Prof. Per Hofvander Per.Hofvander@slu.se

Plant properties:

Pre Harvest

Post Harvest

# Developing a new oil crop by domestication of field cress (Lepidium campestre) using conventional and biotechnology approaches

Contacts for more information:
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Li-Hua.Zhu@slu.se

# Domestication of field cress (Lepidium campestre)

**Field cress *Lepidium campestre***

- **It shares the Brassicaceae family with *Brassica & Arabidopsis***

***Arabidopsis***

***Brassica***

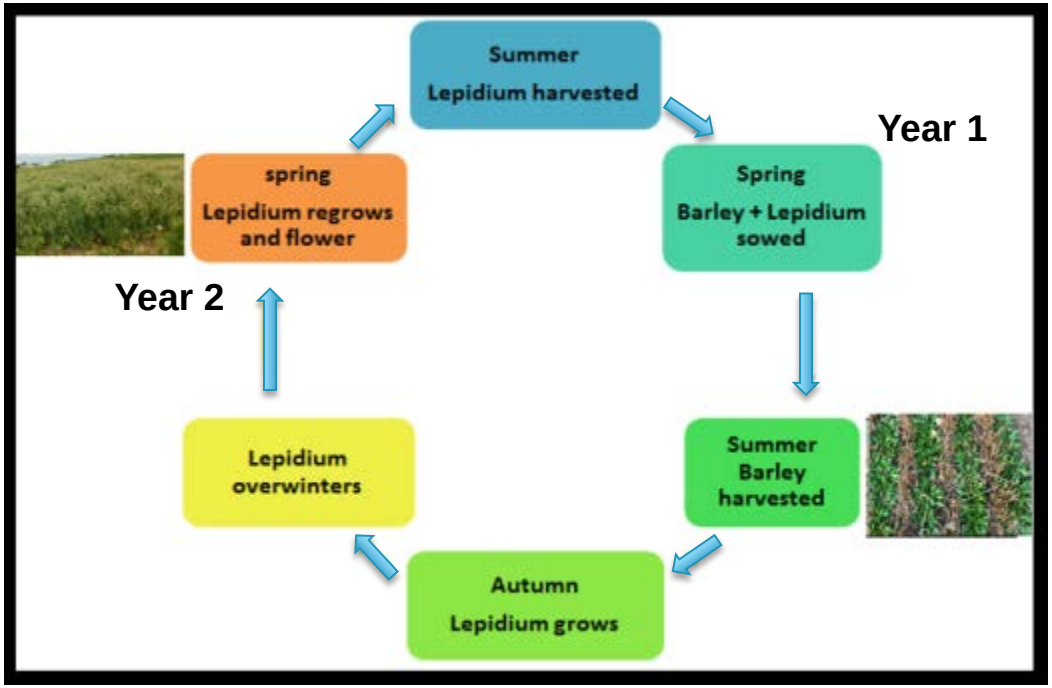
# Field cress (Lepidium campestre)

- **2n=2x=16**
- **biennial**
- **winter hardy**
- **potential for high seed yield**
- **industrial quality oil**

