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Article

Hydroponic production of fireweed for biomass and phytochemicals

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Abstract

Fireweed (*Chamerion angustifolium* (L.) Holub.) is utilized worldwide in traditional medicine, due to its high levels of phenolic compounds that possess bioactive properties with a wide range of therapeutic effects on human health. However, there is little work on the direct production of fireweed for these beneficial phytochemicals. To examine the effects of nutrient availability on fireweed biomass production and metabolomic profile, fireweed plants were grown in a hydroponic greenhouse system with varying concentrations of Hoagland's nutrient solution. As the concentration of the nutrient solution increased, shoot dry mass increased, and root:shoot ratio decreased. Variation in untargeted metabolomic profiles were detected in leaf, stem, and root tissues of hydroponically produced fireweed in response to nutrient level. Several metabolic features were identified, most notably the therapeutic compounds oenothein B and miquelianin. Relative abundances of oenothein B and miquelianin were largely unaffected by nutrient treatments, while several other phytochemicals increased in abundance as nutrient content decreased. This work demonstrates the potentiality of hydroponically cultivated fireweed to supply raw material for phytochemical demand, but the effects of mineral nutrient amendment must first be reconciled with biomass production.

Key words: fireweed, untargeted metabolomics, hydroponics, phytochemistry, oenothein B

Résumé

Partout dans le monde, on utilise l'épilobe à feuilles étroites [*Chamerion angustifolium* (L.) Holub.] comme plante médicinale en raison de sa teneur élevée en composés phénoliques, dont les propriétés bioactives ont de nombreux effets thérapeutiques sur la santé humaine. Malheureusement, peu de recherches portent sur la culture de cette plante expressément pour la récolte de ces substances phytochimiques bienfaisantes. Les auteurs ont cultivé l'épilobe en serre, dans des conditions hydroponiques, avec une solution nutritive Hoagland de concentration variable, pour déterminer les effets de la quantité d'éléments nutritifs disponibles sur la biomasse végétale. La biomasse sèche des pousses augmente avec la concentration d'éléments nutritifs, mais le rapport pousses:racines diminue. Les auteurs ont noté une variation des profils métabolomiques secondaires dans les feuilles, la tige et les racines de l'épilobe cultivé de façon hydroponique, selon la concentration d'éléments nutritifs. Ils ont aussi identifié plusieurs particularités métaboliques, principalement la présence d'oenothéine B et de miquélianine, deux substances thérapeutiques. L'abondance relative d'oenothéine B et de miquélianine est peu touchée par la variation de la teneur en éléments nutritifs, mais la concentration d'autres substances phytochimiques augmente avec l'appauvrissement du régime nutritif. Ces travaux montrent qu'on pourrait cultiver l'épilobe dans des conditions hydroponiques pour fournir la matière première destinée à la production de principes phytochimiques, mais on devra d'abord réconcilier les effets de l'amendement minéral avec la production de biomasse. [Traduit par la Rédaction]

Mots-clés : épilobe à feuilles étroites, métabolomique secondaire, hydroponique, phytochimie, oenothéine B

Introduction

Fireweed (*Chamerion angustifolium* (L.) Holub.) is a perennial herbaceous plant native to the Northern Hemisphere used in traditional North American, European, and Asian societies as a medicine and food source (Rogers 2014). Historic and current use of fireweed is due to the presence of phytochemicals shown to be beneficial for human health (Baert et al. 2015; Schepetkin et al. 2016). Extracts of fireweed possess a wide range of bioactivity including anti-inflammatory, antioxidant, antimicrobial, antiproliferative, and immunomodulatory properties (Juan et al. 1988; Vitalone et al. 2001; Borchardt et al. 2008; Schepetkin et al. 2009; Kiss et al. 2011; Onar et al. 2012; Kosalec et al. 2013). This bioactivity is attributed to the presence and prevalence of polyphenolic com-

pounds, most notably flavonoids, phenolic acids, and ellagitannins (Schepetkin et al. 2016; Agnieszka et al. 2018). Abundance of various polyphenolics within fireweed and tissue-specificity of these compounds have been previously described (Shikov et al. 2010; Granica et al. 2012; Baert et al. 2015, 2017). Oenothein B is the most abundant compound overall and is found in higher concentrations in flowers than in leaves. Oenothein B as well as the flavonoid miquelianin (quercetin-3-glucuronide) have been suggested as a basis for commercialization of fireweed extracts due to evidence of their bioactive properties and their abundance in fireweed tissue (Schepetkin et al. 2016; Baert al. 2017), however, there is little information available on the production of fireweed specifically for its bioactive compounds.

Field cultivation of medicinal plant species can lead to large variation in beneficial compounds due to interactive environmental factors such as temperature and soil fertility and the presence of pests (Canter et al. 2005; Macias et al. 2007; Figueiredo et al. 2008). Hydroponic production, systems in which nutrients are delivered to the plant via liquid solutions in lieu of soil, of medicinal plants is an increasingly attractive solution to quantity and quality issues in the production of marketable phytochemicals. Medicinal plants have been successfully grown in hydroponic culture for harvest of aboveground tissue (Kiferle et al. 2011; Prasad et al. 2012; Kaul et al. 2017; Surendran et al. 2017), root and rhizome tissue (Akiyama et al. 2017; Lu et al. 2018), and multiple tissues simultaneously (Kim et al. 2010; Pedneault et al. 2014). In situ production of phytochemicals is strongly influenced by temperature, light and nutrient availability, pH, and interaction with soil microorganisms and herbivores (Canter et al. 2005), and these factors can be difficult to manage in agricultural fields. Hydroponic systems allow for greater control of environmental conditions that influence phytochemical production and therefore provide opportunities for improving the bioactivity and consistency of phytochemical end products (Hayden 2006) .

