

Bioassay-Directed Isolation and Identification of Phytotoxic and Fungitoxic Acetylenes from *Conyza canadensis*

Sonia C. N. Queiroz,[†] Charles L. Cantrell,^{*,‡} Stephen O. Duke,[‡] David E. Wedge,[‡] Vijay K. Nandula,[§] Rita M. Moraes,^{||,⊥} and Antonio L. Cerdeira[†]

[†]EMBRAPA Environment, C.P. 69, Jaguariúna, SP, 13820-000, Brazil

[‡]National Products Utilization Research Unit, United States Department of Agriculture, Agricultural Research Service, University, Mississippi 38677, United States

[§]Crop Production Systems Research Unit, United States Department of Agriculture, Agricultural Research Service, 141 Experiment Station Road, P.O. Box 350, Stoneville, Mississippi 38776, United States

^{||}National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, United States

[⊥]Center for Water and Wetland Resources, The University of Mississippi, 15 County Road 2078, Abbeville, Mississippi 38601, United States

ABSTRACT: *Conyza canadensis* (L.) Cronquist syn. (horseweed) is a problematic and invasive weed with reported allelopathic properties. To identify the phytotoxic constituents of the aerial parts, a systematic bioactivity-guided fractionation of the dichloromethane extract was performed. Three active enyne derivatives, (2*Z*,8*Z*)-matricaria acid methyl ester, (4*Z*,8*Z*)-matricaria lactone, and (4*Z*)-lachnophyllum lactone, were identified. The lactones inhibited growth of the monocot *Agrostis stolonifera* (bentgrass) and the dicot *Lactuca sativa* (lettuce) at 1 mg mL⁻¹, while the (2*Z*,8*Z*)-matricaria acid methyl ester was less active. In a dose–response screening of the lactones for growth inhibitory activity against *Lemna paucicostata*, (4*Z*)-lachnophyllum lactone was the most active with an IC₅₀ of 104 μM, while the (4*Z*,8*Z*)-matricaria lactone was less active (IC₅₀ of 220 μM). In a fungal direct bioautography assay, the two lactones at 10 and 100 μg/spot inhibited growth of the plant pathogenic fungi *Colletotrichum acutatum*, *Colletotrichum fragariae*, and *Colletotrichum gloeosporioides*. In a dose–response screening of the lactones against six different plant pathogenic fungi, (4*Z*,8*Z*)-matricaria lactone was more active than the commercial fungicide azoxystrobin on *Col. acutatum*, *Col. fragariae*, and *Col. gloeosporioides* at 30 μM and about as active as the commercial fungicide captan against *Col. gloeosporioides*, while (4*Z*)-lachnophyllum lactone was less active.

KEYWORDS: horseweed, acetylenes, *Conyza canadensis*, phytotoxic, antifungal, fungicide, herbicide, allelopathy

■ INTRODUCTION

Conyza canadensis (L.) Cronquist, syn. *Erigeron canadensis* L., belonging to the Compositae (or Asteraceae) family, is a plant species indigenous to America, now distributed globally. *Con. canadensis* is a common weed found in more than 40 crops. *Con. canadensis* infests orchards, roadsides, vineyards, field crops such as corn, soybean, and cotton, hay crops, pastures, and rangeland throughout North, Central, and South America.^{1,2} *Con. canadensis*, also known as horseweed, is also a common weed in no-till crop production systems in the United States. It is problematic because of the frequent occurrence of biotypes with evolved resistance to glyphosate, paraquat, and acetolactate synthase (ALS)-inhibiting herbicides and the weed's ability to complete its life cycle as a winter or summer annual weed.^{3–5} This weed has caused economic losses to farmers due to the decrease in quality and quantity of crop production.^{3,6} To combat the herbicide-resistant weed biotypes, there is a need for new herbicides with new modes of action.⁷

