Environmental Technology & Innovation 20 (2020) 101154

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Potential of pyrolysis liquids to control the environmental weed *Heracleum mantegazzianum*

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ARTICLE INFO

Article history:
Received 26 May 2020
Received in revised form 8 September 2020
Accepted 8 September 2020
Available online 10 September 2020

Keywords:
Pyrolysis acid
Pyroligneous acid
Wood vinegar
Slow pyrolysis
Giant hogweed
Invasive species

ABSTRACT

Replacement of synthetic pesticides with biochemical alternatives and other biological and mechanical control methods represents a future need in plant protection. We investigated if slow pyrolysis liquids (PL) originating from hardwoods, which contain a wide range of organic compounds, can be used to control giant hogweed (*Heracleum mantegazzianum*) either by (i) spraying directly on the seeds (*Carum carvi* seeds used as substitute) and seedlings or by (ii) covering seedlings with PL-containing mulching material (PLM). The effectiveness of the methods was evaluated in laboratory and greenhouse experiments using seedlings of various ages, PLs produced from aspen (*Populus* sp.), birch (*Betula sp.*) and willow (*Salix* sp.) and various PL/PLM application doses. In addition, the biodegradation of birch-derived PL was investigated. All tested liquids inhibited *C. carvi* seed germination effectively when used at > 20% concentrations and only slight differences existed among PLs produced from different biomasses. Direct spraying of PL on *H. mantegazzianum* seedlings was ineffective. PLM (containing 7.5%–40% of PL) inhibited seedling development effectively. Birch PL was readily biodegradable. Further product development is needed because the chemical composition of the PLs in PLM and their modes of action are poorly understood. The weed-inhibiting effect of PLM likely results from the PL and the mechanical barrier constituted by PL-bound peat fibers.

1. Introduction

Herbicides are widely used to manage environmental weeds. However, due to increased concern about environmental and human risks linked to synthetic herbicides (Nicolopoulou-Stamati et al., 2016) several steps to reduce their use have been conducted in the European Union (EC, 2009; EPRS, 2019). Recently, for example, the use of glyphosate – the most used herbicide in the world – has been restricted in public parks, playgrounds and gardens and a total ban of its use may follow in the near future. Because glyphosate plays a key role in the control of both arable and environmental weeds worldwide, there is an urgent need for novel compensatory weed control methods.

Abbreviations: PL, Pyrolysis Liquid; BPL, Pyrolysis Liquid Produced from Birch; APL, Pyrolysis Liquid Produced from Aspen; WPL, Pyrolysis Liquid Produced from Willow; PLM, The Mulching Material Comprised of Peat Fibers, Water and Pyrolysis Liquid; PAH, Polyaromatic Hydrocarbon

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https://doi.org/10.1016/j.eti.2020.101154

One of the most challenging weed problems is represented by the giant hogweed (*Heracleum mantegazzianum*) (Sommier and Levier), which is a highly invasive weed whose presence may lead to a reduction in local plant biodiversity (Page et al., 2006), causing considerable economic damage and sometimes also representing a health hazard to humans (burns) (CABI, 2019; Nielsen et al., 2005). *H. mantegazzianum* is native to the southern slopes of the western Greater Caucasus in southern Russia and Georgia. However, during recent decades it has spread widely throughout northern Europe, with a continually increasing distribution explained partly by rising winter temperatures in Europe (Collingham et al., 2000; Nielsen et al., 2008; Pysek and Prach, 1993). Effective control methods are still under development to restrict its spread and to reduce its adverse impacts. In the EU, *H. mantegazzianum* is included in the recently launched list of Invasive Alien Species of Union concern (EU, 2017), which obliges EU countries to restrict the distribution or eradicate a species. This calls for innovations for appropriate novel control methods (Barzman et al., 2015).

One promising innovation is the development of biopesticides that rely on plant-based molecules. However, the number of practical applications is limited to date. Pyrolysis liquids (PL) are by-products of slow pyrolysis, where various biomasses are converted at elevated temperatures in an oxygen-poor atmosphere into fuel gases, chemicals and carbon-rich products (Fagernäs et al., 2012). PLs collected at pyrolysis reactor temperatures below 280 °C that contain, for example, a wide range of organic compounds like acids, but a low content of tars (oils), constitute a potential plant-based biopesticide for weed control (Hagner et al., 2020b). Earlier studies showed that PLs can be used as a snail repellent (Hagner et al., 2020b; Lindqvist et al., 2010; Tiilikkala et al., 2010) and to control insects, bacteria and fungi (Hossain et al., 2015; Ibrahim et al., 2013; Oramahi and Yoshimura, 2013; Yatagai et al., 2002). Another way to utilize PLs in weed management is to mix them with some organic material, e.g. peat, to create a novel mulching material. In our previous study the efficiency of PL containing mulching material (PLM) for controlling various agricultural weeds was demonstrated in the greenhouse and under field conditions (Hagner et al., 2020a). We suggested that the effect is at least partly due to the high acid content of PLs (Hagner et al., 2020b). A PL containing mulch was recently patented (Finnish patent no. FI127775 (B), international application WO2018108681 (A1)). However, its usability and efficacy under field conditions against various weeds requires more scientific evidence.

To be accepted as a biopesticide, the efficacy of the compound against the target pests must be ensured. A herbicidal effect of PL on *H. mantegazzianum* has not been tested previously. As the used feedstock material largely affects the composition of a PL, also its efficacy against weeds can vary significantly (Hagner et al., 2020b). Consequently, there is a need to establish the best available feedstock material to optimize the quality and ensure the effectiveness of PLs on weeds. Before commercialization and large-scale use, the environmental safety, including residue management and biodegradability of any developed biopesticides, must be demonstrated. Few studies have focused on biodegradation of PLs. Blin et al. (2007), Campisi et al. (2016) and Oasmaa et al. (2012) reported 40%–62% biodegradation of fast or intermediate pyrolysis bio-oils (most also including the tar component) after 28 days. However, only very few degradation data are available for slow PLs (Campisi et al., 2016). All the previously cited studies were conducted under laboratory conditions in an aerobic aqueous medium with optimal conditions for microbes to degrade pyrolysis oils (OECD, 1992). To our knowledge, degradation studies conducted under realistic field conditions and using the liquids collected at low temperatures (without the oily fraction) are almost absent.