Nutrient amendment in a hydroponic system has potential to alter the phytochemical profile and potentially enhance polyphenol content of fireweed. Additionally, higher nutrient availability, especially N and P, increases biomass production of fireweed which would be beneficial in a production system (Bennett et al. 2004; Pinno et al. 2013; Bales and Hirsch-Green 2019). The effects of nutrient manipulation on polyphenol content have been examined in other medicinal plants species. Higher levels of rosmarinic and caffeic acid were found in basil at low N treatments (Nguyen and Niemeyer 2008). Flavonoid yield of *Scutellaria lateriflora* L. increased in response to P (Shiwakoti et al. 2016), although total flavonoids decreased with increasing N fertilization in the medicinal plants *Chrysanthemum morifolium* Ramat. and *Labisia pumila* Benth (Liu et al. 2010; Ibrahim et al. 2011). There are no reports of the effects of nutrient addition on ellagitannin content of medicinal plant species, but N fertilization does not affect levels of hydrolysable tannins in woody plant species (Haukioja et al. 1998). The existing literature provides strong support for the presence of desirable phytochemicals in fireweed, but little on production practices that may enhance the yield of important bioactive compounds in fireweed. This work is the first examination of the effects of nutrient manipulation on the phytochemical content of fireweed utilizing an untargeted metabolomics approach, which allows for a comprehensive analysis and relative quantification of phytochemicals. The objective of this work was to examine the effects of varying mineral nutrient solution concentration on fireweed biomass and phytochemical production in a greenhouse hydroponic system.

Materials and methods

Preparation of hydroponic solutions and experimental design

Nutrient treatments consisted of six strengths of Hoagland's solutions (Hoagland and Arnon 1950): full-strength, half-strength, quarter-strength, eighth-strength, and sixteenth-strength. Stock solutions were first prepared (Table S1) and then combined for the final nutrient solutions (Table S2). Each experimental unit consisted of a 1 L amber Nalgene HDPE Plastic Wide Mouth Leakproof Bottle (Berlin Packaging, Chicago, IL, USA) filled with nutrient solution. Hydroponic bottles were organized in a randomized complete block design across the greenhouse bench and replicated eight times. Each experimental unit contained a single plant.

Preparation of plant materials and greenhouse growth conditions

Fireweed seeds collected from northwestern Wisconsin were purchased from Prairie Moon Nursery in Winona, MN. Seeds were germinated and grown for 3 weeks on the moistened surface of general potting soil. Seedlings were then transferred to 1.5 inch × 1.5 inch × 2.25 inch plug trays and grown for an additional 6 weeks. Seedlings with a 2 cm rosette diameter and maximum root length of 5 cm were carefully extracted from the soil using forceps and soil was rinsed away from the roots using distilled water. Seedlings were transplanted into hydroponic net pots (Growneer) filled with sterilized perlite and placed in nutrient solutions (Fig. S1). Nutrient solutions were maintained at constant levels below the net pot and completely renewed weekly. Fireweed plants were established in an east–west-oriented greenhouse at the University of Minnesota Plant Growth Facilities in St. Paul, MN (44°59′17.4″N, −93°10′51.4″W). Minimal temperature set points controlling air heating were 15/18 °C night/day, and maximal temperature set points were 18/20 °C night/day. Vent opening temperatures were 20/25 °C night/day. Plants were grown under hydroponic conditions in the greenhouse from June to August 2017. The greenhouse environmental conditions were 16 h photoperiod (0600–2200), 400 W high-pressure sodium high-intensity discharge lamps (Gavita, Vancouver, WA; GE Lighting, Cleveland, OH) at a minimum of 150 μmol m^{-2} s^{-1}.

Biomass and metabolomics harvest

Fireweed plants were generally harvested at flowering throughout the experiment, as the flowering period is reportedly the richest source of bioactive compounds (Agnieszka et al. 2018). The experiment was terminated at 18 weeks and

all remaining plants were harvested when no further plants showed signs of flowering (i.e., development of buds). At harvest, individual plants in net pots were removed from their hydroponic bottles, perlite was rinsed away, and roots were teased out of the net pot. Five tissue types were then separated: anther, flower, leaf, stem, and root. Tissue-specific harvest details are described in Table 1. Approximately 200 mg of fresh biomass of each tissue type were harvested, placed in a 1.5 mL microcentrifuge tube, and immediately placed on dry ice. All samples were stored at −80 °C in the dark pending extractions. Following tissue harvest for metabolomics, the remaining aerial and root tissues were separated and placed in paper envelopes, dried in forced-air ovens for 72 h at 60 °C, and weighed.

Phytochemical extraction and analyses

Extractions of plant samples were completed at the Plant Metabolomics Laboratory, University of Minnesota using standard extraction procedures (Martin et al. 2014). Harvested samples in microcentrifuge tubes were removed from the freezer, weighed, and a 2.5 mL tungsten bead was added to each sample along with 70% isopropanol at a rate of 1 mL per 200 mg of frozen sample. Isopropanol and ultra-pure water were purchased from Sigma–Aldrich (St. Louis, MO, USA). Samples were ground and homogenized using a SPEX SamplePrep model 2010 Geno/Grinder® for 5 min at 1500 r/min. Microcentrifuge tubes containing homogenized sample tissues were centrifuged for 4 min at 14 000 r/min. Following centrifugation, the supernatant (extract) was removed from the original tube and transferred to a sterile tube. Extractions were carried out at room temperature (approximately 25 °C). Extracts were stored at −80 °C in the dark prior to liquid chromatography–mass spectrometry analysis.