Several secondary metabolites produced by certain types of plants can affect the germination and the development of other nearby competing species. The identification of these compounds can lead to the discovery of new, natural herbicides

which can be more environmentally friendly and potentially have new modes of action when compared to the currently used synthetic herbicides.⁸ Allelopathy of *Con. canadensis* has been reported and may be a significant factor in its success as a weed.^{9,10} Allelopathic effects of aqueous extracts from *Con. canadensis* have been examined, resulting in the identification of phytotoxic phenolic compounds gallic acid, vanillic acid, catechol, and syringic acid.¹⁰ These are fairly ubiquitous phytochemicals found in many nonallelopathic plant species. However, no studies on the isolation and identification of the phytotoxic compounds from *Con. canadensis* have been done using a bioassay-directed isolation of active compounds, a process more likely to discover highly bioactive, novel compounds that would not be found by looking for compounds that are simple to find. Furthermore, natural phytotoxins often have novel modes of action. The objective of this study was to identify the phytotoxic compounds of *Con. canadensis* by systematically performing bioassay-directed isolation and

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subsequent identification of the bioactive constituents. The activity of the isolated compounds is also reported against various species of plant pathogenic fungi.

MATERIALS AND METHODS

Instrumentation. ^1H and ^{13}C NMR spectra were recorded in CDCl_3 on a Varian ANOVA 400 MHz spectrometer (Palo Alto, CA). All ^{13}C multiplicities were deduced from 90° and 135° distortionless enhancement by polarization transfer (DEPT) experiments. Column chromatography was performed using a Biotage, Inc. Isolera pump (Charlottesville, VA) equipped with a Horizon flash collector and a dual-wavelength (254 and 280 nm) detector.

Fractions and purified compounds were analyzed by GC–MS on a Varian CP-3800 gas chromatograph coupled to a Varian Saturn 2000 MS/MS system. The gas chromatograph was equipped with a CP Sil 8 CB capillary column (30 m \times 0.25 mm, with a film thickness of 0.25 μm) operated using the following conditions: injector temperature, 240°C ; column temperature, 60 – 240°C at $3^\circ\text{C}/\text{min}$ and then held at 240°C for 5 min; carrier gas, He; injection volume, 1 μL (splitless). The MS mass ranged from 40 to 650 m/z , with a filament delay of 5 min, a target TIC of 30 000, a prescan ionization time of 100 μs , an ion trap temperature of 150°C , a manifold temperature of 60°C , and a transfer line temperature of 170°C .

Raw Material. *Con. canadensis* plant raw material (aerial part) was collected in Stoneville, MS, in May 2011. A voucher specimen number of MISS 79456 for *Con. canadensis* has been deposited at The University of Mississippi Pullen Herbarium.

Plant Extraction. A 251.7 g sample of fresh aerial parts of *Con. canadensis* was air-dried in a fume hood, at room temperature, for a week. After grinding, the material was extracted in a beaker with 500 mL of dichloromethane (DCM), providing 11.38 g of extractables.

