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Experimental Investigations into the Insecticidal, Fungicidal, and Bactericidal Properties of Pyrolysis Bio-oil from Tobacco Leaves Using a Fluidized Bed Pilot Plant

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Tobacco bio-oil, gases, and char were produced through pyrolysis of tobacco leaves using a fluidized bed pilot plant under varying temperature (350, 400, 450, 500, 550, and 600 °C) and residence time (5, 10, and 17 s) conditions. The optimized condition for the production of bio-oil was found to be at 500 °C at a vapor residence time of 5 s, giving a bio-oil yield of 43.4%. The Colorado Potato Beetle (CPB) *Leptinotarsa decemlineata* L. (Coleoptera: Chrysomelidae), a destructive pest toward potato crops, and three microorganisms (*Streptomyces scabies*, *Clavibacter michiganensis*, and *Pythium ultimum*), all problematic in Canadian agriculture, were strongly affected by tobacco bio-oil generated at all pyrolysis temperatures. Nicotine-free fractions of the tobacco bio-oil were prepared through liquid−liquid extraction, and high mortality rates for the CPB and inhibited growth for the microorganisms were still observed. A potential pesticide from tobacco bio-oil adds value to the biomass as well as the pyrolysis process.

1. Introduction

Pyrolysis is one of the thermo-chemical processes that is used extensively worldwide to convert biomass into liquid bio-oil, char, and gases. This process is carried out in the absence of oxygen.[1] However, the pyrolysis oil normally contains a high proportion of oxygenates, reflecting the oxygen content of the original substrates. With the current focus on environmentally friendly energy prospects and renewable energy resources, significant research is being directed toward bio-oils. Bio-oil is considered a CO_2 neutral alternative to fossil fuels with low emissions of the undesirable components SO_2, NO_x, and soot.[2] Despite these advantages, bio-oil has several undesirable properties as a fuel, including high viscosity, low heating value, poor volatility, and coking. Refining bio-oil to a satisfactory level for commercial use has been performed, but currently uses too much energy and occurs at too high a cost to be economically viable.[3]

An additional, potentially lucrative prospect for bio-oil is as a source for valuable chemicals. These chemicals could be found in the original biomass, such as nicotine in tobacco bio-oil, or could be created during the pyrolysis process, such as phenols or new chemicals yet to be identified. One of the many potential applications of these chemicals is as a pesticide. The search for effective and safe pesticides is a continuing challenge as species quickly adapt to most pesticides that are applied.

In this Article, tobacco bio-oil is generated through pyrolysis under a wide range of operating conditions and analyzed for pesticide properties toward a variety of species of concern in Canada. One of the reasons this biomass was selected for analysis is that tobacco farmers across the world, and in particular in Canada, are suffering as demand for their crop continues to decline. It is well-known that smoking tobacco has a significant, negative impact on human health. Transitioning out of tobacco farming, however, is difficult due to the specified nature of the equipment used, and therefore many farmers are left with excess crop every year, which currently goes to waste. Thus, finding alternative, healthy, high value applications to this highly abundant product is an important research area. Already, tobacco biomass is being investigated for unique, high value applications, such as for medical or industrial proteins,[4−6] and in the case of this research, as a natural pesticide. Because tobacco's pesticide properties are well-known, converting tobacco leaves to natural pesticides in the form of bio-oil could provide additional income to farmers.

Tobacco biomass has been characterized,[7−9] but very limited work has been published on the pyrolysis of tobacco for the production of bio-oil. One study concentrated on the production of fuel gases but did not perform liquid analysis,[10] while another study performed liquid analysis but failed to analyze the bio-oil for nicotine.[11]

The potential pesticide activity of bio-oil is an exciting research area that has yet to be fully explored. Recently, bio-oil has been studied for its wood preservative qualities[12] and specifically for its antifungal properties.[13] Two species of fungi were tested and found to have inhibited growth patterns in the presence to bio-oil from wood biomass. In contrast, this research Article investigates the pesticide characteristics of bio-oil from tobacco biomass, not only for antifungal activity, but also for antibacterial and insecticidal activity. The pyrolysis of this tobacco biomass is also investigated.