An untargeted metabolomics approach was utilized in this experiment, involving simultaneous measurement and relative quantification of all known and unknown metabolites. Relative abundance, wherein within each sample all detected ions are scaled to the most abundant ion, was utilized to quantify phytochemical content. Metabolomic profiles were obtained using C_{18} reversed-phase ultra-performance liquid chromatography–electrospray ionization–hybrid quadrupole–orbitrap mass spectrometer (Ultimate® 3000 HPLC, Q Exactive™, Thermo Fisher Scientific, Waltham, MA, USA) with an autosampler and with a sample vial block maintained at 4 °C. Chromatographic separations were carried out on an Acquity reversed-phase C_{18} HSS T_3 1.8 μm particle size, 2.1 mm × 100 mm column (Waters, Milford, MA, USA) with column temperature 40 °C, flow rate 0.40 mL/min, and 0.5 μL injected. A 19.5 min gradient using mobile phases A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile was run according to the following gradient elution profile: initial 2% B, 2 min 2% B, 0.5 min 4% B, 1 min 20% B, 4 min 29% B, 1 min 55% B, 9 min 98% B, 1 min 2% B, 1 min 2% B. The MS conditions used were full scan mass range 115–1000 *m/z*, resolution 75 000, desolvation temperature 350 °C, spray voltage 3800 V, auxiliary gas flow rate 20, sheath gas flow rate 50, sweep gas flow rate 1, S-Lens RF level 50, and auxiliary gas heater temperature 300 °C. Xcalibur™ software version 2.1 (Thermo Fisher Scientific, Waltham, MA, USA) was used for data collection and chromatogram visualization. Sample analysis order was randomized across the entire sample set.

Biomass yield statistical analyses

Biomass data were analyzed using the R programming language (R Core Team 2018). Nonlinear regression was used to determine the relationship between shoot biomass and nutrient solution strength, and root:shoot ratio and nutrient solution strength. Growth curve parameters for shoot biomass production in relation to nutrient solution strength were estimated with a logistic growth function (SSlogis). The relationship of the root:shoot ratio in relation to nutrient solution strength was estimated using exponential decay and the SSasymp function.

Metabolomics data processing and statistical analyses

The ProteoWizard tool msconvert (Chambers et al. 2012) was used to convert raw format files to the open mzML format. Due to excessive noise overwhelming the signal, data from the first 4 min and the last 9 min were eliminated from analyses. Thermo Scientific Xcalibur software was used for centroid fitting. All mzML files were compressed into ZIP format using the 7-Zip software prior to uploading to Galaxy. The Galaxy platform Workflow4metabolomics (W4m) was used to preprocess, annotate, and perform statistical analyses of the metabolomic data collected from the Q Exactive™. Principal components analyses (PCA) using the W4m Multivariate tool (Guitton et al. 2017) were conducted to determine differences in metabolomic fingerprints of the different tissues. To identify features that changed over the nutrient treatments, the W4m Univariate tool (Guitton et al. 2017) with Benjamini–Yekutieli-corrected significance p value less than 0.01 was used. Features were provisionally identified using elemental composition assignment to accurate mass measurements, and by comparing UV absorption spectra and MS/MS fragmentation patterns with those from previously published data sets.

Results

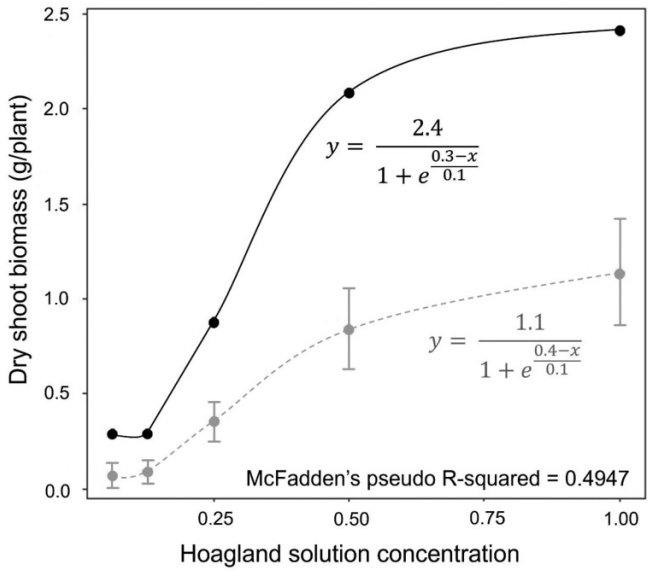
Biomass production

Strength of the Hoagland's nutrient solution influenced dry shoot biomass yield and root:shoot ratio of hydroponically cultivated fireweed. A nonlinear logistic growth model $y = \frac{asym}{1+e^{\frac{xmid-x}{scal}}}$ was used to estimate the relationship between nutrient solution strength and shoot biomass production for the mean and maximum values. Mean shoot biomass production increased as the nutrient solution strength increased, and there was greater variation in the data at higher nutrient levels (Fig. 1). Fireweed biomass production was maximized at a Hoagland's nutrient solution strength near the full-strength (approximately 0.9-strength). Due to the large variation in the data at higher nutrient treatments, a second model was developed to examine the maximum biomass production, with data points based on the largest measure-

Table 1. Tissue-specific details on the harvest of tissues from hydroponic fireweed.

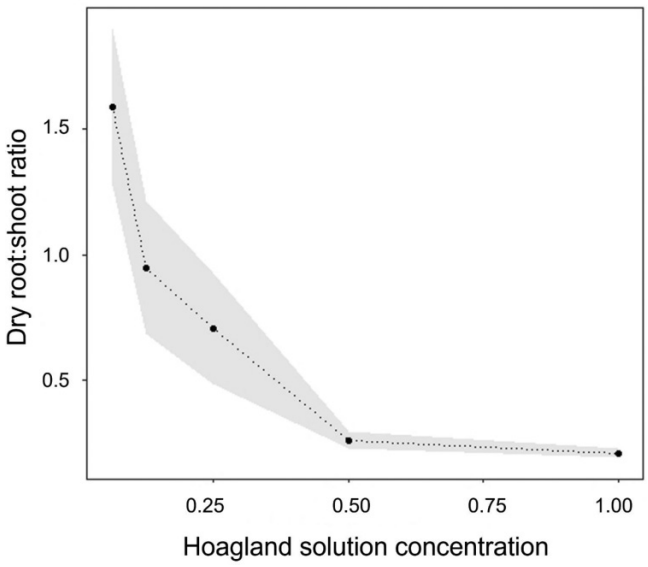
Tissue type	Harvest procedure
Anther	Anthers producing pollen were cut away from their filaments from two flowers (for a total of 16 anthers) using sterile scissors
Flower	Two flowers, not yet producing pollen or possessing a receptive stigma, were cut from the plant at the base of the ovary using sterile scissors
Leaf	The seventh through twelvth leaves, from top to bottom, were cut from the stem using a sterile razor blade. A sterile 8 mm diameter leaf punch was used to sample from directly the middle of the leaves and through the midvein, for a total of six leaf discs
Stem	Using a sterile razor blade, stem pieces 4 mm in length were cut from directly below where the leaves were sampled, for a total of six stem pieces
Root	Using sterile scissors, a 1 cm cross-section of roots 3 cm from the base of stem was cut after roots were thoroughly rinsed to remove all perlite