Phytotoxicity-Guided Fractionation. Initially, 4.45 g of DCM extractables from the aerial parts was dissolved in $\text{MeOH}/\text{H}_2\text{O}$ (90:10, v/v) and subjected to liquid/liquid partitioning with 150 mL of hexane (3 times), obtaining 2.91 g of hexane extractables. To the remaining partition ($\text{MeOH}/\text{H}_2\text{O}$, 90:10, v/v) was added 57 mL of H_2O to make it 70:30 (v/v) $\text{MeOH}/\text{H}_2\text{O}$. Then it was partitioned with 150 mL of chloroform (3 times), obtaining 1.19 g of CHCl_3 extractables. The remaining MeOH was removed using rotary evaporation, followed by the addition of 100 mL of ethyl acetate, obtaining 51 mg of ethyl acetate extractables. The water extract was rotary evaporated to remove any residual organics, and the water was lyophilized, obtaining 37.5 mg of water extractables. The chloroform extractables were separated on a Biotage XP-Sil, 100 g, SNAP cartridge (40–63 μm , 60 \AA , 40×150 mm) running at 40 mL min^{-1} using a hexane:acetone gradient beginning with 100:0 to 90:10 over 1600 mL followed by 90:10 to 80:20 over 700 mL, then 80:20 to 50:50 over 400 mL and 50:50 over 200 mL, and finishing with 0:100 over 350 mL. Portions of 22 mL volume were collected in 16×150 mm test tubes. Six test tubes were combined and concentrated on the basis of thin-layer chromatography (TLC) similarities, providing 0.2037 g of pure compound 1. Six fractions were collected and recombined on the basis of TLC, providing 59 mg of a mixture of compounds 2 and 3. Three fractions were obtained and provided 0.1351 g of pure compound 2. The fraction containing the mixture of compounds 2 and 3 was further purified using a Biotage XP-Sil, 100 g, SNAP cartridge (40–63 μm , 60 \AA , 40×150 mm) running at 40 mL min^{-1} using a hexane:diethyl ether gradient beginning with 90:10 to 50:50 over 1400 mL followed by 50:50 to 0:100 over 300 mL and finishing with 0:100 over 216 mL. Five fractions were collected and recombined on the basis of TLC similarities, providing 8.7 mg of pure compound 3. Three fractions were combined, providing 12.2 mg of a mixture of compounds 2 and 3. Five fractions were combined, providing 21 mg of pure compound 2.

(2Z,8Z)-Matricaria Acid Methyl Ester (1). The molecular formula is $\text{C}_{11}\text{H}_{10}\text{O}_2$ on the basis of EI-MS analysis (M^+ , m/z 174). ^1H NMR ($\text{DMSO}-d_6$, 400 MHz): δ (ppm) 6.36 (1H, m, H-9), 6.54 (1H, d, $J = 12$ Hz, H-3), 6.40 (1H, d, $J = 12$ Hz, H-2), 5.77 (1H, d, $J = 8$ Hz, H-8), 1.90 (3H, d, $J = 4$ Hz, H-10), 3.69 (3H, s, H-11). ^{13}C NMR (DMSO -

d_6 , 100 MHz): δ (ppm) 164.0 (C-1), 145.0 (C-9), 131.5 (C-3), 122.0 (C-2), 108.5 (C-8), 84.3 (C-5), 83.4 (C-7), 78.7 (C-7), 77.4 (C-4), 51.5 (C-11), 16.5 (C-10). The spectroscopic data agree with the published values.¹¹

(4Z,8Z)-Matricaria Lactone (2). The molecular formula is $\text{C}_{10}\text{H}_8\text{O}_2$ on the basis of EI-MS analysis (M^+ , m/z 160). ^1H NMR (CDCl_3 , 400 MHz): δ (ppm) 7.40 (1H, br d, $J = 4$ Hz, H-3), 6.13 (1H, br d, $J = 4$ Hz, H-2), 6.02 (1H, m, H-9), 5.57 (1H, d, $J = 4$ Hz, H-8), 5.43 (1H, s, H-5), 1.81 (3H, d, $J = 4$ Hz, H-10). ^{13}C NMR (CDCl_3 , 100 MHz): δ (ppm) 168.5 (C-1), 155.6 (C-4), 142.6 (C-3), 141.3 (C-9), 119.8 (C-2), 109.4 (C-8), 98.7 (C-7), 94.1 (C-5), 87.8 (C-6), 16.0 (C-10). The spectroscopic data agree with the published values.¹²

(4Z)-Lachnophyllum Lactone (3). The molecular formula is $\text{C}_{10}\text{H}_{10}\text{O}_2$ on the basis of EI-MS analysis (M^+ , m/z 162). ^1H NMR (400 MHz, CDCl_3): δ (ppm) 0.97 (3H, t, $J = 6$ Hz, H-10), 1.57 (2H, m, H-9), 2.37 (2H, dt, $J = 2$ and 7 Hz, H-8), 5.28 (1H, t, $J = 6$ Hz, H-5), 6.17 (1H, d, $J = 8$ Hz, H-2), 7.35 (1H, d, $J = 8$ Hz, H-3). ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 168.9 (C-1), 156.0 (C-4), 142.7 (C-3), 120.0 (C-2), 104.5 (C-6), 95.0 (C-5), 74.7 (C-7), 22.0 (C-8), 21.8 (C-9), 13.4 (C-10). The spectroscopic data agree with the published values.¹³