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2. Experimental Section

2.1. Materials. Finely ground tobacco leaves were provided by Agriculture and Agri-Food Canada, London, ON. Tobacco leaves were obtained from tobacco crops in 2006 and dried at 60 °C. Dried tobacco leaves were then ground using a blender/mixing mill and sieved. The Sauter mean diameter of the tobacco particles used for pyrolysis was 60 μm.

2.2. Methods. 2.2.1. Pilot Plant Design for Pyrolysis. All pyrolysis experiments were carried out using a fluidized bed pilot plant[14] (Figure S1, Supporting Information). The heart of the plant was an atmospheric fluid bed reactor, 0.078 m in diameter, with a 0.52 m long cylindrical section, and equipped with an expanded section made up of a 0.065 m long truncated cone with an upper diameter of 0.168 m, topped by a second, 0.124 m long, cylindrical section. The total volume of this configuration was 6.09×10^{-3} m^3. This assembly provided the lowest vapor residence time (5 s). Two different freeboard extensions were used to increase the vapor residence time to 10 and 17 s. A filter capable of withstanding high temperatures was installed at the gas exit of each of the extensions. Each filter was made up of a perforated pipe connected to the gas exit covered by a fiberglass pad and wrapped inside a fine stainless steel mesh cover. The resulting filter was, in all cases, 0.076 m in diameter and 0.178 m long. Although not ideal, these hot filters have been used in the initial phase of the project with the objective of avoiding the use of a hot cyclone for the char separation, which would be impossible to properly size due to the variety of physical characteristics of the chars expected from the different feedstocks.

The fluidizing nitrogen was injected through a perforated copper distributor plate with 33 holes, 0.5 mm in diameter, equally spaced across the cross section. The reactor was equipped with 18 thermowells for temperature measurements and control (type K thermocouples).

An innovative pulsating automatic feeder was used for biomass injection to the reactor. It quickly dispersed the injected biomass into the core of the fluidized bed.

2.2.2. Bio-oil Production. Tobacco, when injected into the reactor, produced vapors that exited at the top of the reactor through the hot filter section and flowed into three condensers in series through a line traced with Raychem Chemelex heating cable to avoid early, undesirable condensation (as shown in Figure S1). Persistent aerosols were then separated in a cylindrical demister packed with cotton wool. The demister was weighed before and after the experiment. The exact yield of tobacco bio-oil was obtained from the mass of oil collected in the three condensers and the demister.

Pyrolysis was initially carried out at six different temperatures from 350 to 600 °C and at three different residence times (5, 10, and 17 s). Each test was conducted with 700 g of tobacco leaves. Fluidizing and atomizing nitrogen volumetric flow rates were precisely controlled using "Mass Trak" flow-meters from Sierra Instruments Inc., to keep the nominal vapor residence time constant at all temperatures. Tobacco bio-oils produced at all these temperatures separated into two separate phases, an organic and an aqueous one.

Pyrolysis of tobacco leaves was subsequently carried out under the best reactor conditions for high bio-oil yield (discussed in Results and Discussion section and found to be at a temperature of 500 °C and a vapor residence time of 5 s) to determine the accurate liquid, gas, and char yields.

2.2.3. Characterization of Product Gases. Gases were sampled in plastic bags at three different time intervals. To measure the product gas composition, a Hewlett-Packard 5890 series II gas chromatograph (GC) was used. A RESTEK Shin Carbon ST (micro packed), 2 m length column with 1 mm i.d. and 1.58 mm o.d., was used to separate the gas mixture. A thermal conductive detector (TCD) was used to detect the composition of the gas mixture, which consisted of N_2, H_2, CO, CO_2, and CH_4. To measure product gas yields accurately, N_2 was selected as an internal standard gas. Argon was selected as the GC carrier gas. A standard gas mixture with a fixed composition of H_2, CO, CO_2, and CH_4 was used to calibrate the system. The injector was maintained at 150 °C, and the TCD was maintained at 275 °C. A gas sample volume of 0.5 μL was injected with a 100 μL Hamilton syringe. Upon injection, the oven temperature was held at 35 °C for 180 s, then increased at 10 °C/min to 150 °C, and finally increased at 20 °C/min to 250 °C. The temperature was then held constant at 250 °C for 330 s.

