

Characterization of the Pesticide Properties of Tobacco Bio-oil

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Introduction



Pinewood killed by Beetles



Coffee Grounds



Grape Skins/Seeds



Apple Pumice



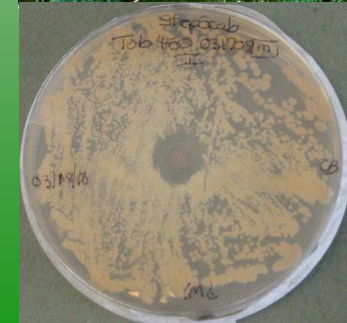
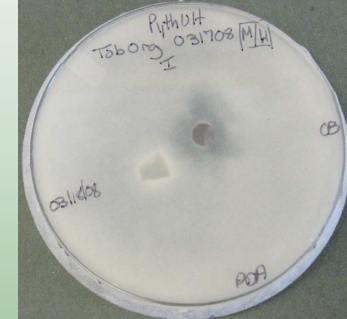
Tobacco Leaves

- Bio-oil from pyrolysis of agricultural and forestry waste
- Potential, alternative use as a pesticide



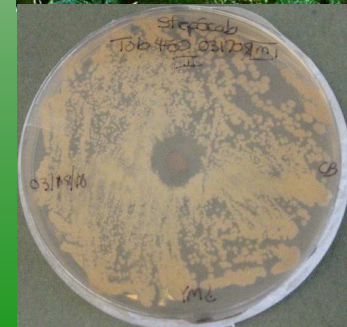
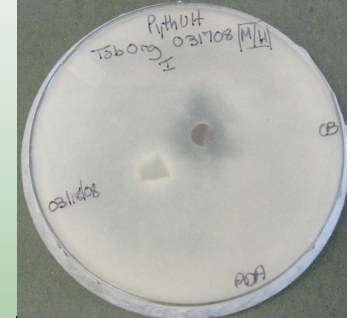
Initial Interest in Pesticides

- Agriculture and Agri-Food Canada
 - Southern Crop Protection and Food Research Centre
- Colorado Potato Beetle
 - Insect pest of potatoes in North America
- Tobacco Bio-oil
 - Nicotine (maintained during pyrolysis)
 - Nicotine-free phase