Fig. 1. Logistic growth curves for maximum (solid black line) and mean (dashed gray line) dry biomass production of hydroponic fireweed under nutrient treatments, with equations for the determined nonlinear logistic growth models. Standard error bars are shown for the mean biomass production.



ments from each nutrient treatment. Growth curve parameters were estimated for both models using the SSlogis function, where *asym* is the asymptote, *xmid* is the *x*-value at the inflection point, and *scal* is the angular coefficient of the tangent line at the point of inflection. All parameters were significant at the 0.05 level. For the mean shoot biomass values, *xmid* was estimated to be 0.4, *scal* was 0.1, and *asym* was 1.1. For the maximum shoot biomass values, *xmid* was estimated to be 0.3, *scal* was 0.1, and *asym* was 2.4. The estimated asym value of 2.4 suggests that there is potential of fireweed to produce more than twice as much shoot biomass than the value reported for the mean. The mean and maximum models determined similar values for both the *xmid* and *scal* parameters, indicating that the optimal Hoagland solution strength for biomass production is similar to the full-strength solution.

Fig. 2. Dry root:shoot ratio of hydroponically cultivated fireweed in response to strength of Hoagland solution. Shaded cone around mean values indicates standard error.

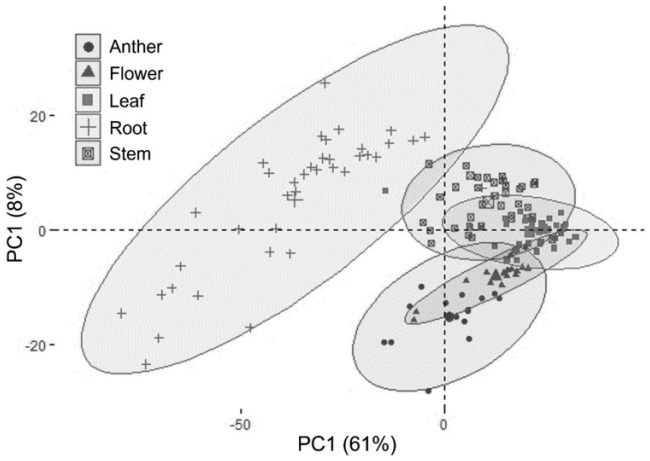


Root biomass increased from eighth-strength to full-strength, although it initially decreased from sixteenth-strength (Fig. S2). There was no statistical difference between the half- and full-strength treatments. As nutrient solution strength increased, the root:shoot ratio and variation around the mean decreased (Fig. 2). A nonlinear exponential decay model $y(x) = y_f + (y_0 - y_f)e^{-\exp(\log \propto)x}$ best explained the relationship between root:shoot and nutrient solution strength. Model parameters were estimated using the SSasymp function, in which the measured *y*-value starts at y_0 and decays towards y_f at a rate of α. Residuals were evenly distributed and all parameters were significant at the 0.001 level. y_0 was estimated to be 2.2, y_f was 0.2, and α was 1.9. The root:shoot ratio of fireweed in a basic hydroponic system plateaus near a solution concentration of 0.5.

Metabolomic profiles of different tissues

To investigate metabolite differences among tissues, samples of five tissue types were harvested separately from all nu-

Fig. 3. PCA score plot for five tissues harvested from hydroponically cultivated fireweed. Data were log (base 2)-transformed, centered, and pareto-scaled. Ovals correspond to 95% confidence region for each tissue type.



trient treatments for LC–MS analyses. Subsequent data processing with Galaxy-M detected 1129 features from positive- and negative-ionization mode together. Unsupervised multivariate statistical analyses by PCA were performed to visualize the changes in features among tissues (Fig. 3). The first principal components (PC1) explained 61% of the total variability, whereas the second principal components (PC2) accounted for 8%. PCA plots showed a separation of root tissue from the other tissues mainly along PC1. The metabolomic profiles of anther and flower tissues appear similar and are separated from leaf and stem tissue along PC2.

Presence of bioactive natural products

Seventeen compounds of commercial interest (Table 2), due to medicinal and therapeutic effects, were identified from hydroponic fireweed samples through literature and MS/MS library searches, as well as molecular ion accurate mass measurements, and UV absorbance data (Table S3). Oenothein B and quercetin-3-glucuronide (miquelianin), compounds of commercial importance, were detected in all tissues across nutrient treatments. Leaf and stem tissue had, on average, the greatest relative intensities of oenothein B (Fig. 4*a*). Miquelianin was a major component of flower, leaf, and stem tissue (Fig. 4*b*).

Nutrient solution strength treatment effects

Mineral nutrient solution concentration had no effect on the amounts of compounds identified in flower or anther tissues. Oenothein B and quercetin-3-glucuronide did not vary in response to nutrient solution strength in any tissue. Additional unidentified features varied significantly over the nutrient treatments in three tissue types: 136 in leaf tissue, 11 in root tissue, and 192 in stem tissue. Of these features, 103 were shared in common between stem and leaf, 1 between root and stem, and 1 shared among all three tissues (Table S4). Overall, only 8% of the features determined to vary significantly in response to increasing nutrient solution concentration increased, and the majority of these features were in the root tissue. When responses to nutrient solution concentration were significant for a specific feature in more than one tissue, it showed the same trend in those tissues. For example, compounds 12 and 16 (provisionally identified as caftaric acid and tellimagrandin II) decreased in intensity in leaf and stem tissue as the strength of the nutrient solution increased (Fig. S3).