Bioassays against *Lactuca sativa* and *Agrostis stolonifera*. Liquid–liquid partitioning and silica gel column chromatographic fractionation of the DCM extract of the aerial parts guided by *La. sativa* (lettuce) and *A. stolonifera* (bentgrass) bioassay data were performed according to Dayan et al.¹⁴ and used to isolate the phytotoxic compounds. A filter paper (Whatman no. 1) and 5 mg of *La. sativa* seeds or 10 mg of *A. stolonifera* seeds were placed in each well of a 24-well multiwell plate (Corning Inc., Corning, NY). Test fractions were dissolved in acetone, CH_2Cl_2 , or water, depending on the solubility of extractables, and mixed with distilled deionized (DDI) H_2O . The final concentration of acetone was 3%. To each test well was added 250 μL of the DDI H_2O mixture. Only solvent and DDI H_2O were added to each control well. The plates were covered, sealed with Parafilm, and incubated at 26°C in a Conviron growth chamber at $173 \text{ mol s}^{-1} \text{ m}^{-2}$ continuous PAR. Phytotoxicity was qualitatively evaluated by visually comparing the amount of germination of the seeds in each well with that of the untreated controls after 7 days. The qualitative estimate of phytotoxicity was evaluated by using a rating scale of 0–5, where 0 = no effect and 5 = no growth or no germination of the seeds. Each experiment was repeated in duplicate.

Bioassays against *Lemna paucicostata*. The method of Michel et al.¹⁵ was used. Briefly, *Le. paucicostata* stocks were grown from a single colony consisting of a mother and two daughter fronds in a beaker on modified Hoagland medium containing 1515 mg/L KNO_3 , 680 mg/L KH_2PO_4 , 492 mg/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 20 mg/L Na_2CO_3 , 1180 mg/L $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.5 mg/L MnCl_2 , 0.025 mg/L CoCl_2 , 0.025 mg/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and 18.355 mg/L Fe-EDTA. The medium was adjusted to pH 5.5 with 1 M NaOH and filtered through a 0.2 μm filter. Each well of nonpyrogenic polystyrene sterile six-well plates (CoStar 3506, Corning) was filled with 4950 μL of the Hoagland medium mixed with 50 μL of DDI water, 50 μL of acetone with the appropriate concentration of test compound, or 50 μL of EtOH with the appropriate concentration of test compound. The final concentration of acetone or EtOH was 1%. Two three-frond colonies from 4–5 day old stock cultures were placed in each well. The total frond area per well was recorded by the image analysis system Scanalyzer (LemnaTec, Würselen, Germany) from day 0 to day 4. The percent increase at days 1–4 was determined relative to the baseline area at day 0.

Statistical Analysis. Data from dose–response and time-course experiments were analyzed using the dose–response curve module¹⁶ of R version 2.2.1 (R-Development-Core-Team, 2009). This software calculates IC_{50} values. Analysis of the means was performed using the version 10 SAS statistical software program (SAS Institute Inc., Cary, NC).

Bioassay against Fungal Plant Pathogenic *Colletotrichum* Spp. Isolates of *Colletotrichum acutatum* Simmonds, *Colletotrichum fragariae* Brooks, and *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. were obtained from Barbara J. Smith, U.S. Department of

Agriculture, Agricultural Research Service, Poplarville, MS. The three *Colletotrichum* species were isolated from anthracnose lesions of strawberry (*Fragaria x ananassa* Duchesne). Fungi were grown on potato dextrose agar (PDA, Difco, Detroit, MI) in 9 cm Petri dishes and incubated in a growth chamber at 24 ± 2 °C under cool-white fluorescent lights (55 ± 5 ($\mu\text{mol}/\text{m}^2$)/s) with a 12 h photoperiod.