2.2.4. Characterization of Char. The differential pressure drop across the fluidized bed was measured at minimum fluidization conditions before and after each experiment. The increase in the reading of the differential pressure drop was proportional to the increase in bed weight. This system was calibrated for very accurate measurement of the char yields.

2.2.5. Characterization of Bio-oil. The bio-oil was characterized through GC−MS analysis of the various fractions examined for biological assays (see below). A HP 6890 Series gas chromatography system with a mass selective detector was used to analyze the bio-oil fractions. All experiments were performed on an HP-5MS, 30 m column obtained from Agilent Technologies with an i.d. of 0.25 mm and a film of 0.25 μm. The injector temperature and auxiliary temperature were maintained at 300 °C. The oven temperature began at 60 °C for 2 min, and then increased at 10 °C/min to 280 °C and was held for 6 min. A threshold of 150 was used, with a mass to charge scan range of 50−300 at a rate of 2.98 scans/s.

2.2.6. Bio-oil Pesticide Characterization. Pesticide activity tests with the bio-oil were performed on a variety of problematic species of microorganisms and one insect. All tobacco bio-oil samples used for the biological tests were produced at a vapor residence time of 5 s and at different pyrolysis temperatures, as specified for each assay.

2.2.6.1. Bio-oil Sample Preparation for Pesticide Analysis. To initially determine which microorganisms were negatively affected by the tobacco bio-oil, a cocktail of naturally separated, organic phases and a cocktail of the aqueous phases of the bio-oils produced from 350 to 600 °C were prepared in acetone (375 mg/mL, one solution of all pyrolysis temperatures). Bio-oil samples from each pyrolysis temperature were then prepared separately in acetone (375 mg/mL, one solution for each pyrolysis temperature). Raw tobacco bio-oil at each pyrolysis temperature was used for the CPB tests.

Two different liquid−liquid extraction techniques were used to generate nicotine-free and nicotine-containing fractions of the tobacco bio-oil. One method was used for the microorganism assays and generated six unique fractions (also analyzed through GC-MS), while the other method was used for the insect assays and generated two distinct fractions. The reason for the two methods was that two separate researchers performed these respective tests. Even so, the end result successfully allowed for nicotine-free fractions to be tested on both the microorganism and the CPB.

The fractionation method used for the microorganism tests, which generated six unique fractions, is illustrated in Figure 1. The organic phase of the tobacco bio-oil pyrolyzed at 450 °C was dissolved in ether at a concentration of 175 mg/mL. This

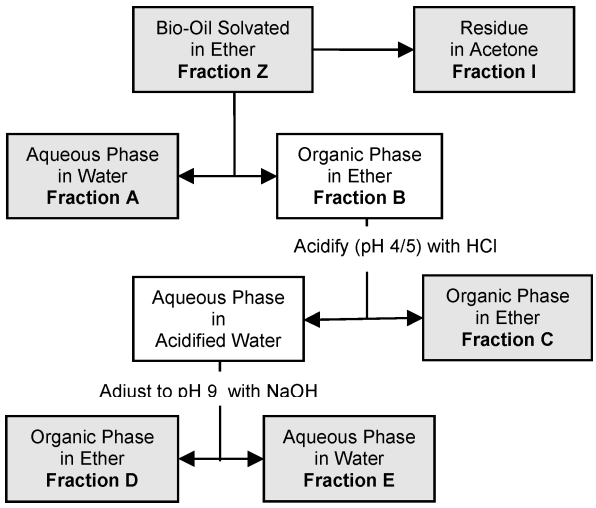

Figure 1. Bio-oil fractionation scheme for microorganism assay testing and GC−MS analysis. Shaded boxes indicate fractions tested in microorganism assays.