The main idea behind this study was to test the efficacy of PL – sprayed as such or applied as a component of novel mulching material (PLM) – for controlling of environmental weeds. As the control of *H. mantegazzianum* populations requires elimination of seeds and seedlings, the effect of PL was tested on both. In the laboratory experiment, we investigated the efficacy of three PLs produced from different feedstocks [birch (*Betula* sp.), aspen (*Populus* sp.) and willow (*Salix* sp.)] on germination of caraway seeds (*Carum carvi*) used to substitute for *H. mantegazzianum*. In the greenhouse, the efficacy of PL derived from birch was tested against *H. mantegazzianum* seedlings of various ages. Two different approaches were used, either direct spraying or application as a component of PLM (PL concentration 0%–40%, peat as mulching material). Due to the absence of *in situ* degradation studies, we also investigated the degradation and residues of mulch containing PL under laboratory and field conditions. We hypothesized that (1) herbicidal efficacy of PLs derived from different feedstocks varies; based on our earlier findings (Hagner et al., 2020b) we assumed that (2) the acid concentration of PLs dominate the weed control capacity, (3) the efficiency of PLM in weed control increases with increasing concentration of PL and (4) the main components of PL are quickly degraded in the soil. *H. mantegazzianum* was selected as a model species because it is the strongest competitor among invasive herbaceous environmental weeds in Finland.

2. Materials and methods

2.1. Composition of the slow pyrolysis liquids

Slow pyrolysis liquid used in the experiments (see below) was produced from birch (*Betula* sp.) wood (BPL) in a commercial producers' continuous retort C (450 °C, holding time 2 h) as described by Fagernäs et al. (2012). During the slow pyrolysis, a composite sample of the total distillate was collected and after settling for two weeks, the aqueous and tar fractions were separated by 30% water addition and decanting. Only the aqueous fraction was used in the experiments (Table 1). The BPL used in the greenhouse and biodegradation experiments was from the same batch. The BLP used in the germination experiment (see below) originated from a later but similarly produced batch. In the germination

Table 1
Characteristics of birch pyrolysis liquid (BPL) used in seedling experiments and biodegradation analyses.

	BPL
Density (g ml^{-1})	1.13
Pyrolytic lignin (weight %)	4.3
Brix densimetry (weight %)	49
N (weight %)	0.12 ± 0.02
C (weight %)	22.2 ± 0.2
Total acids (weight %)	11.2
Phenols (weight %)	2.4
Furans (weight %)	0.5
pH	2.8

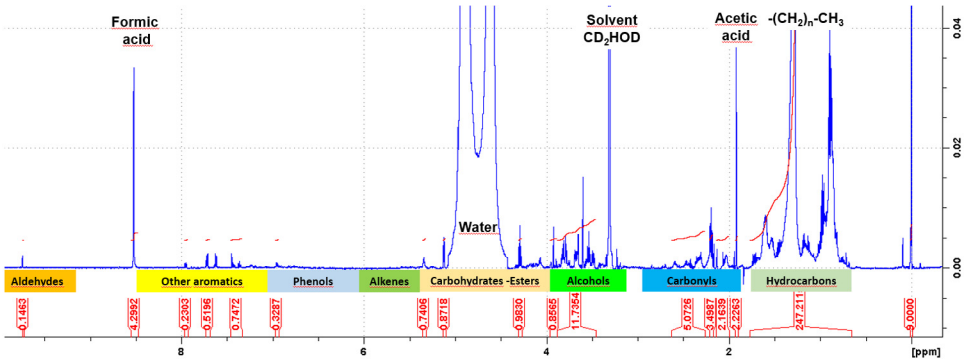

Fig. 1. A representative 1H NMR spectrum from a soil sample taken from 5–10 cm depth at four weeks after mulching the soil surface with PLM3.5. Based on chemical shifts type of compounds (functional groups) present in the sample is possible to determine, e.g. aldehyde signals are at 9–10 ppm. Based on TSP (25 μl, 20 mM, nine protons) reference signal integral at 0 ppm concentrations of each selected compounds, e.g. formic acid (integral 4.3, volume 500 μl, one proton) is possible to calculate.

experiment, also slow PLs produced from willow (*Salix* sp.) and aspen (*Populus* sp.) were included. Willow (WPL) and aspen (APL) PLs were produced in a laboratory scale batch-type slow pyrolysis unit where the reactor was indirectly heated electrically in an oven. Before processing, the raw materials were dried (70 °C). Oven temperature was controlled to rise at approximately 8 °C min^{-1}. To obtain liquids with a high content of organic acids and a low content of tars (and polyaromatic hyrdocarbons (PAHs), see below), reactor temperature was maintained in 270–280 °C for 54 min, and liquids were collected at reactor temperatures below 280 °C. Thereafter pyrolysis process was continued to higher temperatures in order to produce biochar.

Total acidity of the BPL, APL and WPL was determined by titration with 1 M sodium hydroxide (NaOH) that was added in aliquots of 25 meq l^{-1} to continuously magnetically stirred samples, while recording the pH of the solution after each addition. The equivalence points of the obtained titration curves were determined by finding the inflection point using a second-derivative method. The total acidities were calculated as acetic acid equivalents (weight%) (Table 2).