Direct Bioautography. Bioautography techniques were used as primary screening systems to detect antifungal activity. Matrix, one-dimensional protocols on silica gel TLC plates along with *Colletotrichum* spp. as the test organisms were used to identify the antifungal activity according to published methods.^{17,18} Matrix bioautography was used to screen the isolated substances at 10 and 100 $\mu\text{g}/\text{spot}$. Each plate was subsequently sprayed with a spore suspension (10^5 spores/mL) of the fungus of interest and incubated in a moisture chamber for 4 days at 26 °C with a 12 h photoperiod. Clear zones of fungal growth inhibition on the TLC plate indicated the presence of antifungal constituents in each extract. Antifungal substances were readily located on the plates by visually observing clear zones where the active compounds inhibited fungal growth.¹⁹

Ninety-Six-Well Microbioassay. A standardized 96-well microtiter plate assay developed for discovery of natural product fungicidal agents was used to evaluate purified compounds. A 96-well microtiter assay was used to determine the sensitivity of *Col. acutatum*, *Col. fragariae*, *Col. gloeosporioides*, *Fusarium oxysporum*, *Botrytis cinerea*, and *Phomopsis viticola* to the antifungal agents in comparison with commercial fungicides. Cultures of *Ph. viticola* and *Phomopsis obscurans* were obtained from Mike Ellis (Ohio State University, Columbus, OH), and *B. cinerea* Pers. and *F. oxysporum* Schlechtend were isolated in our laboratory. *F. oxysporum* identification was confirmed by Wade H. Elmer (Connecticut Agricultural Experiment Station, New Haven, CT), and the identity of *B. cinerea* was confirmed by Kenneth Curry (University of Southern Mississippi, Hattiesburg, MS). The commercial fungicides azoxystrobin and captan (Chem Service, Inc., West Chester, PA) with different modes of action were used as standards in these assays. Each fungal species was challenged in a dose-response format so that the final test compound concentrations of 0.3, 3.0, and 30.0 μM were achieved (in duplicate) in the different columns of the 96-well plate.

Fungal growth was evaluated by measuring the absorbance of each well at 620 nm at 0, 24, 48, and 72 h except for *Ph. viticola*, for which data were recorded until 120 h. Differences in spore germination and mycelial growth in each of the wells in the 96-well plate demonstrate sensitivity to particular concentrations of compound and indicate fungistatic or fungicidal effects.

RESULTS AND DISCUSSION

In a preliminary study, the aerial parts of *Con. canadensis* were sequentially extracted using DCM, methanol, and water, providing three unique extracts which were subjected to phytotoxicity screening. Evaluation against bentgrass and lettuce at 1 mg mL⁻¹ indicated that the DCM extract caused phytotoxicity, while the remaining extracts were inactive.

The DCM extract prepared from the plant collected in Stoneville, MS, caused a phytotoxicity ranking at 1 mg/mL of 4 against both bentgrass and lettuce, where 0 = no effect and 5 = no growth or no germination of the seeds. The essential oil of the aerial part obtained by steam distillation was also tested for phytotoxicity and was found to have a low activity rating of 1 against both bentgrass and lettuce.

Using phytotoxic activity against both bentgrass and lettuce as a guide, bioassay-directed fractionation of the DCM extract was performed. A modified Kupchan²⁰ liquid-liquid partition using the solvents hexane, chloroform, ethyl acetate, and water was performed with the dried DCM extract at 1 mg/mL. The hexane extract caused phytotoxicity rankings of 3 and 3, chloroform 5 and 5, ethyl acetate 5 and 5, and water 3 and 3 against bentgrass and lettuce, respectively. Due to the highest