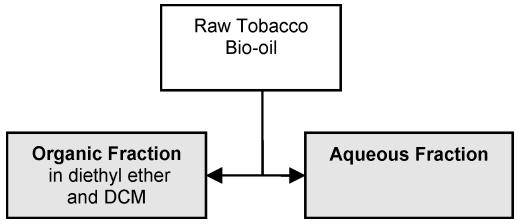

Figure 2. Extraction scheme for nicotine-free tobacco bio-oil fractions for insect assays.

fraction was sterile filtered with a 2.5 cm diameter, 45 μm pore size, syringe filter with a nylon membrane (Whatman, NJ) (Fraction Z). The remaining residue was dissolved in acetone (approximately 102 mg/mL) and was also sterile-filtered, giving a very dark brown solution (Fraction I). Fraction Z was then fractionated into its aqueous (Fraction A) and organic (Fraction B) components with a water/ether extraction. An additional water/ether separation was then performed with Fraction B where the water phase was acidified with HCl to a pH of 4−5. This step caused some components, such as the compound nicotine, to become charged and move into the aqueous phase. An organic, ether phase (Fraction C) and a charged, aqueous phase were generated. The acidic phase was then adjusted to pH 9 (to move the majority of nicotine back into an organic phase) and a final aqueous/ether extraction made an organic phase (Fraction D) and an aqueous phase (Fraction E). Dilution factors were calculated for each fraction, and the volume of sample used for the biological assays was appropriately adjusted. Each fraction was analyzed using GC−MS (Figure S2).

To generate a nicotine-free and a nicotine-containing fraction for the insect tests, liquid−liquid extraction was performed with diethyl ether and dichloromethane (DCM) (Figure 2). The procedure outlined by Oasmaa et al.[15] was used as it closely matched past literature methods for nicotine extraction from tobacco plants.[16,17] A bio-oil mixture from all pyrolysis temperatures (15−20 g) was first passed through a filter paper (Whatman's #4) to remove the solid lignin residue. This residue was washed with two, 5 mL portions of diethyl ether followed by two, 5 mL portions of DCM. The filtrate was then extracted with 20−30 mL of diethyl ether followed by 20−30 mL of DCM. All organic phases were combined, and the solvent was evaporated using a rotary evaporator (BUCHI R-114). The organic fraction recovered was a moderately viscous brown oil, quite similar to the bio-oil itself. The aqueous fraction was orange and had low viscosity.

2.2.6.2. Biological Assays for Pesticide Activity. 2.2.6.2.1. Microorganism Assays. The disk diffusion assay was used to test 11 fungi and 4 bacteria for growth inhibition in the presence of the tobacco bio-oil samples. All species are problematic microorganisms in Canada. See Table S1 for the list of species, their source, and the type of media on which they were maintained. Samples and control solutions were added to sterile, 6 mm diameter filter paper disks and allowed to air-dry before being placed onto freshly inoculated plates. For bacteria tests, the plates were inoculated by streaking the entire surface with freshly grown bacteria to generate a lawn of growth. One or three paper disks were placed into the center of the plate or in a triangular formation on the plate, depending upon the experiment. For fungi tests, a plug of a fresh culture was added about 1 cm away from the disks on a fresh plate. After the plates were incubated at 24 °C for 3 days, the results were recorded. A region of no growth around the disk indicated inhibition (with a minimum measurement of inhibition being 6 mm, the diameter of the disk). Triplicate experiments were performed.