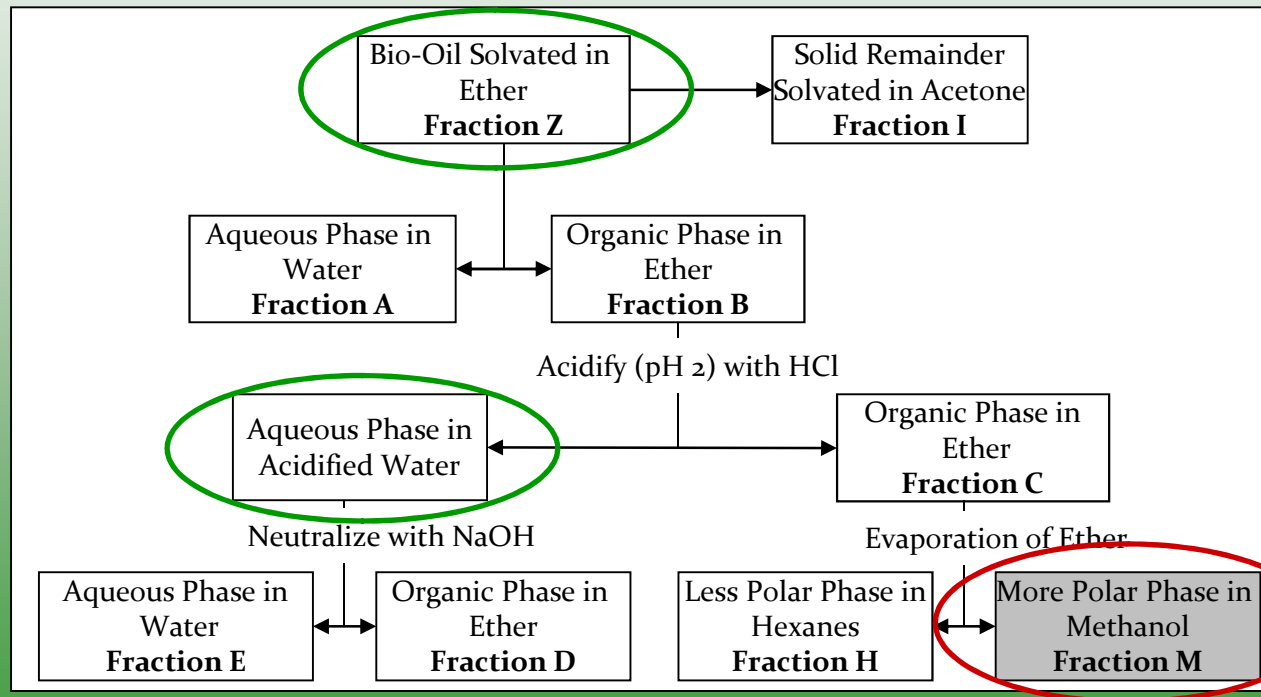
Pesticide Activity of Tobacco Bio-oil

- Bacteria and Fungi
 - 15 species examined, 3 inhibited growth
 - *Pythium ultimum* – seedling damping off disease
 - *Clavibacter michiganensis* – tomato plant disease
 - *Streptomyces scabies* – potato scab disease



Bio-oil Fractionation

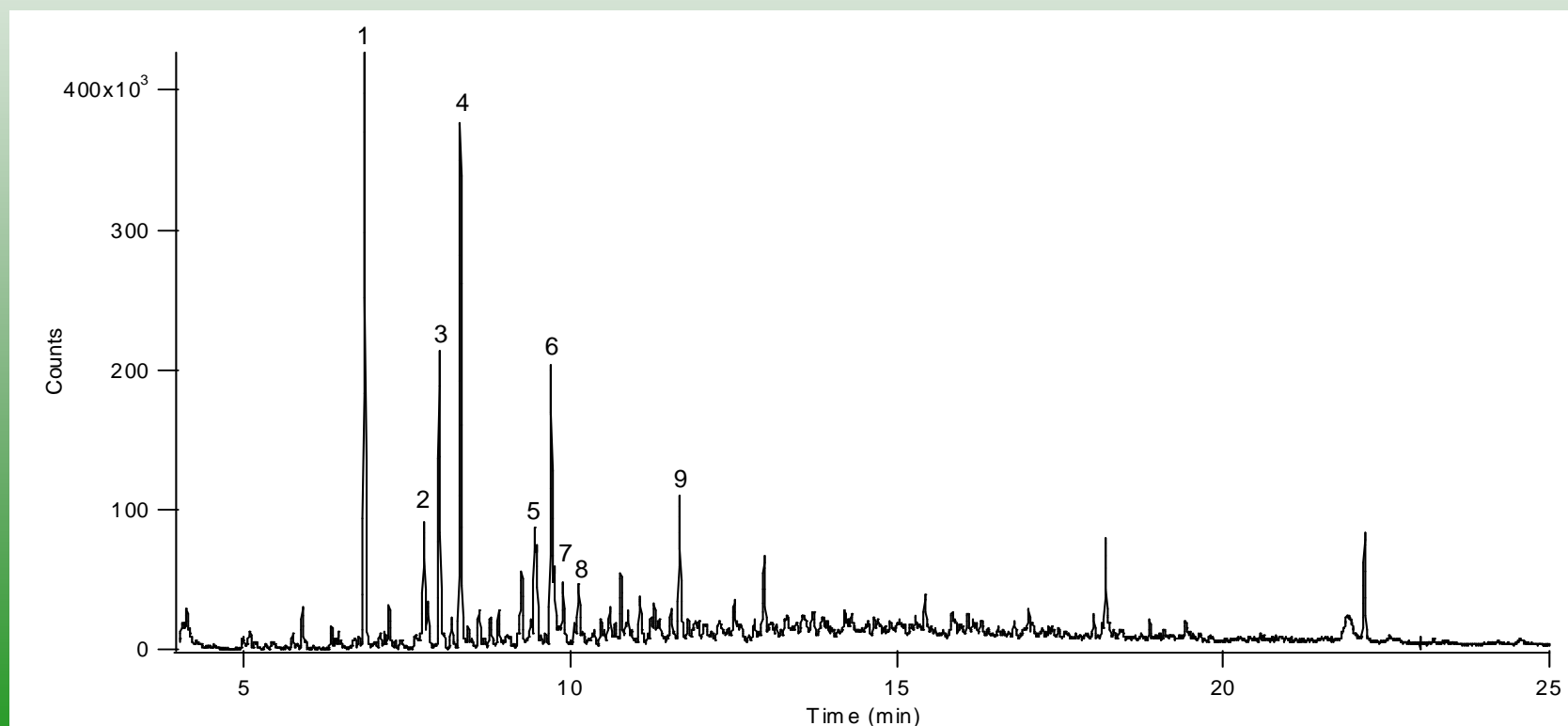
- Liquid-liquid fractionation
 - simplify the chemical composition
 - remove the nicotine from the active bio-oil fraction