The organic compounds in BPL, APL and WPL used in the germination experiment were analyzed by using proton (1H) nuclear magnetic resonance (NMR) spectroscopy (Table 2). NMR spectroscopy is a fast, low cost and reliable method to analyze concentrations of any soluble organic compound. PLs were dissolved into CD3OD (1 ml), 500 μl of this solution was transferred to a 5 mm NMR tube followed by adding sodium salt of 3-(trimethylsilyl)propionic acid-2,2,3,3-d4 acid (TSP, 25 μl, 20 mM) as an internal standard. 1H NMR spectra were measured by using a 600 MHz Bruker instrument equipped with a cryo-probe and standard pulse sequence in which scans numbered 16 (NS) and relaxation delay between pulses was 10 s (D1). After phase and baseline corrections, selected signals from spectra were integrated and concentrations calculated based on TSP signal (Fig. 1). In addition, PLs were analyzed by Eurofins Nab Labs Ltd (Finland) for 16 PAH compounds usually monitored by the US Environmental Protection Agency (EPA). For this, 2 g liquid samples were extracted with hexane, after addition of four deuterated PAH compounds (naphthalene-d8, anthracene-d10, chrycene-d12 and dibenzo[a,h]anthracene-d14) as internal standards. The extracts were further cleaned by DMSO liquid–liquid partitioning and subjected to SIM mode GC/MS analysis. The runs were performed with an Agilent 5973 GC/MS system, equipped with an HP-5MS capillary column (25 m x 0.2 mm, film thickness 0.33 μm). The temperature program applied was 1 min at 60 °C, 8 °C min^{-1} to 300 °C, and 10 min at 300 °C. The splitless injection technique was used. The detection limit for this method is 0.1 μg ml^{-1} sample and the repeatability ± 20%–40%, depending on the concentrations of the individual components. The sums of 16 PAH compounds for BPL, WPL and APL were <0.5, 3.1 and <0.5 mg kg^{-1}(dry matter), respectively.

The BPL used in the biodegradation studies was analyzed for its elemental composition (C, N) using an elemental analyzer (Thermo Scientific, Flash 2000, Organic Elemental Analyzer) and the flash combustion technique, using methionine

Table 2
Concentrations of organic compounds, total acidity as acetic acid equivalent and pH of slow pyrolysis liquids produced from birch (BPL), aspen (APL) and willow (WPL) used in germination experiment.

g L^{-1}	BPL	APL	WPL
Acetaldehyde	0.00	0.09	0.12
1-hydroxy-2-butanone	4.25	6.72	6.82
1-hydroxy-2-propanone	25.0	30.7	28.6
Acetic acid	98.8	167	142
Cathecol	2.67	2.82	3.14
Ethanol	0.00	0.98	2.12
Formic acid	0.16	8.36	5.33
Furfural	0.85	12.4	9.13
Hydroxymethylfurfural	0.00	1.76	1.21
Lactic acid	0.00	3.40	4.50
Methanol	3.74	18.9	26.6
Methyl acetate	0.00	8.77	5.05
Propionic acid	4.01	4.18	5.59
Phenol	0.37	8.40	2.07
Total acids (weight %)	14.1	23.6	20.3
pH	2.8	1.9	2.4

as standard. The analytical characterization was based on the solvent fractionation procedure developed by Oasmaa et al. (2003) and Oasmaa and Kuoppala (2008), slightly modified. Pyrolytic lignin was determined by diluting PL (1 ml) with a 10-fold excess of H_2O (10 ml) and recovering by centrifugation the water insoluble fraction (Campisi et al., 2016). The aqueous supernatant (10 ml) was analyzed with a Brix Refractometer obtaining a fraction that represents an estimation of the total amount of the water-soluble solutes (it was assumed that this fraction was mainly composed by sugar derivatives (Oasmaa et al., 2003).

Gas chromatography–mass spectrometry (GC–MS) analyses of the semi-volatile compounds were performed using a 6850 Agilent HP gas chromatograph connected to a 5975 Agilent HP quadrupole mass spectrometer. Mildly apolar semi-volatile substances (phenols and furans, Table 1) were separated by means of a 5HP-MS (Agilent) fused-silica capillary column (stationary phase: poly[5% diphenyl/95% dimethyl]siloxane, 30 m, 0.25 mm i.d., 0.25 μm film thickness) using helium as carrier gas (constant pressure, linear velocity of 33 cm s^{-1} at 200 °C). The temperature program consisted in a ramp from 50 °C (5 min) to 325 °C (3 min) at 5 °C min^{-1}, and 325 °C for 10 min. The injection port temperature was 280 °C. Characteristics of the BPL used in seedling experiments and biodegradation analyses are listed in Table 1.

2.2. Mulching material

The mulching material comprised peat fibers, tap water and BPL. The peat was homogeneous, unfertilized white *Sphagnum* peat (Kekkilä Natural 630 W) with a pH of 5.9 and EC of 27 mS m^{-1}. The mulching material was produced by mixing 100 l of sieved (2 mm) peat and 100 l of tap water in a 150 l bucket using a twist drill (2 min, 300 rpm). After mixing, the volume of the final mulching substrate was ca. 100 l. In the mulching experiments, various proportions of water were replaced with PL (see below).

2.3. Germination experiment with caraway

As we were unable to get *H. mantegazzianum* seeds to germinate in the laboratory germination spirals, seeds of caraway (*Carum carvi*) belonging in the same family (Apiaceae) as *H. mantegazzianum* were used in preliminary laboratory experiments (referred to as the germination experiment). The herbicidal effect of BPL, APL and WPL on the germination of caraway (*Carum carvi*: Boreal Plant Breeding Ltd.) was tested using the Jacobsen germinator (Rubart Apparate GmBH) (Rumed, 2001) that consists of a germination plate that is temperature-conditioned by means of a water basin below. Germination spirals (filter papers ϕ 6 cm) with a paper wick were placed on the germination plate. The wick was led into the water bath below, supplying the required humidity and temperature (20 °C) to the filter paper. Caraway seeds (20 pcs) were placed on the germination spirals. The tested liquids (1 ml) were evenly sprayed over the seed containing filter paper. The PL concentrations tested were 50, 30, 20, 15, 10 and 5% (v/v). The tests were conducted in three consecutive series (50–30%–10%, 30–20%–10% and 15–10%–5%). Tap water was used as an inert control and BioNeko (acetic acid 125 g l^{-1}, Woodeco Ltd.) was used as an active control. All treatments had three replicates and each consecutive series had its own controls (water and BioNeko). After spaying with tested liquids, the spirals were covered with a glass dome to provide the air humidity required for germination. A little hole in the upper end of the dome ensured a sufficient supply of fresh air and minimum evaporation. After 14 days, the domes were removed and the germinated seedlings were counted.