2.2.6.2.2. Insect Assays. These tests were carried out by the leaf disk application, similar to the procedure outlined by Sengonca.[18] Bio-oil fractions and control solutions were spread on both sides of a potato leaf disk with a cotton-tipped applicator. Three leaves were tested for each fraction; however, most tests were repeated on multiple dates to ensure accuracy. The potato plants (var. Cal White) were grown on site at the Southern Crop Protection and Food Research Centre (SCPFRC), Agriculture and Agri-Food Canada, London, Ontario, with the leaves cut to a diameter of 4 cm. The leaves were allowed to dry after sample application. After drying, the leaves were transferred to a Gelman Petri dish. Five, second instar insecticide susceptible strain Colorado Potato Beetle (CPB) larvae reared at SCPFRC were then transferred to the leaf. Mortality rates were recorded after 24 and 48 h intervals. Adjusted percent mortality values are reported, which take into account the natural mortality levels of the CPB in the control treatments. Control treatments involved simply placing the beetles on leaf disks without any oil present. If a specific test involved dilution of the bio-oil, the control leaf disks were coated with the solvent used.

3. Results and Discussion

3.1. Tobacco Pyrolysis. The effects of pyrolysis temperatures (350−600 °C) and residence times (5, 10, and 17 s) on the liquid yield are as shown in Figure 3. Tobacco bio-oil yields were a strong function of temperature and residence time. The greatest yield peaked at 500 °C for all residence times. It could also be observed that bio-oil yield increased as the residence time decreased, for all temperatures. Comparable results were found when this reactor was used to pyrolyze grape seeds and skins, for at a 5 s vapor residence time, the optimum pyrolysis temperature was also found to be 500 °C.[14]

As shown in Table 1, for a residence time of 5 s and a reaction temperature of 500 °C, the bio-oil yield was the highest (43.4%), followed by the char yield (29.4%) and the gas yield (22.4%). The mass balance on the pyrolysis products was close to 95%, which was within the margin of error. Calculations showed that the heat of combustion of the gases produced was 508 J/g of biomass fed. It was assumed that the water produced by combustion was condensed. The heat of combustion value for tobacco was on the lower side as compared to other feedstocks,

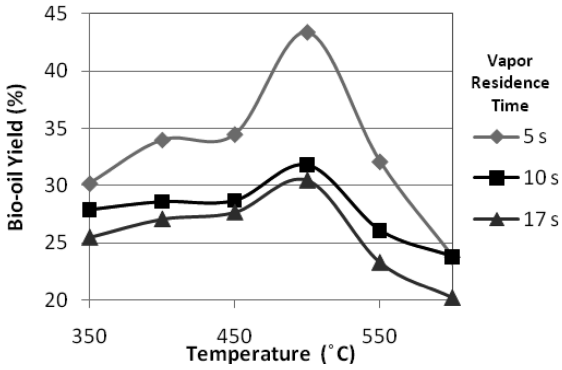

Figure 3. Effect of temperature and residence time on the liquid bio-oil yield. For experimental details, see Methods section.

Table 1. Pyrolysis Product Split at a Vapor Residence Time of 5 s and Pyrolysis Temperature of 500 °C

liquid yield (wt %)	gas yield (wt %)		char yield (wt %)
43.4	22.4		29.4
	H_2	0.7	
	CO	27	
	CH_4	2.8	
	CO_2	69.5	

such as coffee grounds and pinewood, pyrolyzed in the same pilot plant at the same temperature.

The higher liquid yield at lower residence time can be attributed to the fact that lower residence time minimizes secondary reactions[19] such as thermal cracking, repolymerization, and recondensation to maximize liquid yields. It is also very well-known that higher temperature favors gasification (higher gas yields and lower liquid and char yields). Thus, the results obtained are consistent with the existing literature on various other biomass feedstocks.[20]

3.2. Bio-oil Activity toward Pest Species. 3.2.1. Initial Pesticide Discovery. Initial tests with tobacco bio-oil demonstrated clear pesticide activity toward a selection of microorganism species and the Colorado Potato Beetle.

