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Phytochemical Variation in *Echinacea* from Roots and Flowerheads of Wild and Cultivated Populations

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Quantitative phytochemical variation was determined from roots and inflorescences of native plant populations in the genus *Echinacea*. Specimens were collected in situ throughout the natural range of each putative taxon and transplanted to greenhouse cultivation. Ethanolic extracts from individual plants were separated by reversed-phase HPLC to quantify the alkamides, polyenes/ynes, and phenolics, and then grouped by age and taxonomically, according to a recent morphometric taxonomic revision of the genus. Canonical discriminant analysis revealed that cichoric acid, the diene alkamides **1**−**3** and **7**, and ketoalkene **24** were the best taxonomic markers. Mean content for each of 26 phytochemicals revealed useful agronomic information, such as those varieties and organs with the highest accumulations, as well as the optimal age and growth conditions for each variety. The highest amounts of cichoric acid were measured from the older, wild inflorescences of *E. pallida* var. *sanguinea*, whereas the highest quantities of the alkamides **1**−**3** and **7** were present in roots of wild and transplanted *E. purpurea*. Baseline phytochemical data and chromatographic profiles for all types of wild *Echinacea* may be used for protection of wild stands, germplasm identification, and crop improvement.

KEYWORDS: *Echinacea*; phytochemistry; alkamides; polyenes; phenolics; canonical discriminant analysis; chemotaxonomy; HPLC

INTRODUCTION

The native North American plant genus *Echinacea* Moench (Heliantheae: Asteraceae) has recently been reclassified as four species and eight varieties, together with a group of introgressant hybrids, which are associated with specific wild habitats throughout the range (*1*). For medicinal and horticultural purposes, three different taxa are widely cultivated and traded: *E. purpurea* (L.) Moench; *E. pallida* var. *angustifolia* (DC.) Cronq.; and *E. pallida* var. *pallida* (Nutt.) Cronq. However, the other taxa may appear in *Echinacea* medicinal products because of the significant contribution of wildcrafting to commercial *Echinacea* products or through the practice of some growers who introduce wild collected seeds into cultivation. The natural variation inherent in wild population genotypes and environmental factors throughout the range of the genus may contribute substantially to differential expression of medicinal phytochemicals as predicted in the theory of Herms and Mattson (*2*).

The phytochemistry of *Echinacea* was initially characterized by European researchers (*3*−*7*) and was based mainly on the three cultivated taxa. Two major groups of compounds have received attention, the lipophilic compounds including alkamides and ketoalken/ynes (**Figure 1**) and the hydrophilic phenolic compounds (mainly caffeic acid derivatives) (**Figure 2**). There are currently no reports of phytochemical variation among wild populations of native North American *Echinacea* species and varieties. The current study was therefore conducted to determine quantitative phytochemical variation in all *Echinacea* species and varieties. Sampling from wild populations was carefully conducted throughout the natural range of each putative taxon in the genus, tentatively identified in the field according to McGregor (*8*) and later definitively assigned to the new taxonomy (*1*). Transplants and wild germplasm under cultivation were also assessed under uniform growing conditions.

Characteristic lipophilic phytochemical profiles have been previously reported for the three commercial *Echinacea* species, which show that these are good chemotaxonomic characters. The major alkamides in *E. purpurea* were the 2,4-diene type compounds (*4*, *9*). A quantitative comparison within *E. purpurea* revealed the highest levels of the C_{12} diene−diyne alkamides in the roots, whereas the C_{11} diene−diynes were highest in vegetative stems (*10*). *E. angustifolia* roots were characterized by the presence of monoene alkamides and tetraenes (*3*, *9*). In *E. pallida* the major compounds were polyynes/enes, with only