• **Nicotine-free** fractions exhibited pesticide properties

GC-MS of Active Fraction

- Over 100 peaks resolved
- 30 chemicals identified by NIST 2005 library
- Most abundant type of compounds:
 - Phenol derivatives → Known activity towards bacteria/fungi



Chemical Standards

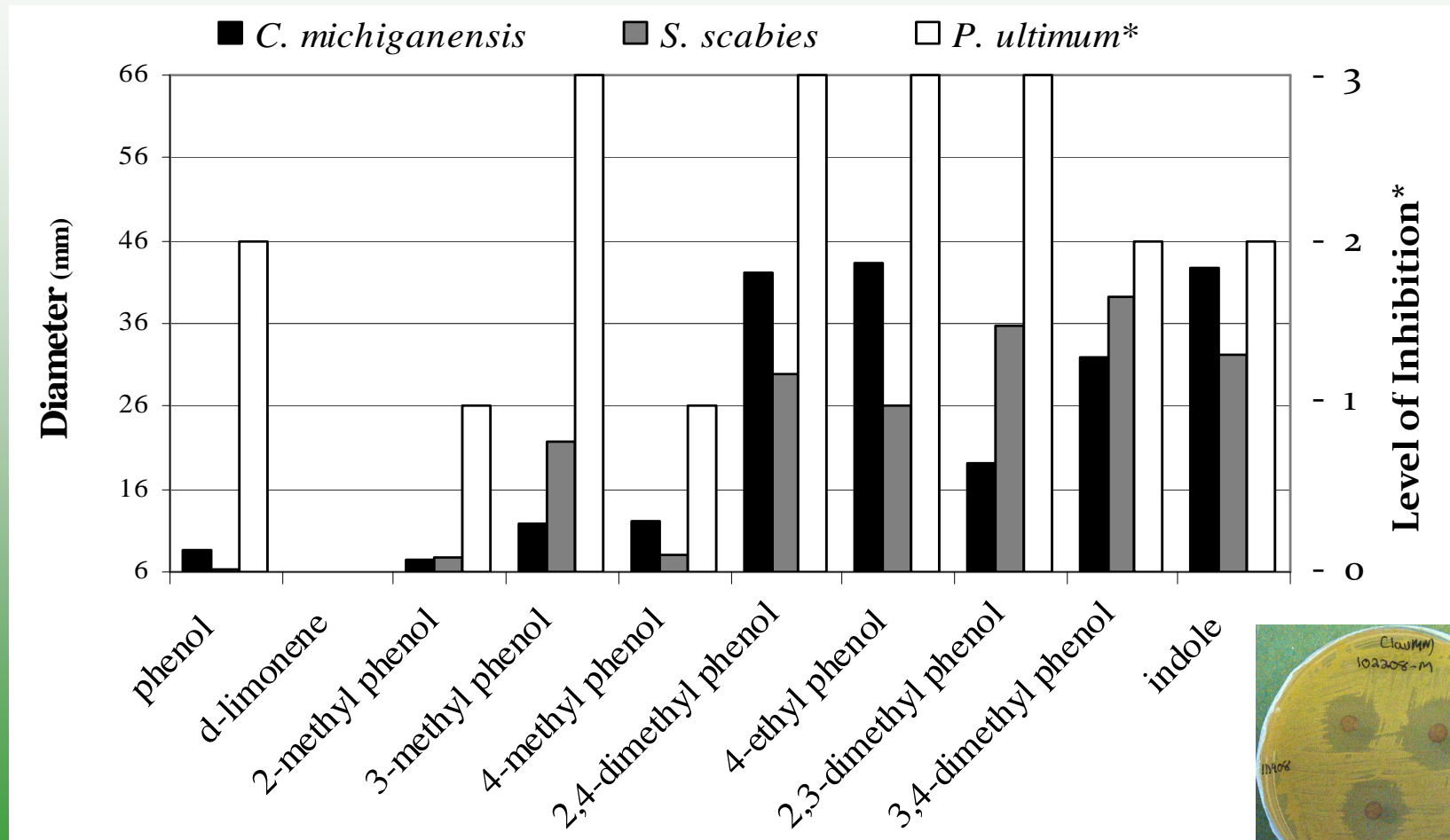
- 10 chemicals account for 54% Peak Area
- Calibration curves (regression > 0.991)

