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

CK - water     JA 30 mg/ml     JA 3 mg/ml

• 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 1st instar CPB – 48 h % mortality

- All three *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
• 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 Factions 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 glucosinolate, 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 an 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
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