2.4. Seedling experiments in greenhouse

Three greenhouse experiments (direct spray experiment, mulching experiment 1 and mulching experiment 2) with seedlings were carried during spring 2019. Before the experiments, pots that were used in the direct spray experiment and mulching experiment 1 (see below) were treated as follows: three recently germinated *H. mantegazzianum* seedlings (age max 7 days) were carefully uprooted and transferred from the field site (Laitiala N: 6737131; E: 487139) in plastic flower pots (ϕ 90 mm) filled with 450 ml of seedling peat (Kekkilä Ltd.). Potted plants were let to stabilize for 7 days before the beginning of the experiments i.e. seedlings were ca. 14 days old when the experiments started. Only pots having three healthy seedlings (height 2–3 cm, 2–3 leaves phase) after the 7 days stabilization were used. 25 pots were used in the direct spray experiment and 35 in the mulching experiment.

The direct spray experiment consisted of five treatments: the pots were sprayed either with 0.5 ml tap water (control), 50% BPL, 75% BPL, 100% BPL (corresponding to 78 ml m^{-2}) or with 1 ml of 100% BPL (157 ml m^{-2}). Each treatment was replicated three times. The pots were placed in a Potter precision laboratory spray tower that sprayed the selected liquid evenly on to the seedlings and surface soil. After spraying, the pots were randomly placed on a greenhouse table in three rows and eight columns.

In the mulching experiment 1 the soil surface and seedlings were covered with a 15 mm layer of PLM (80 ml pot^{-1} i.e. 10 l m^{-2}). The PL concentrations were selected according to pre-experiment work, where PLM with 5% PL was ineffective in killing seedlings (results not shown). The mulching treatments included mixed peat and water only (referred to as control or PLM0) and water replaced by 7.5, 10, 15, 25 or 40% of BPL (PLM7.5, PLM15, PLM25, PLM40). Each treatment had five replicates. In addition, five pots were left as controls without any mulch. The pots were randomly placed on a greenhouse table in 3 rows and 11–12 columns.

In the mulching experiment 2, three *H. mantegazzianum* seeds were sown in pots (ϕ 90 mm) containing 450 ml of seedling peat (Kekkilä Ltd.) in early November 2018. Seeds were collected in summer 2017 from the city of Lahti. The pots were kept for two months at +16 °C after which they were placed outside for cold-treatment to stimulate germination. Mean temperatures at the experimental site (Jokioinen, Finland) in January and February 2019 were −7.7 °C and −1.1 °C, respectively. After two months, the pots were taken in (+16 °C) and the seeds germinated within one week. The pots were irrigated three times a week, and after one month from germination the seedlings were thinned to one seedling per pot. After one month, when the remaining seedlings had reached 20 cm in height, they were cut at 1.5 cm and treated as follows. Five pots were left without mulch and the others were immediately covered with a 1.5 cm layer (80 ml pot^{-1}) of PLM0, PLM15, PLM25 and PLM40 ($n = 5$).

In all greenhouse experiments, the survival and quality (index 1–3: dead, poor, viable) of *H. mantegazzianum* seedlings were recorded after 3, 10, 17, 24 and 31 days from establishing the treatments. The placement of the pots on the greenhouse table was changed at the times of observation. Temperature in the greenhouse was adjusted to 20 °C in 16 h light at and 18 °C in 8 h dark. Pots were irrigated from the base three times a week with 50–100 ml tap water. In addition, the soils were surface irrigated (100 ml) once a week in weeks 2, 3 and 4.

2.5. Biodegradation and residue analyses

The biodegradation of BPL was determined by a ready biodegradability test in an aerobic aqueous medium according to the OECD (1992) guideline 301F, "Manometric respirometry". The test medium was prepared by adding specific concentrations of mineral components from stock solutions (potassium and sodium phosphates plus ammonium chloride, calcium chloride, magnesium sulfate and iron (III) chloride) to distilled water. The bacterial inoculum, derived from an activated sludge taken from a treatment plant receiving domestic sewage located in Ravenna, Italy, was aerated in the mineral medium for 5 days at the test temperature (20 ± 2 °C) before starting the biodegradability tests. The tests were carried out in bottles for 28 days during which time the bottles were placed on an orbital shaker operating at 100 rpm. The BPL and glucose (reference compound) were tested in parallel with a blank (containing only inoculum) and a toxicity control (containing pine wood bio-oil, glucose and inoculum). All treatments were carried out in duplicate.

The theoretical oxygen demand (ThOD) values for the PLs and glucose were calculated on the basis of the carbon content determined by elemental analysis and under the assumption that nitrogen was eliminated as ammonia (no nitrification). The consumption of oxygen was determined by measuring the change in pressure in the apparatus. Evolved carbon dioxide was absorbed in a solution of potassium hydroxide. The amount of oxygen taken up by the microbial population during biodegradation of the test substance (corrected for uptake by blank inoculum) was expressed as a percentage of ThOD.

To assess the BPL residues (main components: aldehydes, formic acid, phthalates, phenols, acetic acid, hydrocarbons and furfural) in the soil, a field experiment was established in Piikkiö in southern Finland (60°25′30″N, 022°31′00″E) in May 2017. Soil in the study site was classified as fine sand, had a pH of 6.5 and at the following soil test nutrient concentrations: Ca 1720, P 22, K 215, Mg 247, S 8, B 0.5, Cu 5.1, Mn 9.6 and Zn 1.95 mg l^{-1} (0.5 M acid ammonium acetate, pH 4.65; Vuorinen and Mäkitie, 1955). The experimental area was fertilized with a NPK fertilizer including micronutrients (Yara Ltd.) at the rate of 600 kg ha^{-1}, to provide 72 kg of N, 24 kg of P and 102 kg of K per hectare. Ten plots of 1 × 1 m were established on the area at 0.5 m distances. Five of the plots were covered with 1 cm layer (10 l m^{-2}) of 0% PLM and five with 3.5% PLM (PLM0 and PLM3.5). After two weeks, 15 onion (*Allium cepa* var. Hylander) seedlings (5 weeks

old) were planted on each plot at ca. 20 cm distances. PL concentration of 3.5% was used in degradation studies as it was shown to be adequate to be used against most agricultural weeds (Hagner et al., 2020a), thus making results more generalizable.