Percent Area	Retention Order	Chemical Name of Standard	Concentration (mg/mL)
14.203	1	Phenol	1.378
14.064	4	3-methyl phenol and 4-methyl phenol	1.340
7.757	6	4-ethyl phenol	0.726
6.631	3	2-methyl phenol	0.646
3.836	9	indole	0.514
2.658	2	d-limonene	0.288
2.480	5	2,4-dimethyl phenol	0.273
1.302	8	3,4-dimethyl phenol	0.248
1.243	7	2,3-dimethyl phenol	0.120

- Standard mixture offered **no inhibition** towards the microorganisms.

Activity of Standards

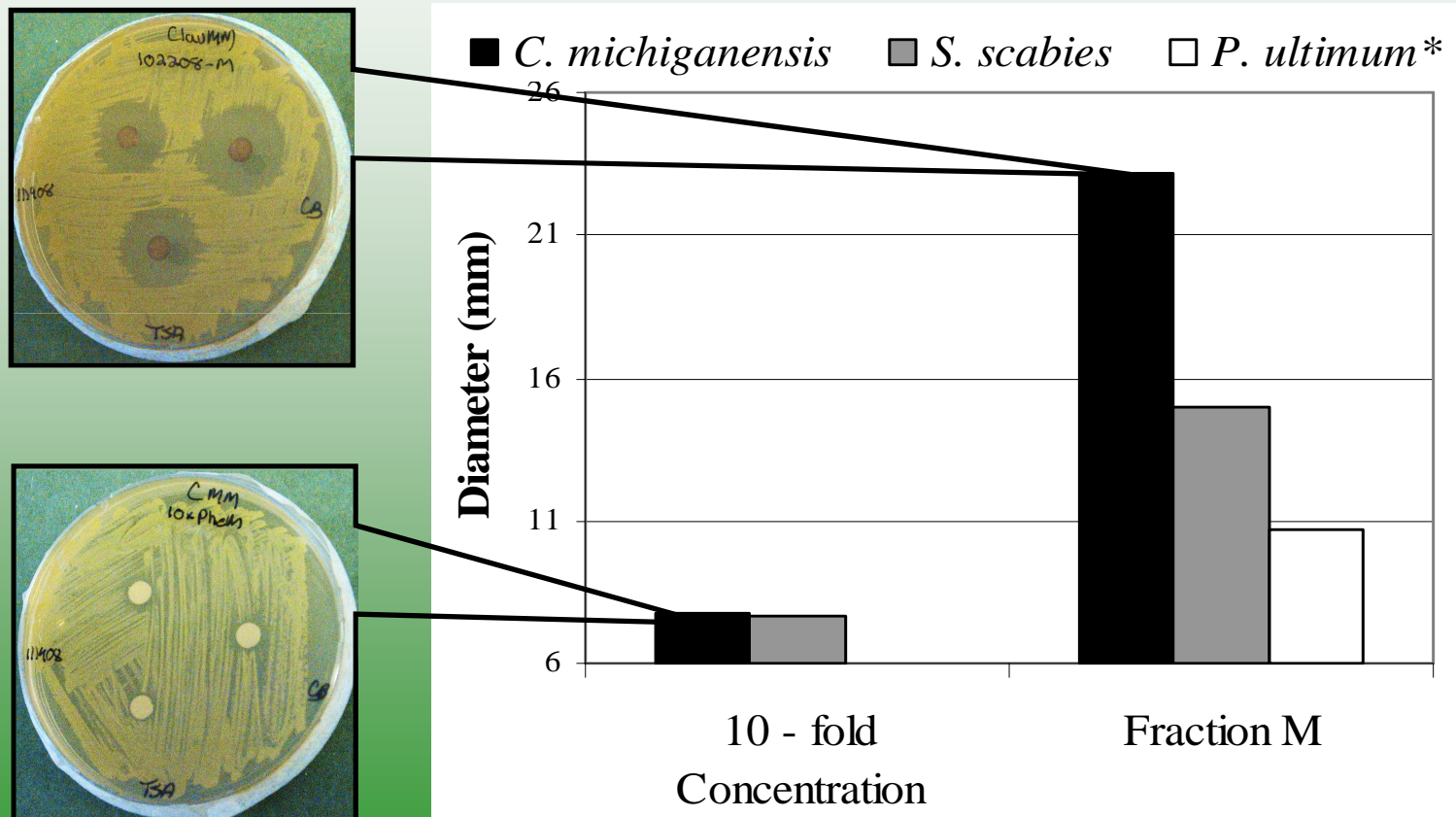
- Concentration increased to 100 mg/mL and tested individually.