Comparison of tissue differences within a nutrient strength treatment

A heat map was used to visualize metabolite differences across the five tissue types across five of the nutrient strength treatments (Fig. 5). The relative abundances of ions detected in each sample ($\log_2$ of the intensity) were averaged across replicates for each tissue and nutrient treatment. Oenothein B was one of the most consistently abundant compounds in all the tissues. Kaempferol and kaempferol 3-rhamnoside were most abundant in anther and flower tissues, while phenolic acids (specifically chlorogenic acid and caftaric acid) were more abundant in leaf and stem tissues. Root tissue had low abundances of most identified chemicals.

Discussion

Effect of nutrient solution strength on biomass production and implications for phytochemical production

It is well established that nutrient availability affects biomass yield of plants in soil and hydroponic systems (Poorter and Nagel 2000; Stewart and Lovett-Doust 2003). In this study, the strength of Hoagland's nutrient solution, and consequently nutrient availability, increased fireweed shoot biomass yield. Modeling of the yield response to overall nutrient strength determined that a near full-strength Hoagland's solution (0.9-strength) is optimal for maximum biomass yield of hydroponic fireweed, confirming the effectiveness of a general Hoagland's solution for hydroponic systems. However, previous work on fireweed yield suggests that covarying levels of specific nutrients may lead to greater yield responses. Fireweed has a strong affinity for assimilating N fertilizer in soil and hydroponic systems (Hangs et al. 2002, 2003), although N addition alone does not affect biomass production (Bennett et al. 2004). Maximum biomass accumulation in fireweed results from fertilizer with added N and P, or N, P and K, and it has been suggested that fertilization with P and K helps to stimulate the uptake of N in fireweed, therefore decreasing the need for excess N fertilization (Pinno et al. 2013). Although nutrient addition can benefit fireweed biomass production, previous research has also shown that N fertilization specifically may limit the production of certain polyphenolic compounds of interest in other medicinal plants (Nguyen and Niemeyer 2008; Kováčik and Klejdus 2014; Guillén-Román et al. 2018). Further work is needed to determine appropriate levels of multiple nutrients in the hydroponic solution to optimize biomass as well as phytochemical production.

Table 2. Provisionally identified features in hydroponically cultivated fireweed.

	Feature	Feature assignment	Observed ion	Molecular formula	Monoisotopic mass	Retention time (sec)	Observed *m/z*	Error (ppm)	Reference(s) for identification
Flavonoids									
	1	Epicatechin or catechin	M–H	$C_{15}H_{14}O_6$	290.0790	298	289.0718	0	Chen et al. 2001; Bark et al. 2011
	2	Kaempferol 3-arabinoside*	M + Na	$C_{20}H_{18}O_{10}$	418.0900	441	441.0790	−0.5	Cai et al. 2005; Schepetkin et al. 2016
	3	Kaempferol 3-rhamnoside	M–H	$C_{21}H_{20}O_{10}$	432.1056	457	431.0983	1.2	Cai et al. 2005; Schepetkin et al. 2016; Baert et al. 2017
	4	Quercetin 3-rhamnoside	M–H	$C_{21}H_{20}O_{11}$	448.1006	401	447.0934	−0.2	Regos et al. 2009; Schepetkin et al. 2016
	5	Kaempferol 3-glucuronide	M–H	$C_{21}H_{18}O_{12}$	462.0798	401	461.0706	−4.3	Schepetkin et al. 2016; Baert et al. 2017
	6	Hyperoside or isoquercetin	M–H	$C_{21}H_{20}O_{12}$	464.0955	366	463.0866	−2.4	Sukito and Tachibana 2014; Schepetkin et al. 2016
	7	Quercetin 3-glucuronide	M–H	$C_{21}H_{18}O_{13}$	478.0747	364	477.0652	−4.8	Vasco et al. 2009; Baert et al. 2017; Agnieszka et al. 2018
	8	Naringenin	M + H	$C_{15}H_{12}O_5$	272.0685	415	273.0755	−0.7	Schepetkin et al. 2016; Vallverdu-Queralt et al. 2010
	9	Kaempferol	M + H	$C_{15}H_{10}O_6$	286.0477	457	287.0548	−0.7	Szostek et al. 2003; Schepetkin et al. 2016
	10	Quercetin	M + H	$C_{15}H_{10}O_7$	302.0427	344	303.0498	−0.3	Chen et al. 2001; Cai et al. 2005; Schepetkin et al. 2016
Phenolic acids									
	11	Chlorogenic acid	M–H	$C_{16}H_{18}O_9$	354.0951	277	353.0878	0	Vasco et al. 2009; Baert et al. 2017
	12	Caftaric acid*	M + H–$2H_2O$	$C_{13}H_{12}O_9$	312.0481	319	277.0341	−4.7	Lee and Scagel 2009
Ellagitannins									
	13	Ellagic acid	M–H	$C_{14}H_6O_8$	302.0063	361	300.9989	−0.3	Szostek et al. 2003; Schepetkin et al. 2016
	14	Oenothein B	M–2 H	$C_{68}H_{50}O_{44}$	1570.1670	284	783.0689	1.8	Granica et al. 2012; Boulekbache-Makhlou et al. 2013; Baert et al. 2015; Agnieszka et al. 2018
	15	Tellimagrandin II (−)	M–H	$C_{41}H_{30}O_{26}$	938.1025	332	937.0922	−3.3	Boulekbache-Makhlou et al. 2013; Yoshida et al. 2018
	16	Tellimagrandin II (+)*	M + Na	$C_{41}H_{30}O_{26}$	938.1025	331	961.0911	−0.6	Boulekbache-Makhlou et al. 2013; Yoshida et al. 2018
Other									
	17	Coumarin*	M + ACN + H	$C_9H_6O_2$	146.0368	279	188.0705	0.5	Schepetkin et al. 2016; Sayik et al. 2017

Note: Feature assignments are based upon multiple criteria, which are summarized in the supplementary materials.
*Upon inspection of the raw data, [M + H] + ion for the feature was identified in addition to the adduct reported here.