To determine which microorganism species were inhibited by the tobacco bio-oil, naturally separated organic (375 mg/mL organic phase in acetone) and aqueous (used directly without dilution) phase mixtures from all pyrolysis temperatures (350−550 °C) were assayed against 11 fungi and 4 bacteria (Table S1). These species were selected for analysis because of their destructive properties toward agriculture in Canada and were available for testing through Agriculture and Agri-Food Canada. No inhibition was found from the aqueous phases of the tobacco bio-oil. In contrast, the organic phases of the tobacco bio-oil showed clear inhibition for two bacteria, *Streptomyces scabies* (*S. scabies*) and *Clavibacter michiganensis* sub. sp. michiganensis (*C. michiganensis*), and one fungus, *Pythium ultimum* (*P. ultimum*).

Pythium ultimum is a fungus that affects plants as a seedling damping-off disease.[21] Plants affected include eggplant, pepper, lettuce, tomato, and cucumber. *Clavibacter michiganensis* kills young plants and deforms fruits, primarily tomatoes.[22] *Streptomyces scabies* is a common potato scab disease that infects potatoes and makes them unmarketable.[23] Finding inhibition for *S. scabies* is particularly exciting because, currently, no safe pesticide exists on the market that can control this widespread disease.

This discovery of tobacco bio-oil affecting only three microorganism species (and not the remaining 12) is particularly

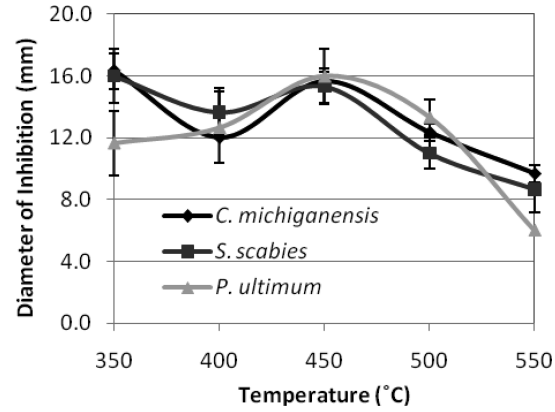

Figure 4. Effect of pyrolysis temperature on the diameter of inhibition for the three affected microorganism species. Error bars indicate ± standard deviation (σ) of replicate measurements within an experiment (total length 2σ).

interesting. This selective inhibition suggests that the active components in the bio-oil are not destructive to all living things, which is an important quality for a potential pesticide.

The Colorado Potato Beetle was also found to be negatively affected by the presence of the tobacco bio-oil. Early tests confirmed high mortality rates for the CPB, and further experiments were performed to investigate one of the key pyrolysis parameters: the pyrolysis temperature.

3.2.2. Investigation into the Effect of Pyrolysis Temperature on Pesticide Activity. Bio-oil produced at each pyrolysis temperature successfully inhibited the growth of each of the three microorganisms (Figure 4).

As the pyrolysis temperature increased to 550 °C, the activity of the bio-oil seemed to decrease. This could be due to the active components being cracked into smaller, inactive components at this high temperature. At 450 °C, the greatest inhibition was observed for all three species. For this reason, as well as the fact that this temperature was close to 500 °C (the pyrolysis temperature with the greatest percent yield of bio-oil), the bio-oil pyrolized at 450 °C was selected for continued investigation. It is important to note that, although these bio-oil samples were prepared to a specific concentration, the observed variations in the activity with pyrolysis temperature could be affected by the amount of water in each bio-oil sample. The water was not removed from the sample to avoid removing other, potentially important chemicals in the process. Nevertheless, each bio-oil sample was found to successfully inhibit the growth of each species.

Similar to the microorganism pattern of inhibition, the CPB was found to be strongly affected by bio-oil produced at all pyrolysis temperatures (Figure 5). The potency of each bio-oil was quite strong given the high mortality levels seen. The 48 h results show that 100% of the beetles tested at each pyrolysis temperature died when in the presence of the tobacco bio-oil. Although the 24 h results seem to demonstrate some changes in toxicity with pyrolysis temperature, these changes are only minor.

