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Bioherbicidal activity of *Sinapis alba* seed meal extracts

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ABSTRACT

Although seed meal from yellow mustard (*Sinapis alba* L.) is a potential tool for controlling weeds as a consequence of produced phytotoxic products, use is limited by batch-to-batch variability and logistical constraints. Our objective was to develop an efficacious bioherbicide by extracting and identifying the active ingredients in *S. alba* seed meal that demonstrate phytotoxicity to greenhouse-grown Powell amaranth (*Amaranthus powellii*) and green foxtail (*Setaria viridis*). Companion bioassays with separate potential active ingredient solutions containing ionic thiocyanate (SCN^-), 4-(hydroxymethyl)phenol (4-OH), or 2-(4-hydroxyphenyl)acetonitrile (Nitrile) at concentrations approximating those in the extract were performed. When applied pre- (PRE) or postemergence (POST), SCN^- and extracts were the most active solutions on both weed species. The highest rate tested of SCN^- of 2.8 kg ha^{-1} controlled Powell amaranth 98% and green foxtail 84% compared to the highest rate of extract (2.8 kg SCN^- ha^{-1}) that controlled Powell amaranth 97% and green foxtail 82%. POST application of the extract was less effective as compared to SCN^- solutions, with SCN^- showing 97% control of Powell amaranth and 71% control of green foxtail as compared to the extract displaying only 46% control of Powell amaranth and 23% control of green foxtail. Little or no herbicidal activity was observed on both weed species following PRE or POST application of 4-OH or Nitrile. Development of a bioherbicide based on extracting and concentrating SCN^- from *S. alba* seed meal is feasible, especially if scale up activities focus on eliminating the need for alcoholic extractants and yield higher active ingredient products.

1. Introduction

Various research groups have shown that yellow mustard (*Sinapis alba* L.) seed meal is phytotoxic (Boydston et al., 2008; Boydston et al., 2011; Handiseni et al., 2011; Shrestha et al., 2015; Wang et al., 2015; Webber et al., 2017; Yu and Morishita, 2014), leading to the proposed commercial utilization of the seed meal as a bioherbicide (Borek and Morra, 2005). The substitution of synthetic herbicides by natural compounds is essential in organic agriculture and potentially beneficial to human and environmental health. Adoption and large-scale use of mustard seed meal bioherbicides are limited by the bulky nature of the seed meals, variability in active ingredient concentration, and unanticipated impacts on the soil ecosystem as caused by the addition of large amounts of carbon and nitrogen (Popova et al., 2017). Increased efficacy and improved transportation and application logistics for a bioherbicide product can be achieved by extracting and concentrating the active ingredients from *S. alba* seed meal.

Mustard plants contain compounds called glucosinolates that are enzymatically hydrolyzed by myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) to form a variety of biologically active compounds (Brown and Morra, 1997; Rosa et al., 1997). *S. alba* seed meal contains the glucosinolate sinalbin (4-hydroxybenzyl glucosinolate; [(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] 2-(4-hydroxyphenyl)-N-sulfooxyethanimidothioate) that when hydrolyzed produces 4-(isothiocyanatomethyl)phenol, an unstable molecule quickly transformed non-enzymatically to SCN^-, 4-(hydroxymethyl) phenol (4-OH), or 2-(4-hydroxyphenyl)acetonitrile (Nitrile) (Fig. 1) (Borek and Morra, 2005). Concentrations of sinalbin as measured in seed meal produced by cold pressing *S. alba* seed range from 90 to 202 μmol g^{-1} meal with a mean value of 148 μmol g^{-1} (unpublished data), making the seed meal a valuable feedstock for herbicidal active ingredients. Based on a correlation of sinalbin and the observed phytotoxic response, as well as the historic use of SCN^- as a herbicide (Ahlgren et al., 1951; Beekhuis, 1975; Brown and Morra, 1997; Stiehl and Bible, 1989), it has been speculated that SCN^- is the responsible active ingredient (Borek and Morra, 2005); however, conclusive evidence demonstrating what compound or compounds in *S. alba* seed meal are responsible is lacking.

Abbreviations: PRE, preemergence; POST, postemergence; DAT, days after treatment; DAS, days after sowing; 4-OH, 4-(hydroxymethyl)phenol; Nitrile, 2-(4-hydroxyphenyl)acetonitrile

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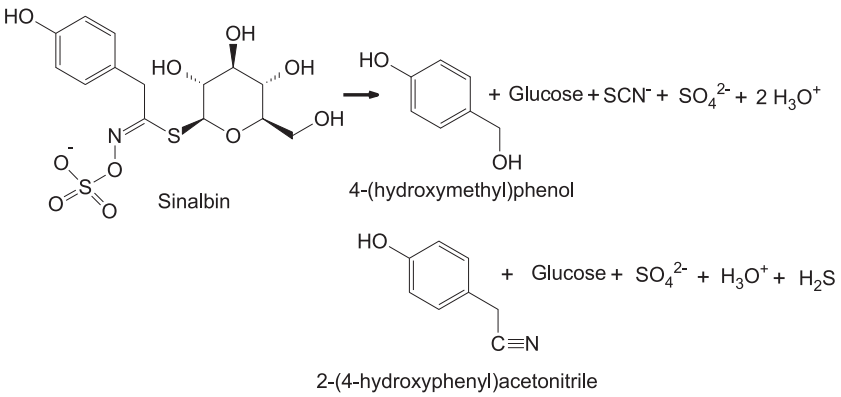

Fig. 1. Pathway for sinalbin hydrolysis to form the three potentially bioactive compounds SCN^-, 4-(hydroxymethyl)phenol, and 2-(4-hydroxyphenyl)acetonitrile.