Fig. 4. Boxplots showing the distribution of oenothein B (*a*) and quercetin-3-glucuronide (*b*) in five fireweed tissues (means displayed include all nutrient treatments other than no nitrogen treatment).

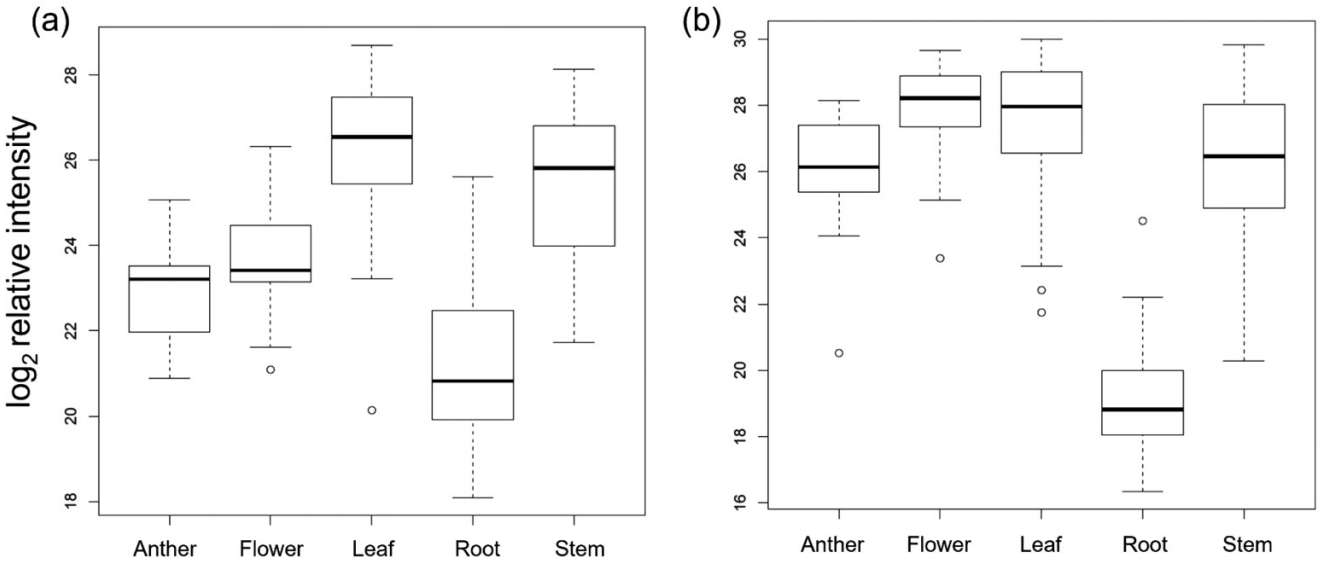
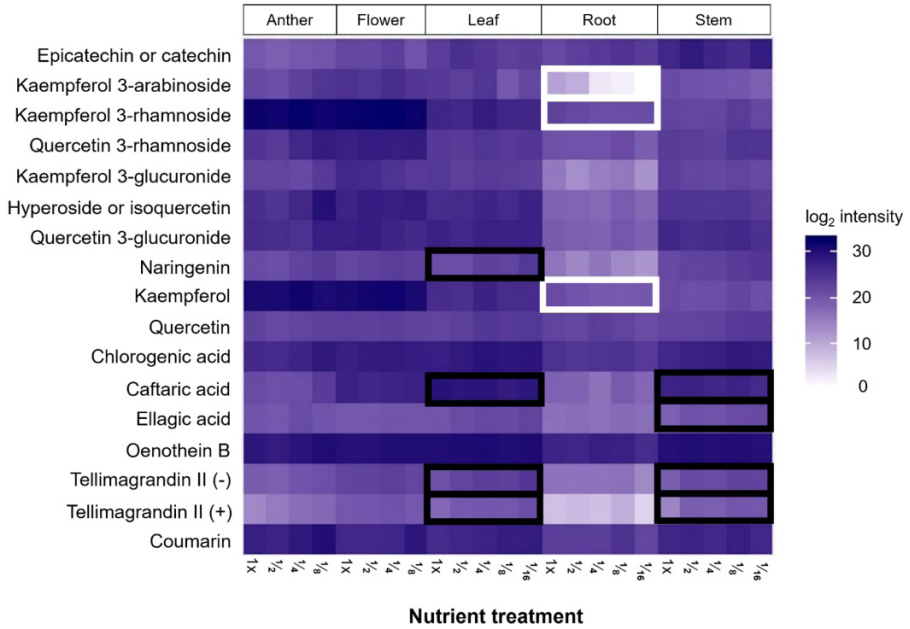


Fig. 5. Heat map of the mean $\log_2$-transformed intensity values of anther, flower, leaf, root, and stem tissue. There were no sixteenth-strength anther or flower samples. Darker shaded tiles indicate a high abundance of the metabolite and white indicates low abundance. White boxes indicate a significant decrease in abundance from high strength to low strength. Black boxes indicate a significant increase in abundance.



Many biotic and abiotic factors can affect the root:shoot ratio, although the most widely addressed factor is nutrient availability (Mokany et al. 2006). Although variation in shoot biomass accumulation among individual fireweed plants increased as nutrient solution strength increased, the variation in root:shoot ratio decreased, indicating a more consistent response to nutrient allocation to root and shoot tissue under higher nutrient treatments. It is generally assumed that, under conditions of high nutrient availability, plants allocate energy to tissues responsible for acquiring the next limiting resource, which is often light (Lambers and Poorter 1992; Agren and Franklin 2003). This results in greater allocation to shoot rather than root growth. The findings of this research are consistent with this theory: as the strength of the solution, and thus the availability of nutrients, increased, the root:shoot ratio decreased. It is important to note that re-

duced root growth is common in hydroponic systems, potentially due to inadequate aeration, and root growth in this study was limited by container volume whereas shoot growth was not limited (Trolldenier and Hecht-Buchholz 1984). Fireweed leaf and flower tissues are typically targeted for medicinal and therapeutic use (Kosalec et al. 2013), so a lower root:shoot ratio would be desirable for maximum production. However, in hydroponic systems, the harvest of root tissue is much less labor-intensive than plants grown in soil and root tissue may be a novel source of desirable phytochemicals.