- **suitable as under-sown crop**

# Undersown field cress

## Undersowing field cress with spring cereals



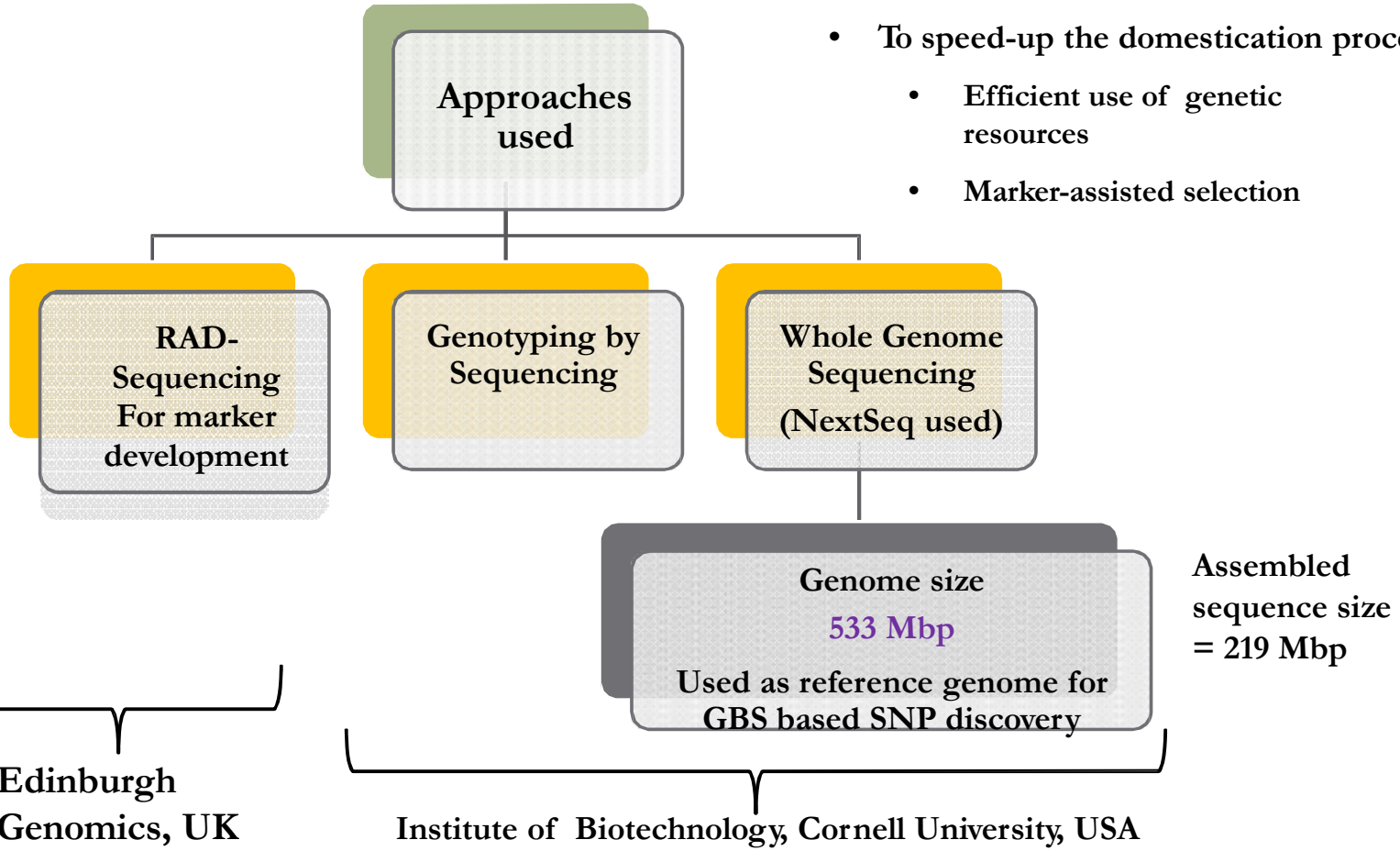

- **In a separate line we are developing a perennial type that can provide better ecosystem services**
  - **higher carbon storage**
  - **reduced input costs**
  - **better energy use efficiency**
  - **better soil and water management**

- **It serves as cover/catch crop during the first year (autumn/ winter)**
- **and harvested as an oil crop the second year**

# Major traits targeted

- **Pod shattering**
- **Seed yield**
- **Oil content**
- **Synchronous maturity**
- **Seed dormancy**
- **Perenniality**
- **Pathogen resistance**

# Developing genomic tools and resources




- To speed-up the domestication process
  - Efficient use of genetic resources
  - Marker-assisted selection

# Non-GM based breeding of *Lepidium campestre* and developing its genomic tools and resources

. **Major traits targeted for breeding** :