Soil samples for the analysis of the BPL remains were taken from each plot 4 and 12 weeks after mulching (n=5). Three soil samples were taken from each plot at two depths 0–5 (including PLM) and 5–10 cm using soil corer (ϕ 3.5 cm) and pooled to achieve one composite sample per plot per depth. Samples (50 g) were put on an extraction thimble (33 × 100 mm Whatman Cat No. 2800-330) and extracted using Soxhlet apparatus and 200 ml methanol (VWR HiPerColv Chromanorm Methanol for HPLC LC-MS grade, product code 83638.320) as a solvent with 6 h extraction time. After extraction the solvent was removed and stored at +4 °C until the analysis. Organic compounds from the methanol extracts were analyzed by proton (1H) nuclear magnetic resonance (NMR) spectroscopy similarly as for PLs. After phase and baseline corrections, selected signals from spectra were integrated and concentrations calculated based on TSP signal (Fig. 1). Assuming that PLM become mixed in the upper 5 cm soil layer and using soil bulk density of 1500 g l^{-1} produces a PL concentration of 4.6 ml kg^{-1} in the topsoil. Thus, in the initiation of the experiment the concentrations of main components of PL in the soil after covering with PLM3.5 exceeded the detection limit of NMR (1 ppm, see below).

2.6. Statistical analyses

The normality of data was analyzed using the Kolmogorov–Smirnov and Shapiro–Wilk tests and the homogeneity of variances with Levene's test. First, the similarity of control treatments in the germination experiment (water only and BioNeko) conducted during various days was tested with a one-way ANOVA. The Tukey post hoc test was used for paired comparisons. As there were no differences in the numbers of non-germinated seeds in controls (water and BioNeko) conducted on separate days, the data from the three germination experiments were combined and analyzed together by comparing the number of dead seedlings in control, BioNeko and various PL treatments (APL, WPL and BPL: 5%–50%) using one-way ANOVA.

Data concerning the herbicidal effect of various liquids on seedlings (Direct spray experiment, Mulching experiments 1 and 2) were analyzed using repeated-measures ANOVA with treatment as predictor variable and number of non-germinated seeds or eliminated seedlings as the response variable. If there were interactions between time and treatment (only in mulching experiment 2), each timing was analyzed separately using one-way ANOVA. PL residues in the soil were also analyzed using ANOVA models for repeated measures, where the plot treatment was treated as a fixed factor and the repeated measures were comprised of either soil layers (vertically repeated measure of plot treatment effects) or samplings (temporally repeated measure of plot treatment effects). All analyses were conducted using IBM SPSS Statistics 25.

3. Results

3.1. Effect of PLs on seed germination

In the germination experiment, the herbicidal effect of three different PLs was tested using *C. carvi* as the test species (used to mimic *H. mantegazzianum*, which did not germinate in the spirals). In the control spirals that received water only, the germination of seeds was ca. 70% after 14 days. Active controls sprayed with BioNeko eliminated ca. 90% of the seedlings. A similar reduction in germination to the BioNeko was reached with all the PLs at 20, 30 and 50% concentrations (significance for control $p < 0.01$ in all cases) (Fig. 2). At 15% APL, WPL or BPL concentration, the share of non-germinated seeds (70%) was 20% less than with BioNeko, but the difference was not statistically significant (significance of water control $p < 0.01$ in all PL treatments). At 10% concentrations, all PLs performed significantly more weakly than BioNeko and at 5% concentration the PLs were comparable with water (Fig. 2). Even though the APL seemed to be the most effective PL when used in 5% concentration, no discernible differences between the PLs within the tested application rates were found ($p > 0.05$).

3.2. Impact of PLs on seedlings

In the direct spray experiment the 7 day old *H. mantegazzianum* seedlings sprayed with 0.5 ml of 50%–100% BPL (corresponding to 39–78 ml m^{-2}) were not damaged. Only the highest dose (1 ml 100% BPL corresponding with 157 ml m^{-2}) eliminated 40% of the seedlings (Repeated-measures ANOVA; $DF = 4$, $F = 6.27$, $p = 0.02$) (Fig. 3).

The effect of mulching material containing various proportions of BPL was studied in the mulching experiments 1 and 2. In mulching experiment 1, all seedlings without mulch survived through the 31 day study. However, application of a 1.5 cm layer of control mulch, containing only peat and water (PLM0), eliminated on average 53% of seedlings. Further, BPL application (7.5%–40%) on the mulching material resulted in 100% elimination of seedlings, the difference being significant between the control (PLM0) and all the PL-containing treatments, between the PL containing treatments and controls without mulching and between PLM0 and controls without mulching (Repeated-measures ANOVA; $DF = 6$, $F = 30.85$, $p < 0.01$ in each case) (Fig. 4).

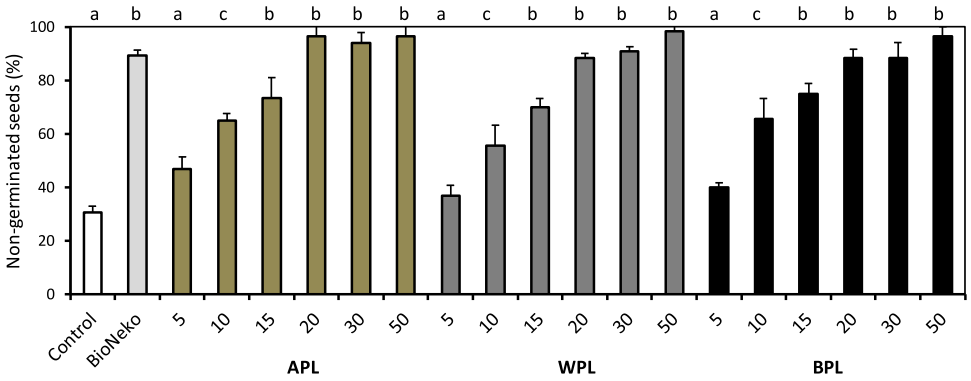

Fig. 2. Percentage (%) of non-germinated *Carum carvi* seeds after 14 days of exposure to various concentrations of slow pyrolysis liquids (5%–50%) derived of willow (WPL; gray bars), aspen (APL; brown bars) and birch (BPL; black bars), commercial herbicide BioNeko (active control) and water (inert control) (mean ± SE, n=3–9, see Methods). Different letters indicate significant difference ($p < 0.05$) of treatment compared with the H_2O (a) and BioNeko controls (b).