Fireweed tissues possess distinct metabolomic fingerprints and contain commercially relevant compounds

Previous work on phytochemical production of fireweed has identified numerous compounds with medicinal and therapeutic value (Schepetkin et al. 2016). In this study, several of these compounds were provisionally identified in hydroponically cultivated fireweed (Table 2) and tissue comparisons of phytochemical content were visualized through a PCA plot (Fig. 3). Tissues with similar functions shared similar metabolomic fingerprints. Flower and anther tissue, both reproductive tissues, were found to have similar metabolomic fingerprints. Leaf and stem tissue also shared similar metabolomic fingerprints, likely due to the sharing of similar key functions such as photosynthesis leading to the presence of similar metabolites (Fig. S3).

Polyphenolic compounds are the main components of fireweed extracts and include phenolic acids, flavonoids, and ellagitannins, and 17 polyphenolic compounds were identified in various tissues of hydroponically produced fireweed. Information on tissue specificity of phytochemical compounds in fireweed was previously limited to an analysis in which polyphenolic compounds from leaf, flower, and stem parts were identified and quantified, and ellagitannins constituted the majority of the dry mass of leaves and flowers (Baert et al. 2017). We found that in hydroponically grown fireweed, the relative abundance of oenothein B (feature 16) was highest in leaf and stem tissue, and it was the third most abundant feature in flower and anther tissue. Three flavonoids (kaempferol-3-rhamnoside, myricetin-3-rhamnoside, and quercetin-3-rhamnoside) were previously found to be unique to flower tissue (Baert et al. 2017). Here, quercetin-3-rhamnoside (feature 4) and kaempferol 3-rhamnoside (feature 3) were identified in hydroponically cultivated fireweed samples in tissues other than the flower, suggesting the possible usefulness of tissues other than flower to therapeutic formulations. However, levels were over threefold greater in flower tissue than in leaf, root, and stem tissue, indicating the superiority of flower tissue for certain compounds.

Belowground tissues of many medicinal plants are rich sources of bioactive phytochemicals (Briskin 2000). In this experiment, root tissue was distinctly different from all other tissues and possessed the lowest relative abundances of most provisionally identified features. There are several potential explanations for the reduced content of bioactive compounds in hydroponic fireweed root tissue. First, root growth was limited in this hydroponic system (under 0.3 g root mass per plant) and the architecture of developing roots—mainly higher root branch density when compared to soil-grown fireweed—suggested a strong role in nutrient acquisition rather than storage (Chen et al. 2011). Second, soil microbes are believed to play a strong role in the production of bioactive compounds in medicinal plants and hydroponic systems lack soil that could facilitate the production of these compounds (Solaiman and Anawar 2015; Wu et al. 2009). Finally, attention has historically been given to fireweed leaf and flower tissue, as these are tissues used in traditional practices (Rogers 2014). There is a massive gap in knowledge on bioactive compounds that may reside in the root tissue. Future analyses and comparisons of commercially relevant polyphenolics from hydroponically produced fireweed should include confirmation of feature identification with standards and quantification of these phytochemicals to determine yield per biomass.

Nutrient availability affects relative abundance of features in leaf, root, and stem tissue

Nutrient content of hydroponic solutions affects the phytochemical content in the tissues of plants (Zheng et al. 2006; Giorgi et al. 2009; Senizza et al. 2020). In this study, nutrient solution strength affected various metabolites in leaf, stem, and root tissue but did not appear to affect metabolites in flower or anther tissue. The absence of an effect in reproductive tissues could be potentially due to small sample sizes and lack of representation across the nutrient treatments, as not all individual plants flowered in this experiment. Environmental conditions, especially nutrient availability or stress, influence the phytochemical content of plants and there is a strong relationship between elemental stoichiometry and the plant metabolome (Rivas-Ubach et al. 2012). Nutrient availability and subsequent allocation within the plant have been shown to affect the production of storage (Chapin et al. 1990), defensive (Coviella et al. 2002; de Lange et al. 2019), and stress avoidance compounds (Hale et al. 2005; Galieni et al. 2015) in various plant species. Available published research on environmental drivers of phytochemical production in fireweed is limited to the effects of elevation, and its associated increased UV-B radiation and resource limitation, on phenolic compound content (Monschein et al. 2015). The research presented here serves as a first step in examining nutrient-related changes to the metabolome of fireweed in a hydroponic production system.

Organic and mineral fertilizers tend to decrease the content of phenolic acids in plants (Nørbæk et al. 2003; Nguyen and Niemeyer 2008; Sinkovič et al. 2015). In general, nutrient fertilization negatively affects the concentration of flavonoids in plants (Ibrahim et al. 2011; Deng et al. 2012; Nybakken et al. 2018), although increases in flavonoid content have been reported as well (Naguib et al. 2012). Most research on the production of polyphenolics in response to nutrient availability have focused on modulation of a single nutrient (most commonly N) or includes additional alterations

to environmental conditions that may confound interpretations of results. In this study, most features that changed significantly across nutrient treatments were found to decrease in relative abundance as nutrient solution strength increased. MS library searches of varying features other than the identified features indicate that many are polyphenolic compounds. However, unlike other flavonoids, the relative abundance of quercetin-3-glucuronide (compound 7) was not influenced by nutrient solution strength. Similarly, the record is unclear as to whether fertilization has a direct effect on the level of hydrolysable tannins produced within plant tissues. The relative abundance of the ellagitannin oenothein B, a hydrolysable tannin, was not affected by the strength of the nutrient solution in this experiment. Much of the work on ellagitannin content in response to nutrient availability has been conducted on long-lived perennial woody species, not on herbaceous species like fireweed, with results ranging from no evidence for fertilization influencing levels of hydrolysable tannins to evidence for specific levels of fertilization for improved tannin content (Haukioja et al. 1998; Chabeli et al. 2008; Mudau et al. 2008). The results presented here suggest that increasing nutrient solution strength negatively affects the relative abundance of most, but not all, polyphenolic compounds of fireweed, and further work is required on fireweed to optimize production of desired phytochemicals.