- Pod shattering, Oil content, synchronous maturity, seed yield and perenniality

- **Pod shattering**:
  - Pod-shatter resistant Breeding lines that lose less than 10% of their seeds conditions that wild type genotypes loose more than 90% of their seeds are developed. The resistant lines as good as rapeseed
- **Oil content**:
  - In some breeding lines, oi content has been increased by up to 35% (from ca 20% to ca 27%)
- **Synchronous maturity**:
  - Few perfect synchronously maturing breeding lines have been developed
- **Seed yield**:
  - During yield trial in 2015/2016, up to 4.5 t/ha per breeding line under insufficient rain fall condition
- **Perenniality**
  - Perennial breeding lines have been developed through interspecific hybridization between *Lepidium campestre* and *L. heterophyllum*

# Non-GM based breeding of *Lepidium campestre* and developing its genomic tools and resources

. **Different approaches have been used to develop genomic tools and resources**

- **RAD-Sequencing produced**:
  - More than 190000 DNA sequences (117-567 bp)
    - used to develop 1700 microsatellite markers
  - About 90000 SNPs
  - 9K SNP chip developed and used to genotype 1200 samples
    - For GWAS and QTL mapping
- **Genotyping by Sequencing (GBS) produced**
  - About 126000 SNPs
    - QTL mapping underway
- **Whole genome sequencing**
  - Genome size = 533 Mbp
- **Genetic linkage map developed**
  - contains 10,302 contigs and 1044 SNPs

- **30 genes coding for desirable traits were identified through comparative genomic analysis of Lepidium and Arabidopsis genomes**

# Genetic improvement of *Lepidium campestre* using biotechnology approaches

## Results achieved using GM approach

- **High oleic acid:**
  - increased from 11% in WT to 80% in GM-line
- **High oil content:**
  - increased from 20% in WT to 27% in GM-line by overexpression a transcription factor gene and hemoglobin genes.
- **Reducing pod shatter**:
  - GM-lines with reduced pod shatter are obtained by down-regulating one key gene regulating the pod shatter
- **Wax ester production**:
  - over 25% wax esters were produced in lepidium seeds for industrial applications by overexpressing the jojoba wax synthesis genes

## Current research focus

- **Trait stacking: combine improved traits into single improved lines**
- **Using the CRISPR technique to obtain transgene free improved lines**

*Ivarson et al. BMC Plant Biol., 2013*
*Ivarson et al. Plant Cell Rep. 2016*
*Ivarson et al. Front. Plant. Sci. 2017*

*Mistra Biotech supported by Mistra and SLU*

# Developing Crambe abyssinica as a platform for production of tailor made industrial oils.

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# Crambe abyssinica: industrial crop

Crambe fullfils the criteria for a robust and safe oil crop platform for industrial products

- Not a food crop (60% erucic acid)
- No outcross with other oil crops or wild relatives in northern Europe
- Reasonable oil yield per hectare (same as spring rape)
- Already in production as an agricultural crop
- Excellent fatty acid composition of oil for the conversion into wax esters and ultra-high erucic acid content

*Financial sources: EU-ICON, FORMAS and SSF*

# Achievements so far with crambe

- **Extra high erucic acid (HEA):** increased from 60% in WT to 73% in GM-line by regulating three key genes involved in fatty acid biosynthesis.

- **Wax ester production**: up to 50% of wax esters in the seed oil in GM-lines compared to zero in WT by overexpressing 3 jojoba wax synthesis genes.

- **High oleic acid:** increased from 15% in WT to 80% in GM-line by regulating 2 key genes involved in the seed oil biosynthesis.

*Li et al. Plant Biotech J. 2012, 2016*
*Guan et al. Plant Biotech J., 2013*
*Zhu et al. Sci. Rep. 2016.*

# Field trials on HEA and wax GM lines

**The results showed reduced seed setting and seed oil for both types of GM lines. For the wax lines, reduced germination.**

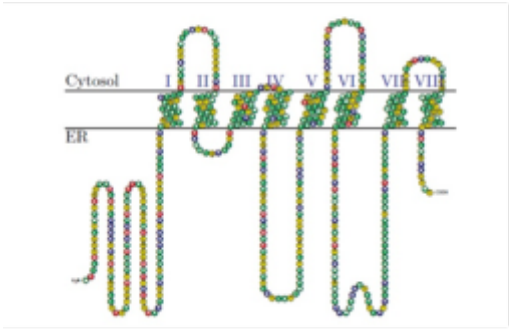
# Ongoing research

Studies on molecular mechanisms limiting further increase in erucic acid level and investigating the mechanisms underlying the altered agronomic traits in the different GM-lines through functional analysis of some important genes involved in oil biosynthesis