- The concentration in Fraction M is **not sufficient**

Activity of Standard Mixture

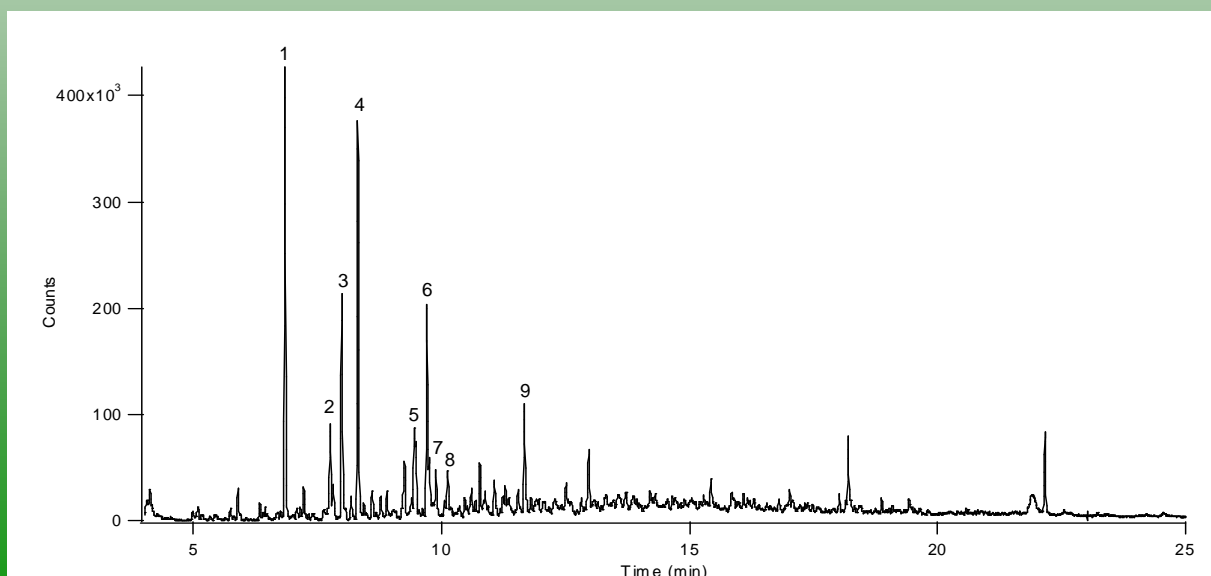
- The standard mixture concentration was increased 2, 5, and 10-fold
- Only minimal inhibition was observed at a 10-fold concentration.



- The identified standards provide a **very small percentage** of the inhibition observed from Fraction M.