Although both above- and belowground tissues have previously been shown to increase production of phenolics in response to environmental stress (Muzika 1993; Kraus et al. 2004; Caretto et al. 2015; Galieni et al. 2015), there are few reports where stress responses of both of these tissues are examined simultaneously. An "opposite metabolic response"—corresponding decreases and increases in shoot and root metabolites—has been demonstrated for drought and temperature stress, but such studies on single or multiple stressors remain scarce (Gargallo-Garriga et al. 2014, 2015; Mundim and Pringle 2018). In this research, eight of the provisionally identified features (2, 3, 8, 9, 12, 13, 15, and 16) were found to vary in mean intensity across nutrient treatments in leaf, stem, and root tissue. In this research, three putatively identified flavonoids (features 2, 3, and 8) and 8 of the 11 unidentified root features increased in intensity in response to increasing nutrient solution strength, whereas most identified and unidentified features in leaf and stem tissue decreased in response to nutrient availability. When plants are exposed to environmental stress, there is an upregulation of both root growth (Berendse and Moller 2008) and metabolism in the root system (Gargallo-Garriga et al. 2014), which may account for the increased abundance of some features in root tissue shown here. Many popular botanical-based products are derived primarily from the root and rhizome tissue (e.g., coneflower, licorice, and turmeric), and fireweed root contains polyphenols, tannins, and flavonoids and has been used around the world to treat various ailments (Adamczak et al. 2019; Dreger et al. 2020). Fireweed root tissue is easily harvestable in a hydroponic system, but further research on the production of phytochemicals of potential medicinal and commercial value must be conducted to determine its usefulness.

There is need to reconcile phytochemical production with biomass production

Whether in the field or in a hydroponic system, there is a need to optimize phytochemical production in medicinal plants such as fireweed if the beneficial compounds are to be commercialized. In the case of polyphenolic compounds, such as those prevalent in fireweed, a trade-off between investment in plant defense versus growth has been proposed and studied through the lenses of the carbon-nutrient balance and growth-differentiation balance hypotheses (Massad et al. 2012). These hypotheses state that plant growth and polyphenolic production are competing processes. Although not directly addressed in this research, there is a hint of this phenomenon in the production of fireweed, as biomass production was positively correlated with increasing nutrient availability whereas many phytochemicals decreased in abundance. Application of environmental stress has been utilized to influence polyphenolic production in medicinal plants (Fonseca et al. 2006; Reilly et al. 2008; Chrysargyris et al. 2016). However, nutrient deficiencies often negatively affect yield, and there must be an awareness of the effect of an applied environmental stress on biomass production. Nutrient starvation, specifically N, has been recommended for the production of polyphenolics in medicinal plants (Giorgi et al. 2009). Instead of strictly reducing nutrient availability and risking reductions in yield, recently there has been interest in short-term limitation of nutrients (Zhou et al. 2018) and balancing nutrient supply with yield (Radusiene et al. 2019) in plant production to optimize both biomass and phytochemical yields. This could be accomplished in a hydroponic system by changing nutrient solutions at a critical time, but would be difficult in a field setting. The trade-off between the production of biomass and phytochemicals with commercial value must be acknowledged and explored in medicinal plant species prior to large-scale production operations.

Conclusion

Fireweed is a widely known medicinal plant with an extensive history of use in traditional medicine (Schepetkin et al. 2016). Recent work on fireweed has focused mainly on phytochemical content (Baert et al. 2017; Agnieszka et al. 2018) and fitness, growth, and resource allocation strategies in relation to ploidy level (Bales and Hirsch-Green 2019; Walczyk and Hersch-Green 2019). In this research, fireweed biomass yield and metabolomic response to nutrient availability in a hydroponic system were examined. Fireweed grown with full strength Hoagland's nutrient solution produced the most harvestable biomass, and logistic modeling suggests that biomass production is maximized near the full-strength solution. Fireweed grown under hydroponic conditions produced several therapeutic polyphenolic compounds, and an untargeted metabolomics approach allowed for detection of the effects of nutrient solution strength on relative metabolite abundance. Oenothein B and miquelianin, two phytochemicals of potential commercial interest, were detected in fireweed under hydroponic conditions, and their abundances

were not affected by the level of minerals in the nutrient solution. Tissues varied in abundances of provisionally identified features, with higher abundances of features found in leaf tissue for most compounds, followed by flower and stem tissue. Abundance of several features decreased when nutrient strength increased, suggesting that nutrient stress may elicit the production of the corresponding phytochemicals. Based on these results, future research should address the trade-off between nutrient availability and biomass production to optimize growing conditions for fireweed as a medicinal crop. In future studies, a combination of targeted in addition to untargeted metabolomics approaches should be utilized to examine compounds of importance, but also to address the possibility of synergistic or additive actions of co-occurring constituents. Compounds of interest, such as oenothein B and several flavonoids, should be quantified on a content per biomass basis to provide appropriate recommendations for fireweed production.

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Data availability

Raw metabolomic data files will be made available through the MetaboLights data repository.

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Author contributions'

All authors conceived and planned the experiments. KFS carried out the experiments, and took the lead in writing the manuscript. All authors provided critical feedback during the experiment, analysis, and manuscript preparation.

Competing interests

The authors declare there are no competing interests.

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Supplementary material

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