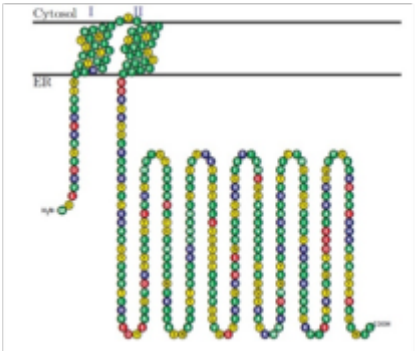
- DGAT (Specificities of DGATs partly determine the oil quality)

Two isoforms of DGAT

DGAT1 (MBOAT superfamily) - many transmembrane regions




DGAT2 (LPLAT superfamily) - few transmembrane regions




- Transcriptome analysis for wax ester synthesis in transgenic crambe, etc.

# Production of plant oils containing sex pheromones for targeting insect pests

Contacts for more information:
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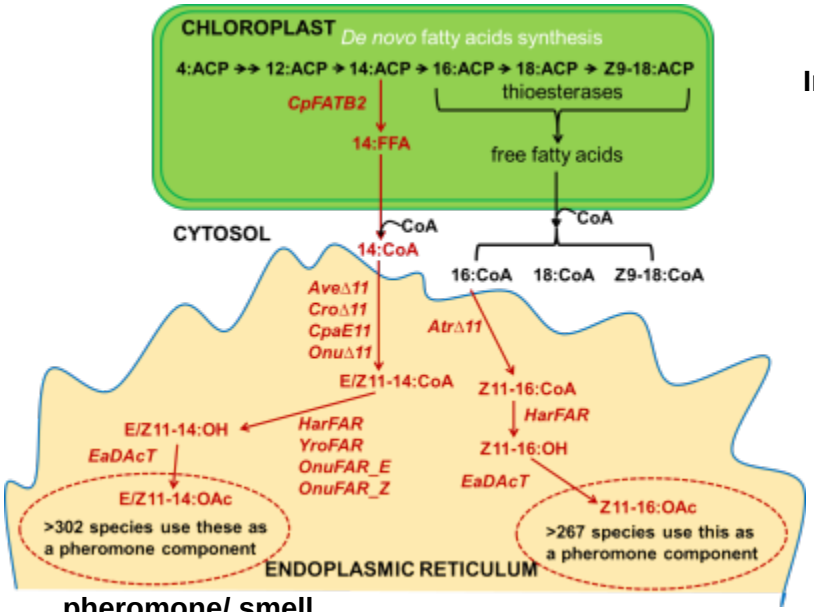
In collaboration with:

# Plant oils containing pheromone components for use in insect pests traps




**pheromone/ smell**

**KLICKA**

**Insect pest**


nature COMMUNICATIONS

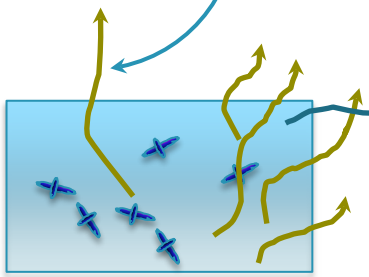
ARTICLE

Received 5 Nov 2013 | Accepted 30 Jan 2014 | Published 25 Feb 2014 OPEN

## A plant factory for moth pheromone production

Bao-Jian Ding, Per Hofvander, Hong-Lei Wang, Timothy P. Durrett, Sten Stymne & Christer Löfstedt

Moths depend on pheromone communication for mate finding and synthetic pheromones are used for monitoring or disruption of pheromone communication in pest insects. Here we produce moth sex pheromone, using *Nicotiana benthamiana* as a plant factory, by transient expression of up to four genes coding for consecutive biosynthetic steps. We specifically produce multicomponent sex pheromones for two species. The fatty alcohol fractions from the genetically modified plants are acetylated to mimic the respective sex pheromones of the small ermine moth *Yponomeuta evonymella* and *Y. padella*. The...