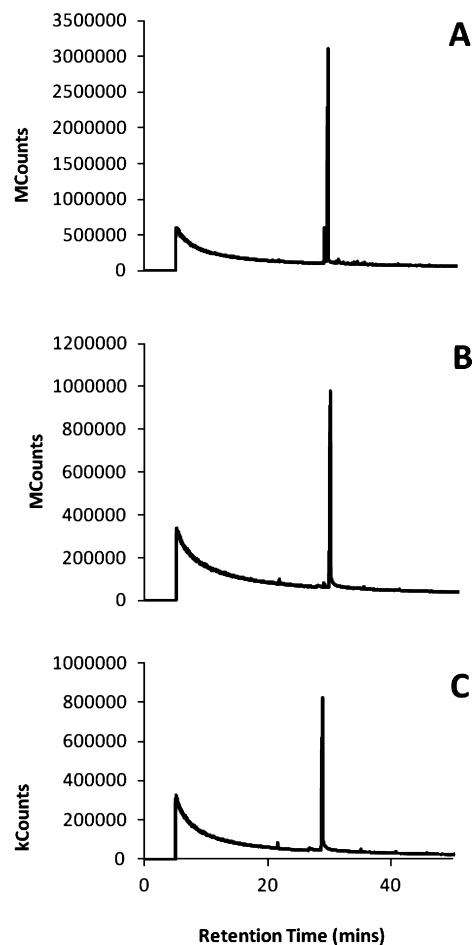


Figure 1. TIC of the isolated compounds (A) (2Z,8Z)-matricaria acid methyl ester, (B) (4Z,8Z)-matricaria lactone, and (C) (4Z)-lachnophyllum lactone.

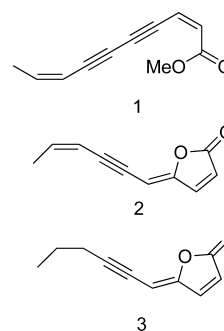


Figure 2. Chemical structures of the (2Z,8Z)-matricaria acid methyl ester (1), (4Z,8Z)-matricaria lactone (2), and (4Z)-lachnophyllum lactone (3).

activity and larger quantities of the chloroform extract, it was further purified using silica column chromatographic fractionation. The silica gel column chromatographic purification of the extract produced pure compounds 1 and 2 and a mixture of compound 2 and another unknown. This mixture was submitted to the silica gel column chromatographic purification, providing pure compounds 2 and 3. By a combination of spectroscopic methods (¹H NMR and ¹³C NMR), GC-MS, and comparison with the literature data, compounds were identified as enyne derivatives (2Z,8Z)-matricaria acid methyl ester (1),¹¹ (4Z,8Z)-matricaria lactone (2),¹² and (4Z)-

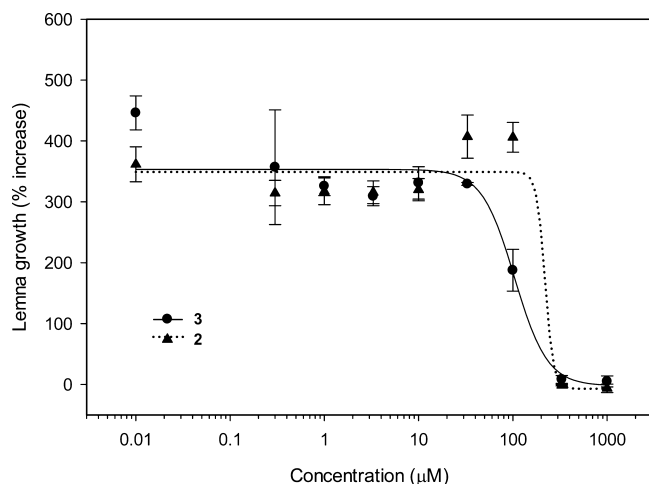


Figure 3. Dose–growth response curve of the effects of the (4Z,8Z)-matricaria lactone (2) and (4Z)-lachnophyllum lactone (3) on *Le. paucicostata* growth 7 days after treatment. The solvent control value is 0.01 µM.

lachnophyllum lactone (3) (Figure 2).¹³ Figure 1 shows the GC–MS total ion chromatogram (TIC) of each of the three compounds 1–3 and provides an indication of the purity for all three compounds.