Our preliminary investigations indicated the possibility that 4-OH and Nitrile may contribute to observed phytotoxicity. For scale up of the extraction procedure and for registration of a commercial product as a biopesticide, it is imperative to definitively identify the phytotoxic active ingredient(s). Our objectives were to 1) extract the active ingredient(s) to produce an efficacious bioherbicide and 2) determine if SCN^- is the primary active ingredient responsible for phytotoxicity. Our eventual goal is to develop protocols for active ingredient extraction from *S. alba* seed meal for the purpose of formulating commercially viable biopesticides.

2. Materials and methods

2.1. Materials

S. alba (IdaGold variety) seed was obtained locally (Latah County, ID, USA). Seed was cold crushed to produce seed meal that contained approximately 15% residual oil (Peterson et al., 1983).

2.2. Chemicals

4-OH, Nitrile, and potassium thiocyanate (SCN^-) standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, water, methanol, and other solvents were of HPLC or LC/MS grade. Solvents and all other chemicals (at least of analytical grade) were purchased from Sigma–Aldrich or ThermoFisher (Pittsburgh, PA, USA).

2.3. Mustard meal extract preparation and analysis

Mustard meals were homogenized and ground to a fine powder. Mustard meal was extracted with 30% (v/v) methanol at a 1:20 v/v ratio using an end-to-end shaker at room temperature for 2 h. Seed debris was separated by filtering, and filtrates were concentrated by rotary evaporation to remove most of the solvent. Concentrated extract was then freeze-dried to obtain a free flowing powder.

For analysis of potentially biologically active compounds, 0.1 g of *S. alba* extract was dissolved in 5 mL of 73% aqueous methanol and agitated on an end-to-end shaker for 1 h. Solutions were centrifuged and the supernatant was analyzed for sinalbin, 4-OH, Nitrile, and SCN^-. For analysis of 4-OH and Nitrile, extracts were diluted 20 times with 73% aqueous methanol. For analysis of SCN^-, extracts were evaporated to dryness under a gentle stream of N_2 and redissolved in water. All analyses were performed immediately prior to the first application, yielding results that accurately reflect extract concentrations at the time of efficacy trials based on preliminary studies indicating that the compounds of interest are stable within the time period of the experiments.

4-OH and Nitrile were analyzed using an Agilent 1200 Series HPLC system equipped with a diode array detection (DAD) system (Agilent, Santa Clara, CA, USA). Separation was performed using a Zorbax SB-Aq (50 mm × 4.6 mm, 3.5 µm) rapid resolution column with a Zorbax SB-Aq (12.5 mm × 4.6 mm, 5 µm) guard column (Agilent, Santa Clara, CA, USA) maintained at 30 °C. The injection volume was 5 µL. The mobile phase consisted of 0.1% formic acid in water (solvent A) and in acetonitrile (solvent B). The gradient program started with isocratic elution at a flow rate of 0.4 mL min^{-1} using 5% B for 3 min followed by a linear gradient to 70% B from 3 to 10 min 4-OH and Nitrile were quantified by monitoring absorbances from 220 to 230 nm. External calibration curves were used for quantification of all the analytes. Limits of detection were 0.11 for 4-OH and 0.21 mM for Nitrile.

SCN^- was analyzed as described previously (Popova and Morra, 2014). Briefly, quantification involved a Dionex Ion Analyzer (Dionex, Sunnyvale, CA, USA) fitted with a Dionex 4 × 210 mm anion-exchange IonPac AS16 column and analyte elution using sodium hydroxide (100 mM) as the mobile phase at a flow rate of 0.9 mL min^{-1}. Detector stabilizer temperature was set at 30 °C with temperature compensation of 1.7% per °C, and anion suppressor current set to 300 mA.

2.4. Weed control efficacy

2.4.1. Preemergence (PRE) assays

Twenty-five Powell amaranth (*Amaranthus powellii*) or 50 green foxtail (*Setaria viridis*) seeds were planted 1- or 2-mm deep, respectively, in 10-cm containers filled with loamy sand soil (pH 7.0, 0.55% organic matter). Soil was maintained at field capacity by watering daily. The following day aqueous solutions of *S. alba* extract, potassium thiocyanate (SCN^-), 4-OH, and Nitrile were applied separately to the surface of pots using a pneumatic bench sprayer equipped with a single flat fan nozzle (Teejiet 80015E, Spraying Systems Co., Wheaton, IL.) calibrated to deliver 234 L ha^{-1}. The three rates of individual compounds tested approximated those applied in the three rates of *S. alba* extract. Extract concentrations as noted in all figures and tables refer to SCN^- present in each respective extract to allow for direct comparison of phytotoxicity with solutions of synthetic SCN^-. The rates were 50, 100, and 200 mM for SCN^- and 4-OH, and 12.5, 25, and 50 mM for Nitrile. Concentrations of the compounds as applied with the described spray system correspond to application rates of: 0.7, 1.4, and 2.8 kg SCN^- ha^{-1}; 23.5, 47.0, and 94.0 kg extract ha^{-1}; 1.45, 2.9, and 5.8 kg 4-OH ha^{-1}; and 0.4, 0.8, and 1.6 kg Nitrile ha^{-1}. A nontreated control that received only water was included.

All solutions were made using distilled water. Due to the relatively high rate of *S. alba* extract required in solution, the 200 mM rate (94 kg ha^{-1}) of extract was achieved by mixing a 100 mM rate (47 kg ha^{-1}) and spraying pots twice. Treatments were replicated five times and each trial was repeated. Pots were placed in a greenhouse with a 14.5-h day length and maintained at 32/19 °C maximum/minimum temperatures. Pots were watered overhead daily by lightly sprinkling using a hose attachment as needed to prevent pots from