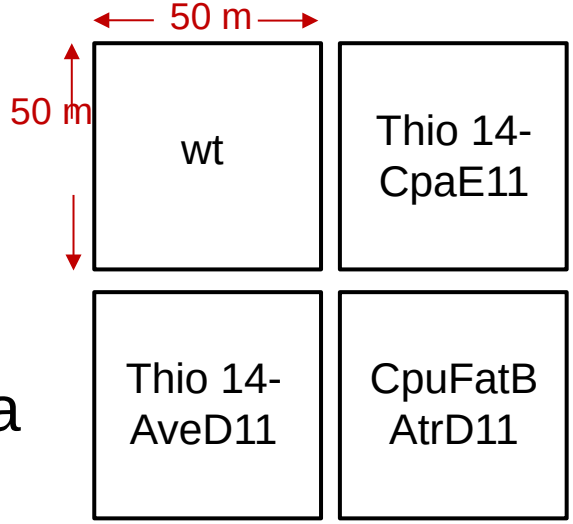
**Pheromone trap = A support covered with a sticky substance and pheromones that attracts males of the insect pest to be stuck on the trap**

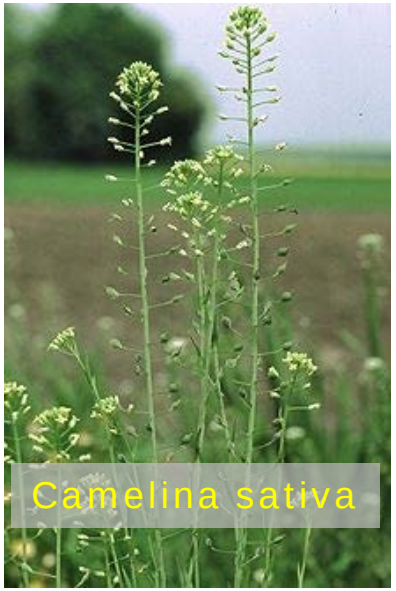
**Genetically modified camelina produce an oil with specific fatty acids. After extraction, these can be modified into unique aldehydes, acetates and alcohols, which are active as sex pheromones for specific insect pests.**

**Crop**

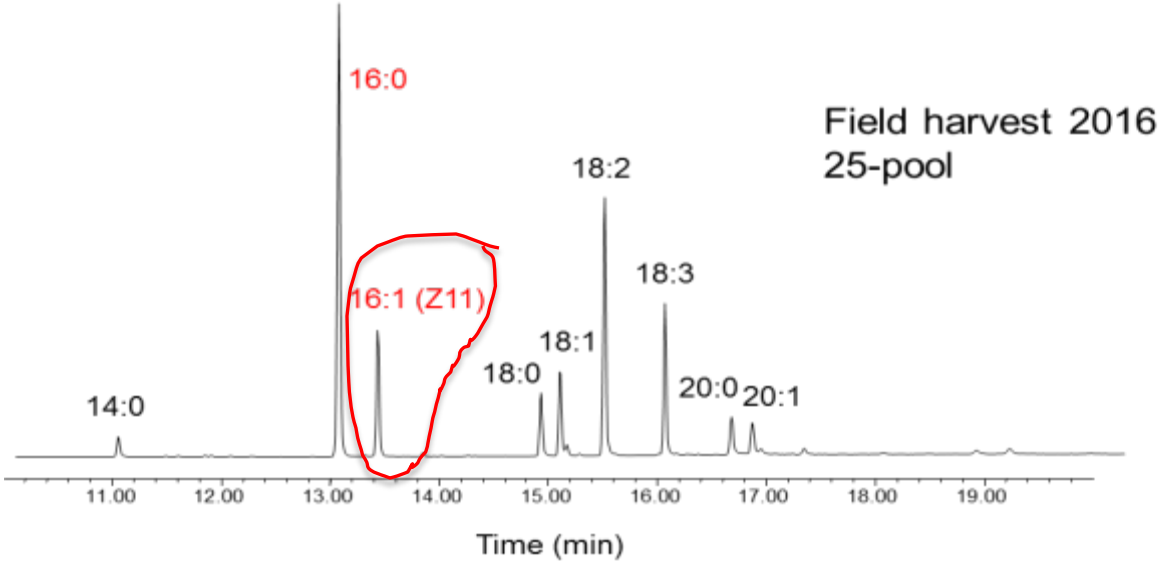
# Camelina sativa (Gold-of-pleasure) with oil containing pheromone precursors