Compound 1 caused phytotoxicities of 4 and 2, compound 2 phytotoxicities of 5 and 5, and compound 3 phytotoxicities of 5 and 5 at 1 mg mL⁻¹ in bentgrass and lettuce preliminary bioassays, respectively.

In a dose–response screening of the lactones for growth inhibitory activity against *Le. paucicostata*, Figure 3, (4Z)-lachnophyllum lactone (3) was the most active with an IC₅₀ of 104 µM, while the (4Z,8Z)-matricaria lactone (2) was less active (IC₅₀ of 220 µM).

Matricaria esters and related compounds are common constituents in members of the tribe Astereae of the family Asteraceae.^{21,22} (2Z,8Z)-Matricaria acid methyl ester (1) had previously been shown to have strong growth and/or germination inhibitory effects on other plant species, such as the two grasses *Schizachyrium soperium* and *Leptochloa dubia*²³ and the two grasses rice and *Miscanthus sinensis*, as well as the dicot *Artemisia artemisiaefolia*.²⁴ In both cases, the authors linked the activity of these compounds to the apparent allelopathic nature of the producing plants. Polyacetylenes such as 1 are known to be phototoxic, producing reactive oxygen species in the presence of light and molecular oxygen.²⁵

Matricaria esters also have antifungal activity against *Col. acutatum*, *Col. fragariae*, and *Col. gloeosporioides*,²⁶ fungitoxic activity against *Pyricularia oryzae*,²⁷ and antibacterial against *Mycobacterium tuberculosis*.²⁸ (4Z,8Z)-Matricaria lactone (2) and lachnophyllum lactone isolated from *Erigeron apiculatus* (*Conyza* and *Erigeron* are in the Conyzinae subtribe of Asteraceae) showed fungitoxic activity against *Py. oryzae*.²⁷ 2 isolated from the roots of *Con. canadensis* showed antiproliferative activities against three different types of human cancer cell lines and low activity against noncancer cell lines.¹²

Since the hexane extract showed a lower activity level than the chloroform extract, it was analyzed using GC–MS to determine if the previously isolated compounds were present. Compound 1 was the major compound in this extract and is likely responsible for the phytotoxic activity observed. Further fractionation of this extract was conducted using silica gel