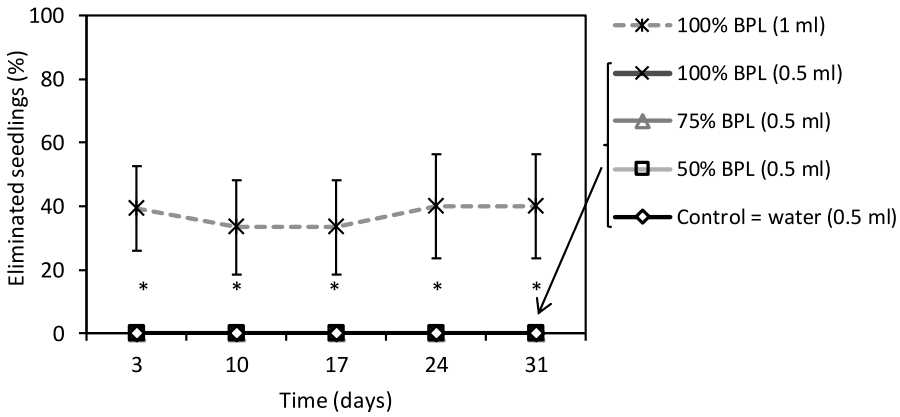

Fig. 3. Proportion of dead seedlings (%) in the pots (ϕ 90 mm) after 3–31 days from spraying with various doses of birch pyrolysis liquid (BPL) (Direct Spray Experiment). Mean ± SE, n=5, each replicate representing mean value of three seedlings. Significant difference compared with control (* $p < 0.05$ or ** $p < 0.01$).

In mulching experiment 2, each plant that was only cut grew new shoots within 3 days (Fig. 5). In contrast, all seedlings (also control i.e. PLM0) that were both cut and covered by mulch were still below mulch 3 days after treatment. However, after 10 days the plants in controls (PLM0) had grown through the mulching material. Addition of 15% PL (PML10) prevented the regrowth of 66% of the cut seedlings. In pots having 25 and 40% PL concentration in the mulch (PLM25 and PLM40), none of the seedlings grew new shoots during the 31 day follow-up (difference to control: Repeated-measures ANOVA; $DF = 4$, $F = 15.06$, $p < 0.01$) (Fig. 5). There were no differences in the quality of seedlings, i.e. each survived seedling was of good quality (viable: class 3) in all experiments.

3.3. Biodegradation and remains in the soil

According to the OECD (1992) guideline 301F "Manometric respirometry", the BPL was classified as readily biodegradable because it achieved 76% biodegradation during first 10 days. In the field, the concentrations of acetic acid, formic acid, aldehydes, phthalates, phenols, and hydrocarbons did not differ significantly between PLM0 and PLM3.5 treated soils after 4 weeks (Table 3) (Repeated-measures ANOVA, $p > 0.05$ in each case). The acetic acid content was higher in one PLM3.5 sample taken 12 weeks after mulching, thus increasing the mean value of PLM3.5 treatment (Table 3) but the difference between it and the control was, however, not statistically significant. No furfural residues were found in any sample.

4. Discussion

We studied whether slow PLs can be used for controlling invasive environmental weeds, either by direct spraying on seeds and seedlings or by using as a component of novel mulching material. Initially, we hypothesized that PLs

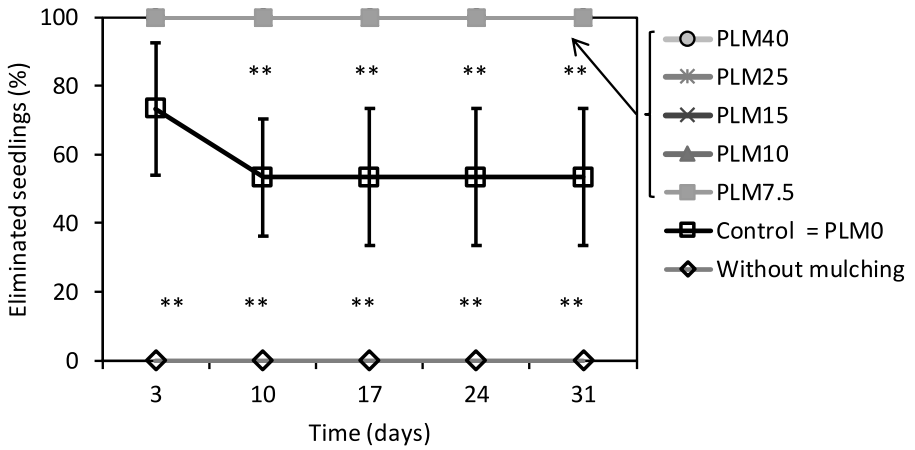

Fig. 4. Proportion of dead seedlings (%) in the pots covered with 1.5 cm layer of mulching material containing various proportions (0%–40%) of birch pyrolysis liquid (PL) (mulching experiment 1). For example, PLM10 contains 10% PL. Mean ± SE, n=5, each replicate representing mean value of three plants. Significant difference compared with control (** $p < 0.01$).