May, 2016—Nebraska




## Oil composition CpuFatB-AtrD11




**After seed oil extraction, fatty acids are isolated and chemically modified into active pheromone compounds that are used to prepare pest insect traps for field testing.**

# Field trapping experiment 2017

Time: early March, 2017

Place: Canton, China

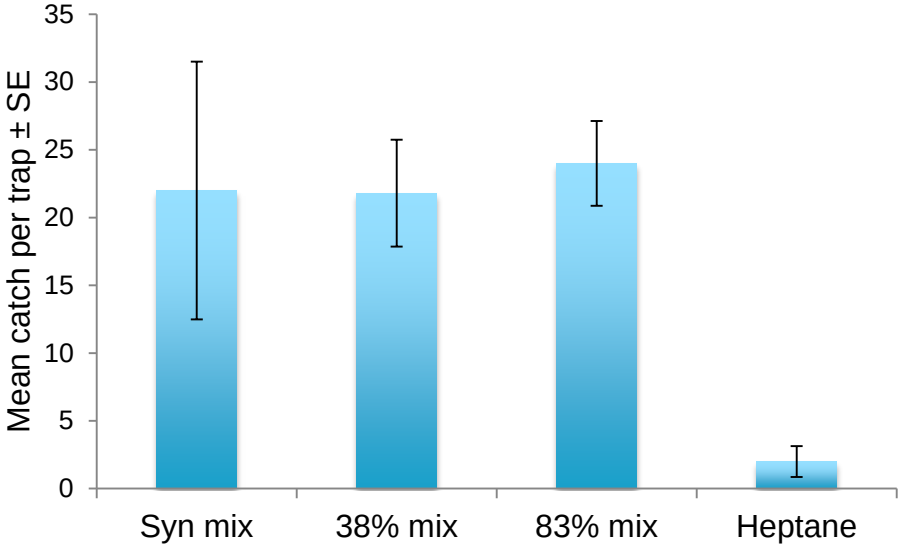
Plants: cauliflower, radish *et al*

Treatments:

- primarily purified product (precursor purity 38%)
- highly purified product (precursor purity 83%)
- synthetic pheromone (optimal)
- negative control (solvent alone)

Cooperator: Guangdong Institute of Applied Biological Resources


**Results from field trapping experiment**



# Identifying the underlying mechanisms for oil composition in rapeseed

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In collaboration with:

UNIVERSITY OF GDANSK

# Identifying the underlying mechanisms for oil composition in rapeseed

- Studying the role of enzymes that are crucial for regulating the composition of seed oil in rapeseed
- Comparing high erucic and low erucic rapeseed
- Identify the genetic background of importance for the mechanism regulating oil quality
- Knowledge achieved from our research on the seed oil metabolism in Crambe abyssincia provide clues in understanding the mechanism regulating seed oil quality in rapeseed

# Precision breeding of rapeseed

Contacts for more information:
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# Precision breeding of rapeseed

- Using CRISPR/Cas technique to produce transgene free
- Target traits: disease resistance, seed yield, herbicide resistance in the first step
- Protoplast approach is used for gene editing by CRISPR and the protoplast approach looks very promising so far as high quality protoplasts can be obtained from rapeseed.
- The project has just started

*Project supported by FORMAS*
*Lantmännen provides the initial working material*

*Source: Li-Hua Zhu, SLU, Alnarp*