It was possible that the toxicity effect of the bio-oils toward the CPB was caused solely by the high quantities of nicotine in the bio-oil. Nicotine is a moderately effective insecticide against the CPB with an LD_{50} of 61 μg per CPB.[24] Sufficient quantities of nicotine could be present in the bio-oil to account for the observed activity. Thus, the bio-oil was separated into nicotine-free and nicotine-containing fractions to determine the effect of nicotine in the observed activity.

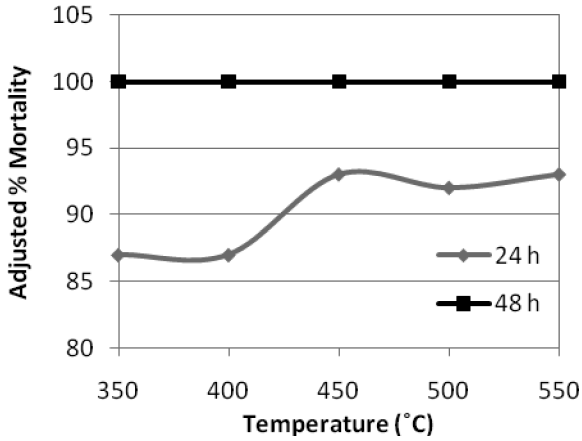

Figure 5. Effect of pyrolysis temperature on the adjusted percent mortality of the Colorado Potato Beetle at 24 and 48 h.

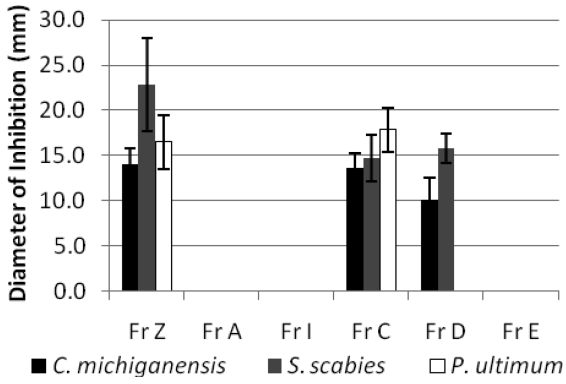

Figure 6. Measured diameters of inhibition for three microorganisms by the six tobacco bio-oil fractions (see Figure 1 for fractionation scheme) after 3 days of growth. Fraction C is nicotine-free. Error bars indicate ± standard deviation (σ) of replicate measurements within an experiment (total length 2σ).

3.2.3. Investigation into the Activity of the Nicotine-Free Fractions of Tobacco Bio-oil. The fractionation scheme shown in Figure 1 was used to generate the six fractions tested on the three microorganisms, as shown in Figure 6. As expected, Fraction Z (the initial fraction) had high activity toward the microorganisms. However, high levels of nicotine were also found in Fraction Z (Figure S1), so much so that few other chemicals could be observed in the chromatograms of this fraction.

The fractionation scheme successfully generated a nicotine-free fraction, Fraction C, which was confirmed by the absence of a nicotine peak in the GC−MS data. This fraction was also strongly active (as shown in Figure 6). Phenol and a variety of its derivatives were found to be in high concentration in this fraction. Although phenolic compounds are known to have pesticide properties,[25,26] 10 of the most abundant compounds in this fraction were quantitatively tested by chemical standards, and it was found that these most abundant phenolic compounds were not present in high enough concentrations to be responsible for the observed activity.[27]

Fraction D, which contains nicotine, was also found to be active. However, when nicotine standards were tested to match and even double the concentration of nicotine found in Fraction D, no inhibition was observed. It is interesting to note that nicotine is the most abundant and almost the only peak detected by GC−MS in this fraction. Therefore, the active components in Fraction D cannot be detected by our GC−MS analysis method. These active components either have a higher boiling point than 280 °C (the highest temperature in our GC program) or cannot be detected by an electron impact MS detector.