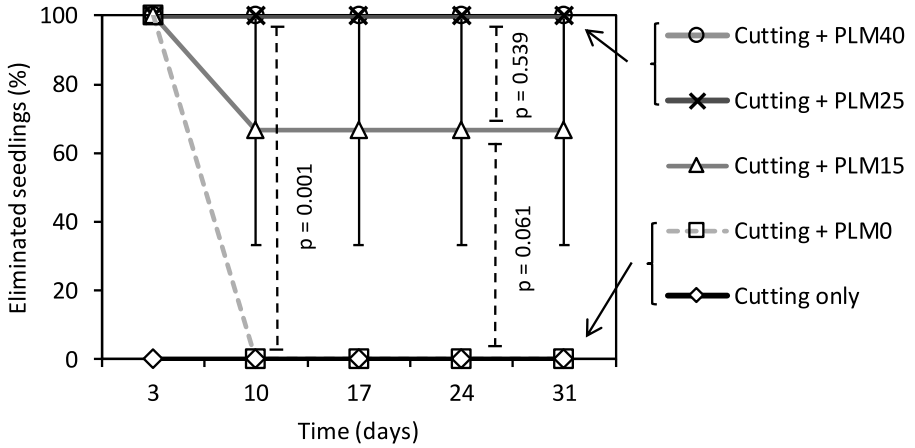

Fig. 5. Proportion of dead seedlings (%) in the pots covered with various doses of birch pyrolysis liquid containing mulching material after cutting the seedlings (mulching experiment 2). For example, PLM10 contains 10% pyrolysis liquid. Mean ± SE, n=5. Least Significant Differences are depicted with a dashed vertical line and *p*-value.

Table 3
Content of aldehydes, formic acid, phthalates, phenols, acetic acid and hydrocarbons (mg kg^{-1} fresh soil sample) in the field soil at 4 and 12 weeks after the mulching with PLM0 (control) and PLM3.5 (3.5% pyrolysis liquid). Mean, maximum and minimum values (n = 5).

Treatment	Layer (cm)	Week	Aldehydes		Formic acid		Phthalates		Phenols		Acetic acid		Hydrocarbons	
			Mean	Min–Max	Mean	Min–Max	Mean	Min–Max	Mean	Min–Max	Mean	Min–Max	Mean	Min–Max
PLM0	0–5	4	0.20	0.00–0.42	3.35	0.28–6.90	0.40	0.00–0.89	0.62	0.00–1.81	0.16	0.00–0.47	60.0	51.4–72.3
PLM3.5	0–5	4	0.07	0.00–0.18	0.95	0.00–2.72	0.28	0.00–0.74	0.48	0.00–1.00	0.36	0.00–0.77	33.7	24.2–53.4
PLM0	5–10	4	0.04	0.00–0.13	5.19	4.00–6.73	0.32	0.00–0.61	0.00	0.00–0.00	1.53	0.80–2.30	28.3	20.9–32.1
PLM3.5	5–10	4	0.07	0.00–0.15	4.67	0.00–12.2	0.27	0.00–0.57	0.03	0.00–0.11	1.02	0.00–3.03	31.3	12.4–48.4
PLM0	0–5	12	0.12	0.00–0.26	4.33	2.51–5.73	0.33	0.00–0.58	0.86	0.00–1.64	1.29	0.00–2.08	56.6	45.7–68.9
PLM3.5	0–5	12	0.09	0.00–0.22	3.25	0.00–5.92	0.28	0.00–0.67	0.82	0.00–2.96	5.94	0.00–18.0	37.1	6.11–64.9
PLM0	5–10	12	0.03	0.00–0.08	3.92	2.23–6.19	0.21	0.00–0.37	0.22	0.00–0.67	1.66	0.80–3.30	52.6	42.1–69.6
PLM3.5	5–10	12	0.09	0.00–0.23	3.53	1.11–8.75	0.33	0.00–0.83	0.17	0.00–0.67	1.22	0.16–3.59	28.5	3.05–39.9

produced from various feedstocks vary in their herbicidal efficacy such that higher acid concentration of the product results in improved weed control. This hypothesis was not confirmed: total acid concentration of the PL produced from willow, aspen and birch varied considerably, but their elimination efficiency regarding seed germination varied only slightly. Secondly, we assumed that the efficiency of PL in eliminating seedlings increases with increasing application dose. This hypothesis could not be confirmed because direct spraying of PLs on 14-day old *H. mantegazzianum* seedlings was ineffective irrespective of the application dose. However, the PL containing mulching material (PLM) eliminated the 14 days old seedlings completely at all concentrations (7.5%–40%). Also, two-month old seedlings were completely

eliminated using a combination of cutting and mulching with PLM (25%–40%). Finally, we hypothesized that the principal PL compounds are quickly degraded in the soil. This was confirmed: BPL reached 76% degradation over 10 days and no residues of the principal compounds were evident one and three months after the PLM application to the field soil.

PLs produced from willow, aspen and birch inhibited the germination of *C. carvi* seeds completely when used at concentrations of > 20%. Previously, we suggested that the potential efficacy and required dose of various PLs, when used against soft-bodied insects and broadleaved-weeds, can be evaluated according to the total content of acids in the product (Hagner et al., 2020b). However, contrary to our hypothesis, this was not apparent in the experiment with caraway seeds because the total acid content of birch-derived PL was somewhat lower (14.1%) than that of aspen (23.6%) or willow (20.3%), but their herbicidal efficacy differed little. We based our assumption on the premise that acetic acid has long been used in plant protection as an approved active compound in Europe and is listed as a biopesticide in the USA (EPA, 2018; EU Pesticides Database, 2019). Also propionic and butyric acids have exhibited phytotoxicity (Lynch, 1980). So it is possible that the germination inhibition effect of PLs on the tested seeds was a joint effect of several organic acids and compounds, including furfural and phenols originating from lignocellulosic biomass (Fagernäs et al., 2012; Hagner et al., 2020b; Hoekman et al., 2011). Experiments dedicated to identifying the active compounds behind the herbicidal effect of PLs are strongly encouraged.