Active Components

- 10 compounds account for 54% peak area (each remaining, <2.8%)
- 11 other similar compounds (phenol, indole derivatives) account for an additional 20% peak area
- Likely a few, highly active components left to identify
- Detection via GC-MS? (40% bio-oil detected via GC-MS)
 - Not recognized by NIST 2005 library?
- Pesticide activity is not exclusive to tobacco bio-oil
 - Common compounds are likely responsible for activity

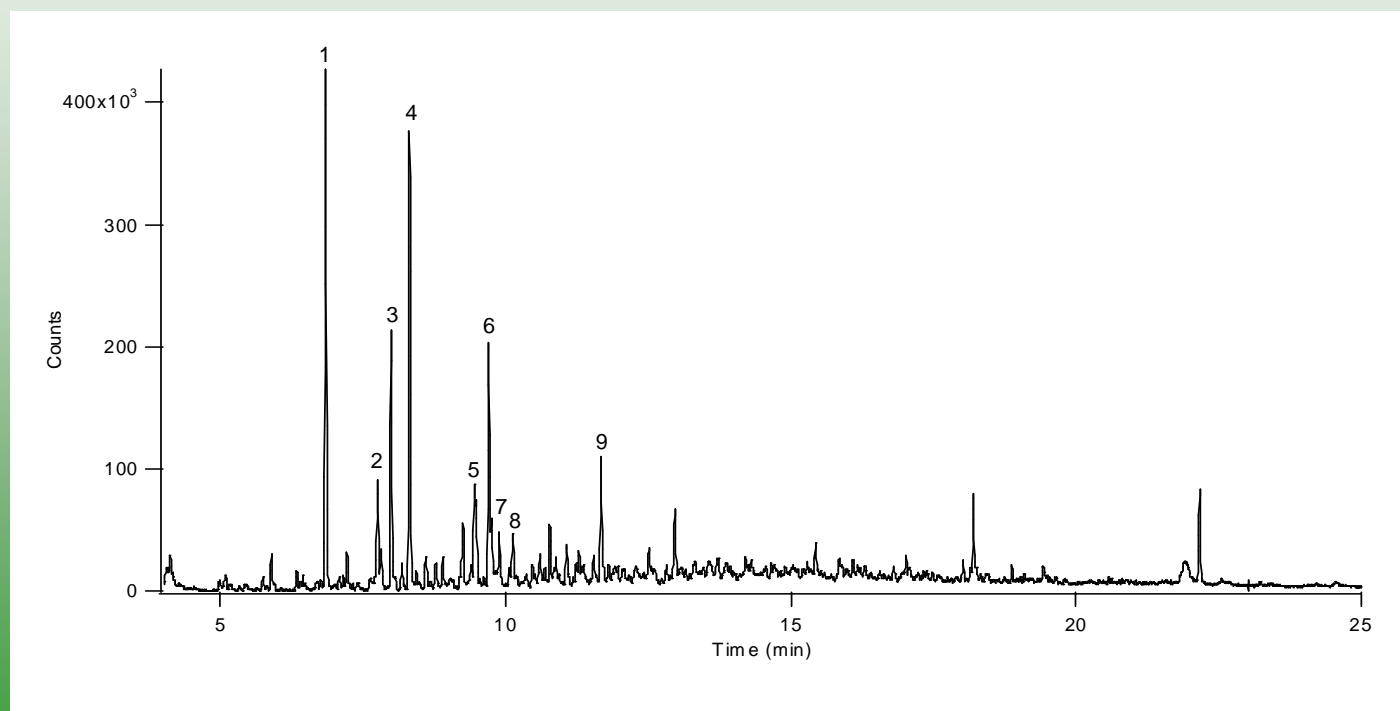


Activity of Other Bio-oils

	<i>Coffee Grounds</i>	<i>Pine-wood</i>	<i>Grape Seed/Skin</i>	<i>Tobacco Leaves</i>	<i>Juncea straw</i>	<i>Carinata straw</i>	<i>Canola Straw</i>	<i>Apple Pumice</i>
Bacteria								
<i>Acidovorax avenae</i>	0	0	0	0	0	x	x	0
<i>Clavibacter michiganensis</i>	X	X	X	X	X	X	X	X
<i>Erwinia cartotovorae pv atroseptica</i>					x	X	x	0
<i>Pseudomonas syringae pv. tomato</i>	0	0	0	0	0	x	0	0
<i>Streptomyces scabies</i>	X	X	X	X	X	X	X	X
