Bio-oil from the pyrolysis of canola, *Brassica napus*, and mustard, *B. carinata* and *B. juncea*, straw: the potential for insecticide development

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Introduction

• Project:
  agricultural crop residue → bio-fuel, chemicals

• This presentation:
  → bio-oils with pesticidal activity

• Alternative to chemical insecticides:
  – Reduce use of fossil fuels:
    • Insecticide fraction extracted
    • Remainder of bio-oil: cheap fuel
  – Prevent development of insect resistance
Introduction

• In Canada:
  – > 200,000 ha of mustard
  – Straw: under-used agricultural residue
  – Mustard seed residue has been applied as a soil amendment that can suppress pathogens and insect pests

• Great potential for:
  – Conversion to liquid bio-oil
  – Pest control application
Objectives

1) Screen bio-oils of canola and mustard straw for insecticidal activity

2) Identify active compound(s) in bio-oil fractions
Methods

• Fast pyrolysis in fluidized bed pilot plant:
  - 300 and 500 °C
  - 2 s vapor residence time

• Straw from:
  – canola *Brassica napus*
  – mustard *Brassica carinata*
  – mustard *Brassica juncea*

→ bio-oils:
  - aqueous phase: *B. napus* (BNA), *B. carinata* (CA), *B. juncea* (JA)
  - organic phase: *B. napus* (BNO), *B. carinata* (CO), *B. juncea* (JO)
Methods

- Tested for insecticidal activity using the Colorado potato beetle *Leptinotarsa decemlineata* (CPB) potato leaf disc bioassay

- Raw bio-oil and separated solutions tested at 3 and 30 mg/ml
Methods

- All fractions redissolved in water and acetone to an equivalent 30 mg/l concentration, for insecticide assays
Methods

• HPLC fractionation method:
  - Agilent 1200 Series HPLC
  - Waters Symmetry C18 column (5µm, 4.6x250mm) for analysis
  - Waters Symmetry semi-preparative C18 column (7µm, 7.8x300mm) for fraction collection

• HPLC analyses of known Brassicaceae compounds:
  - Sinigrin and AITC from seed, straw and bio-oils from *B. juncea* were analyzed by HPLC (Agilent 1200)
Results

Tests with 30 mg/l against 1\textsuperscript{st} instar CPB – 48 h % mortality

- All three \textit{Brassica} aqueous phases (CA, JA, BNA) active
- Mustard bio-oils had higher bio-activity with aqueous versus organic phase (CO, JO)
- 300 °C bio-oils were more active than 500 °C bio-oils
Methods

- All fractions redissolved in water and acetone to an equivalent 30 mg/l concentration, for insecticide assays
Results

- Remaining studies with 300 °C oils
- Aqueous phase of *B. juncea* bio-oil chosen for further purification
- The 2\textsuperscript{nd} aqueous separation retained the greatest activity in fraction D (30 mg/ml)
Methods

- All fractions redissolved in water and acetone to an equivalent 30 mg/l concentration, for insecticide assays
Results

- Bioassay-guided fractionation by HPLC with semi-preparative C18 column
- 10 fractions of D30 were collected
- Fraction 1 was found to be the most active of ten sub-fractions collected and the most polar
Results

- Further HPLC purification of Fraction 1 produced 5 peaks and 9 sub-fractions were collected
- Insecticidal activity was found only in Fractions 1-4 and 1-5
- Fraction 1-5 was the more active
Results

• LC-MS spectra: fraction 1-5 differed primarily in the amount of just one compound
• This probable active compound has a molecular mass of 134
Conclusions

• Insecticidal activity in *B. juncea* bio-oils not associated with glucosinolates, Sinigrin, or isocyanate, AITC

• Active compound
  – molecular mass of 134
  – likely contains an amide group

• The presence of this compound in the other active fractions of bio-oils from mustard and canola needs to be verified

• Separation of a insecticidal compound could provide a “value-added” product from mustard straw
On-going Projects

- Repeat pyrolysis with new mustard straw sample
- Liquid-liquid separation of bio-oil completed
- Clean-up of aqueous phase with Solid Phase Extraction (SPE) and acetonitrile rinse
We greatly appreciate the financial support of The Agricultural Bioproducts Innovation Program (ABIP).

Technical Assistance from R. Chapman, B. Pocs and D. MacArthur is gratefully acknowledged.