We assumed that efficacy of PLs in eliminating seedlings increases with increasing application dose. However, the direct spraying of PLs on 14-day old *H. mantegazzianum* seedlings was ineffective irrespective of dose (max dose 157 ml m^{-2}), even though all the seedlings were soaked by the liquids. In previous studies, the corresponding dose of PL produced from various biomasses (willow, forest residue, pine bark, wheat straw) eliminated over 80% of 7-day old seedlings of rape (*Brassica rapa*) (Hagner et al., 2020b). Unquestionably, the *H. mantegazzianum* seedlings are exceptionally strong, which can be linked the invasive nature, and controlling the seedlings with direct spraying of PLs seems to be ineffective, although the effect of more concentrated PLs is worth testing in the future.

However, when the PL was used as a component of mulching material (7.5%–40% i.e. 750–4000 ml of liquid per m^2), none of 14-day old seedlings survived. Also the mulch containing only peat and water (PLM0) prevented the growth of around half of the 14-day old seedlings. The added PL acted as an adhesive between peat fibers, increased the hardness of the cover and further prevented the growth of seedlings (Hagner et al., 2020a). Also two-month old seedlings were completely eliminated when a combination of cutting and mulch containing 25%–40% of PL was used. The final effect was possibly a result of the hard cover and the compounds arising during slow pyrolysis of lignocellulosic biomass plant constituents. The weaker herbicidal effect of PL on seedlings when sprayed directly in comparison with mulching is also explained by higher PL doses per m^2 after PLM application. In addition, after covering with PLM, the seedlings are assumed to be exposed to the PL-containing compounds for a longer time than after direct spaying. After spraying, some of the volatile organic PL compounds quickly evaporate. The use of adhesive agents to increase the herbicidal efficacy of PLs after spraying treatment would be an interesting topic of investigation.

Finally, we hypothesized that PL-containing compounds are quickly degraded in the soil. This was confirmed. The levels of acetic acid, formic acid, aldehydes, phthalates, phenols, furfural and hydrocarbons in the soil samples taken one and three months after mulching did not differ between soils covered with PL-amended and non-amended mulch. In addition, in the laboratory biodegradation test, BPL was 76% degraded over 10 days and could therefore be classified as readily biodegradable. These results indicate that at least BPL is, for the most part, degraded in the soil in one month. Previously, Blin et al. (2007) focused at the biodegradation of pyrolysis oils and showed the water-soluble part of slow PL (from spruce) reached 62% biodegradation during 28 days and was more easily mineralized by bacteria and fungi than fast pyrolysis oils. Oasmaa et al. (2012) also reported the biodegradability of several lignocellulosic fast pyrolysis bio-oils to be 40%–50% after 28 days. Campisi et al. (2016) studied the biodegradability of three bio-oils produced from fast pyrolysis of pine wood and intermediate pyrolysis of corn stalk and poultry litter. These three bio-oils were also biodegradable, with 40%–60% biodegradation after 28 days. Campisi et al. (2016) concluded that fast pyrolysis bio-oils are less degradable than those produced by slow and intermediate pyrolysis, but only very few data are available for slow and intermediate pyrolysis (Campisi et al., 2016). The potential leaching of PL was not estimated in this study. However, in a previous greenhouse experiment, no remains of the 14 most abundant components of BPL were detected in the leachates 44 days after mixing the liquid (2000 l ha^{-1}) in the soil (Hagner, 2013). The biodegradability of the slow PL analyzed in the present study is in agreement with previous results. Mulching material containing 3.5% PL is sufficient to control typical agricultural weeds (*Chenopodium album* L., *Epilobium angustifolium* L., *Tripleurospermum inodorum*) (Hagner et al., 2020a,b) and was thus selected to be used in degradation studies. However, it must be noted that even though 3.5% PLM is suitable for controlling agricultural weeds and weeds in parks (Hagner et al., 2020a), the concentration of PL may need to be higher when controlling *H. mantegazzianum*, especially if older seedlings are targeted. Using higher PL concentrations in *H. mantegazzianum* control needs to maintain a balance between the highest assumed risks caused by this invasive weed and the potential risks of the control methods.

5. Conclusion

Depending on the application strategy, PLs can be used to control *H. mantegazzianum*. Germination of *C. carvi* (substituting for *H. mantegazzianum*) was effectively restricted by all tested PLs of hardwood origin. The direct spraying of PLs on *H. mantegazzianum* seedlings was an ineffective control method irrespective of dose. However, mulching material

containing PL offered an alternative to control small seedlings of *H. mantegazzianum* when using > 1.5 cm layer (containing >7.5% of PL). Spreading mulch should be done simultaneously with or just after seed germination. Under greenhouse conditions, larger seedlings (< 30 cm) were also successfully controlled using a combination of cutting and mulching. However, further product development is needed as the chemical composition of the liquids from slow pyrolysis can vary significantly and thus their effectiveness as a part of a mulching material may differ. Efficacy of mulching on higher plants should be tested under field conditions and with various PL concentrations and repeated treatments.

CRediT authorship contribution statement

Marleena Hagner: Conceptualization, Methodology, Investigation, Data analysis, Writing - original draft. **Bengt Lindqvist:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Jouko Vepsäläinen:** Investigation, Writing – review & editing. **Chiara Samorì:** Investigation, Resources, Writing – review & editing. **Riikka Keskinen:** Resources, Writing – review & editing. **Kimmo Rasa:** Conceptualization, Writing – review & editing. **Terho Hyvönen:** Supervision, Conceptualization, Methodology, Resources, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Laboratory Engineer Outi Haapala is thanked for the developing and conducting the methanol extraction method for biodegradation studies.

Funding

The study was a part of a research project "Awareness building, surveying and controlling invasive alien species (IAS) in Finland -LIFE+ (LIFE17 NAT/FI/000528)". Finvasive LIFE is financially supported by the European Union in the framework of the European Commission's LIFE Programme. Information and opinions expressed on this [*web-site/publication/whatever*] do not reflect the official opinion of EASME or the European Union. EASME or the European Union are not responsible for any use of the information. Pyrolysis liquids (WPL and APL) were produced under the project "Use of pyrolysis products in securing the fertilizer value of liquid manures" (PYSTI) funded by the Ministry of the Environment (Finland) from the Programme to promote the recycling of nutrients and improve the status of the Archipelago Sea.

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