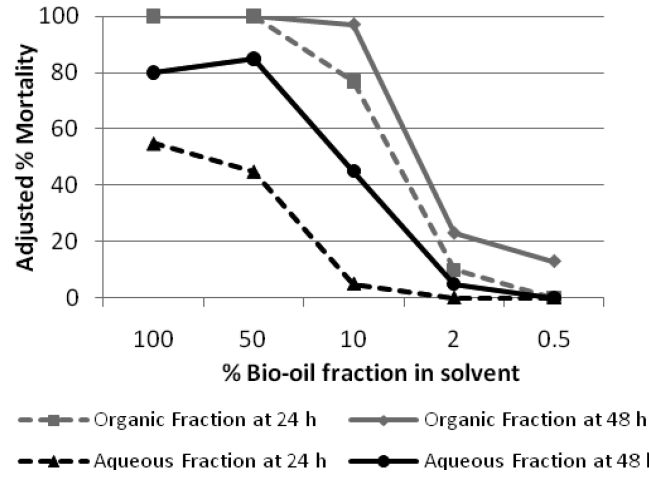

Figure 7. Dilution tests comparing the aqueous (nicotine-free) fraction and the organic (nicotine containing) fraction prepared as illustrated in Figure 2. Results for both fractions were recorded at 24 and 48 h.

A nicotine-free fraction was also found to be active in the CPB assays. The organic fraction showed greater activity over the aqueous (nicotine-free) fraction. After 24 h of testing, the organic fraction obtained 100% mortality rates, while after 48 h of testing, the aqueous fraction obtained a maximum of 80% mortality for the CPB (Figure 7). It is also worth noting that, although the aqueous phase did not result in 100% mortality to the CPB, application of the aqueous phase to the leaf resulted in a greatly reduced appetite for the beetle. Using the aqueous phase at 2% concentration or higher, the beetles would eat little to none of the leaf. Studies have shown that 24 h starvation of the CPB does not prove fatal; however, starvation does cause increased susceptibility to applied insecticides.[28] Whether or not the chemical agent that causes mortality is the same as the agent that is causing starvation is not known, but the starvation is aiding the insecticidal activity of the aqueous fraction.

Further investigation into the nicotine content of the organic fraction was performed. Nicotine standards were tested at the concentration found in the organic fraction. Dilution tests of the organic phase and the equivalent nicotine standard demonstrated that the potency of the samples was the same when measured at 48 h. However, the 24 h results demonstrated that the organic fraction worked faster at causing death in the CPB than the nicotine standards. This indicates that additional, non-nicotine components are acting in the organic fraction.

The assays performed on the CPB and the three microorganisms clearly indicate that tobacco bio-oil contains potent, non-nicotine components with insecticidal and antibiotic activity. Multiple, active components must be present in the tobacco bio-oil as liquid−liquid extraction produced multiple, active fractions. Some of these active compounds cannot be detected by GC−MS.

4. Conclusions

Pyrolysis experiments demonstrated that the liquid bio-oil yield was a strong function of temperature and vapor residence time. The maximum bio-oil yield was found at a reactor temperature of 500 °C and the lowest residence time, 5 s.

Bio-oil was found to have valuable pesticide characteristics toward three problematic microorganisms as well as the Colorado Potato Beetle, a major agricultural pest. Bio-oil produced at all pyrolysis temperatures was effective at inhibiting

the growth or causing mortality in the microorganisms and Colorado Potato Beetle, respectively.

Nicotine was found to be active toward the Colorado Potato Beetle, but had no effect on the microorganisms. Nicotine-free fractions of tobacco bio-oil were found to be active toward the Colorado Potato Beetle and three microorganisms. Multiple components are likely responsible for this activity. These components were not lethal to all of the microorganisms that were examined, demonstrating that these chemicals may only be toxic to selective species, which is a desirable quality in a potential pesticide.

As the demand for tobacco is decreasing, the search for other valuable products from this resource is increasing. A natural pesticide that targets problematic species is a very valuable find. Further investigation into the active components and the potential applicability of using tobacco bio-oil as a natural pesticide will continue.

Acknowledgment

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Supporting Information Available: Additional figures and table. This material is available free of charge via the Internet at http://pubs.acs.org.

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