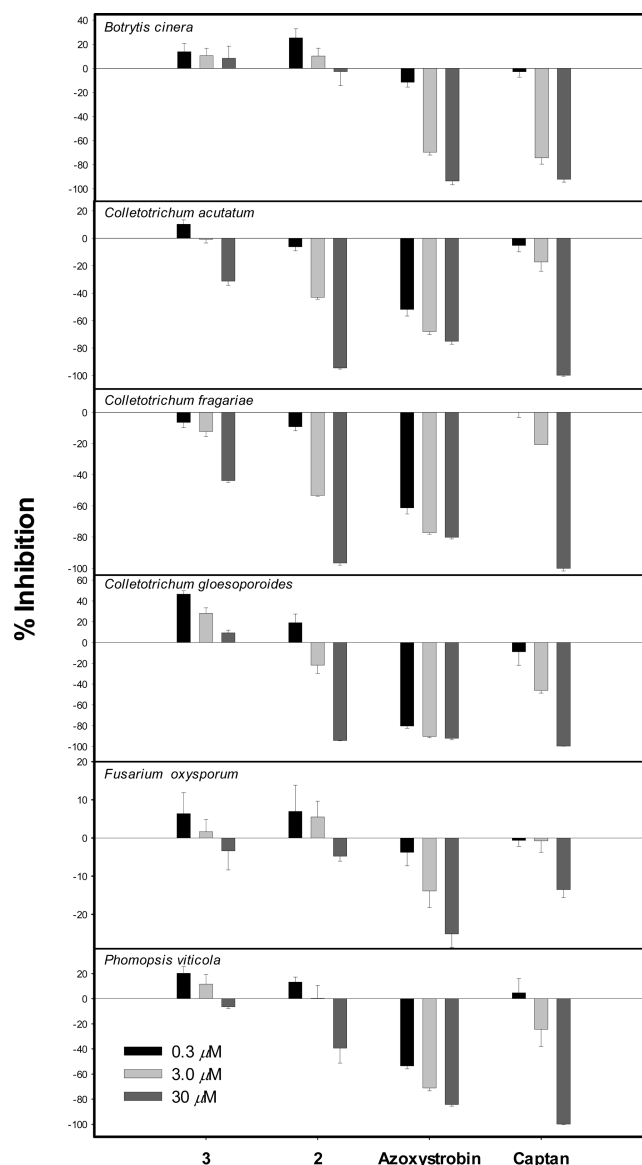


Figure 4. Dose–response screening of the lactones against six different pathogenic fungi: (4Z)-lachnophyllum lactone (3) and (4Z,8Z)-matricaria lactone (2).

chromatography, resulting in no significant activity found in the fractions when tested against bentgrass and lettuce (data not shown). Although the ethyl acetate extract demonstrated activity against bentgrass and lettuce at 1 mg mL⁻¹, it was not submitted to the bioassay-directed fractionation due to the small quantity obtained and a large number of compounds present observed by TLC.

The two lactones (4Z,8Z)-matricaria lactone (2) and (4Z)-lachnophyllum lactone (3) were analyzed in a direct antifungal bioautography assay against the strawberry pathogens *Col. gloeosporioides*, *Col. acutatum*, and *Col. fragariae*. The pathogens *Col. acutatum*, *Col. gloeosporioides*, and *Col. fragariae* can occur singly or in combination and can infect flowers, fruits, leaves, petioles, stolens, and plant crowns.²⁶ In strawberry crops these fungi cause a disease loosely referred to as anthracnose.²⁹ Inhibition zones at 10 µg/spot for compound 2 were 0.98 ± 0.02, 0.70 ± 0.00, and 2.65 ± 0.25 mm in diameter against *Col. gloeosporioides*, *Col. acutatum*, and *Col. fragariae*, respectively. Inhibition zones for 3 were 0.58 ± 0.03, 0.75 ± 0.05, and 0.30

± 0.00 mm in diameter at 10 µg/spot against *Col. gloeosporioides*, *Col. acutatum*, and *Col. fragariae*, respectively.

As an extension to the antifungal bioautography testing above, both compounds were evaluated in a dose–response screening against six different plant pathogenic fungi, *B. cinerea*, *Col. acutatum*, *Col. fragariae*, *Col. gloeosporioides*, *F. oxysporium*, and *Ph. viticola*. (4Z,8Z)-Matricaria lactone (**2**) was active against the three species of *Colletotrichum*, while (4Z)-lachnophyllum lactone (**3**) was much less active (Figure 4). Also against the three *Colletotrichum* spp., **2** was as active as the commercially available fungicides azoxystrobin and captan at 30 µM.

In summary, we have examined *Con. canadensis* for compounds active against plants and fungal plant pathogens. The three isolated acetylenes may be involved in the reported allelopathy of *Con. canadensis*. Since these compounds are found in other plants from the Asteraceae family, they may play a role in allelopathic properties of different species. Allelopathy can be a problem or a weed management tool in sustainable agriculture.^{30,31} Plants produce a vast array of secondary metabolites. Some of these compounds have the potential to be used directly as natural pesticides in agriculture or as structural leads for new synthetic pesticides with the advantages of reduced environmental persistence and accumulation, great target selectivity, and good activity.⁸ The most promising compound as a potential herbicide from our study was (4Z)-lachnophyllum lactone (**3**). (4Z,8Z)-Matricaria lactone (**2**) has promise as a fungicide, since the activity against the three species of *Colletotrichum* was comparable to that of the commercial fungicides azoxystrobin and captan.

AUTHOR INFORMATION

Corresponding Author

*Phone: (662) 915-5898. Fax: (662) 915-1035. E-mail: charles.cantrell@ars.usda.gov.

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Notes

The authors declare no competing financial interest.

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