<i>Xanthomonas gardneri</i> Group D					X	x	x	x
Fungi								
<i>Alternaria panax</i>	0	0	0	0	0	0	0	0
<i>Alternaria solani</i>	0	0	0	0	0	0	0	0
<i>Botrytis cinerea</i>	0	0	0	0	0	0	0	0
<i>Colletotrichum acutatum</i>	0	0	0	0	0	0	0	0
<i>Colletotrichum coccades</i>	0	0	0	0	0	0	0	0
<i>Fusarium oxysporum</i>	0	0	0	0	0	0	0	0
<i>Penicillium expansum</i>	0	0	0	0	0	0	0	0
<i>Pythium ultimum</i>	0	0	0	X	0	0	0	0
<i>Rhizoctonia solani</i>	0	0	0	0	0	0	0	0
<i>Sclerotinia sclerotiorum</i>	0	0	0	0	0	0	0	0
<i>Verticillium dahliae</i>	0	0	0	0	0	0	0	0

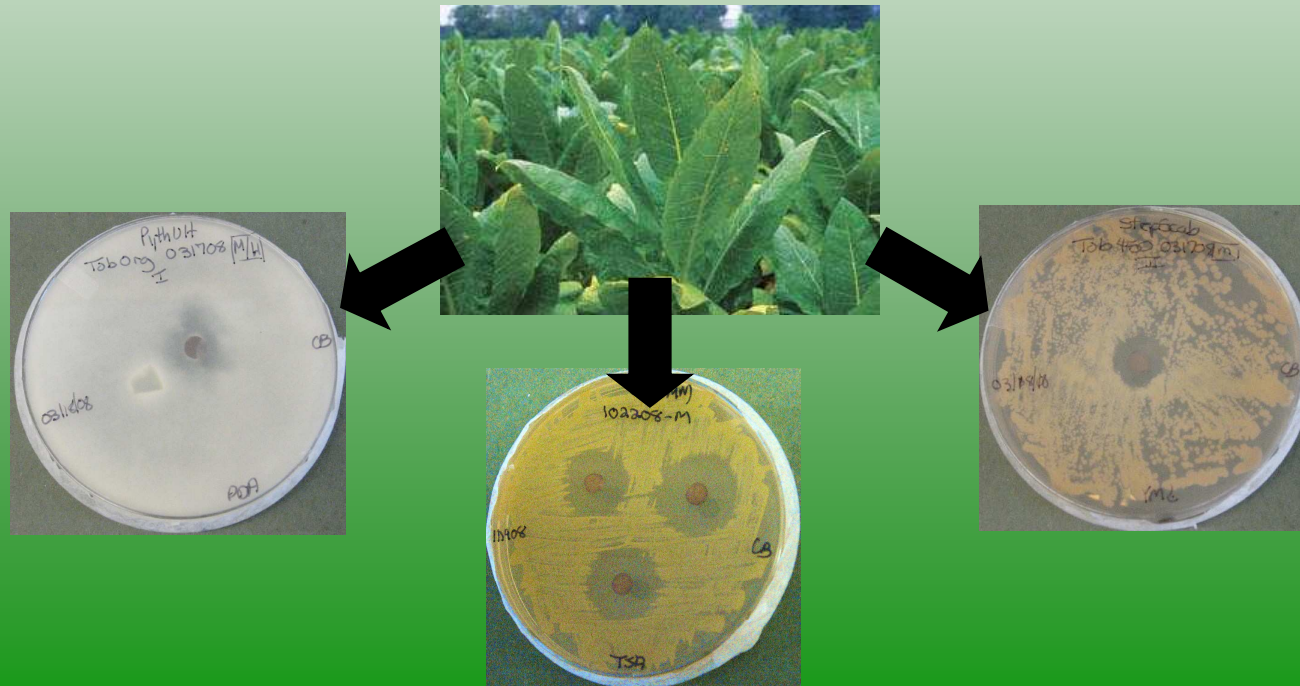
The Search Continues

- Preparation scale liquid chromatography separation with MS detection



Conclusions

- The most abundant types of chemicals in Fraction M are phenol derivatives
 - Known pesticide properties
- The ten standards tested are only slightly responsible for the pesticide activity
- Highly potent chemicals remain to be identified



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