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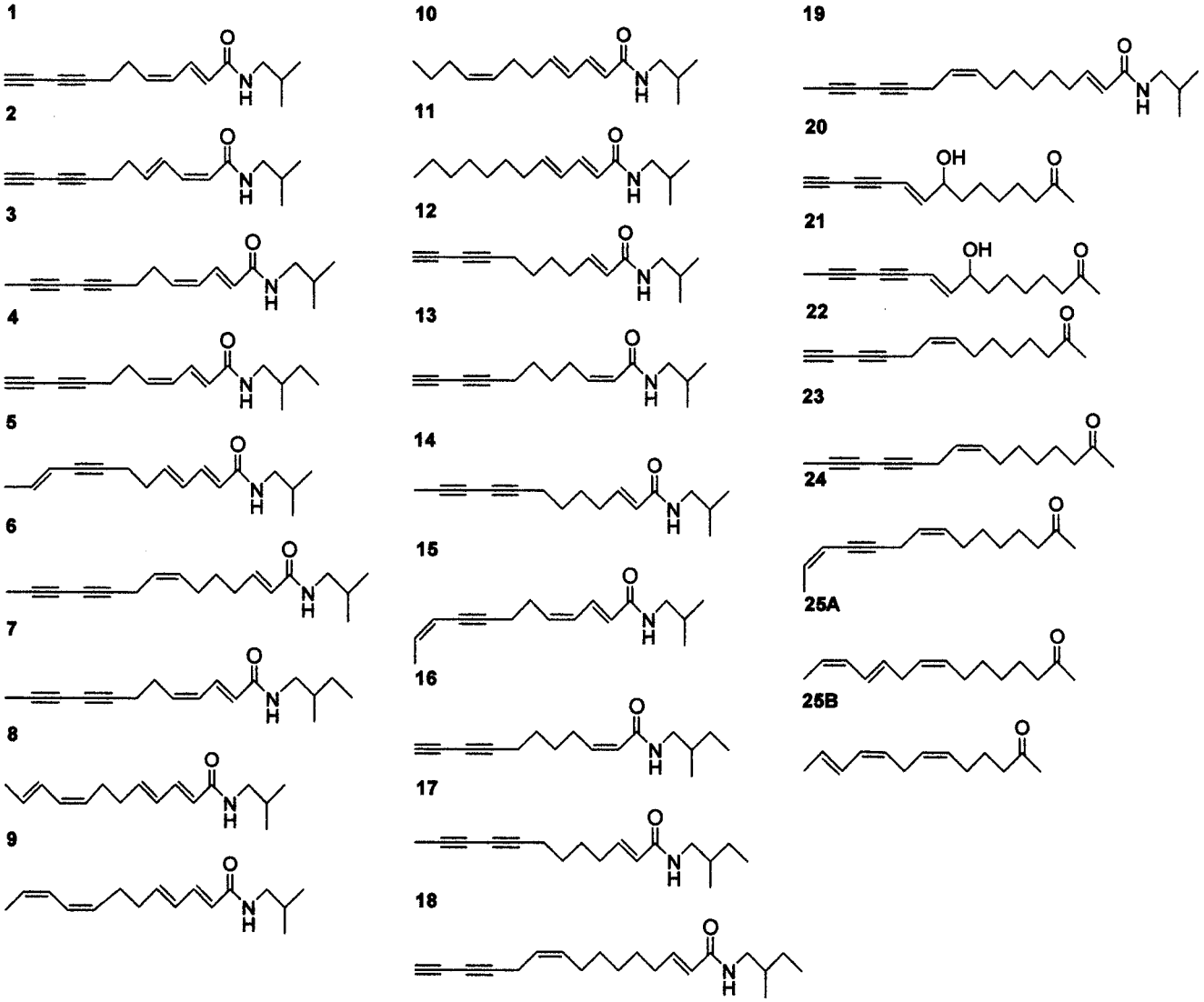

Figure 1. Lipophilic phytochemicals characteristic of the genus *Echinacea* and used in this study: **1**, undeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide; **2**, undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide; **3**, dodeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide; **4**, undeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide; **5**, dodeca-2*E*,4*E*,10*E*-triene-8-ynoic acid isobutylamide; **6**, trideca-2*E*,7*Z*-diene-10,12-diynoic acid isobutylamide; **7**, dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide; **8**, dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide; **9**, dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide; **10**, dodeca-2*E*,4*E*,8*Z*-trienoic acid isobutylamide; **11**, dodeca-2*E*,4*E*-dienoic acid isobutylamide; **12**, undeca-2*E*-ene-8,10-diynoic acid isobutylamide; **13**, undeca-2*Z*-ene-8,10-diynoic acid isobutylamide; **14**, dodeca-2*E*-ene-8,10-diynoic acid isobutylamide; **15**, dodeca-2*E*-ene-8,10-diynoic acid isobutylamide; **16**, undeca-2*Z*-ene-8,10-diynoic acid isobutylamide; **17**, dodeca-2*E*-ene-8,10-diynoic acid isobutylamide; **18**, pentadeca-2*E*,9*Z*-diene-12,14-diynoic acid isobutylamide; **19**, hexadeca-2*E*,9*Z*-diene-12,14-diynoic acid isobutylamide; **20**, 8-hydroxytetradeca-9*E*-ene-11,13-diyn-2-one; **21**, 8-hydroxypentadeca-9*E*-ene-11,13-diyn-2-one; **22**, tetradeca-8*Z*-ene-11,13-diyn-2-one; **23**, pentadeca-8*Z*-ene-11,13-diyn-2-one; **24**, pentadeca-8*Z*,13*Z*-dien-11-yn-2-one; **25A**, pentadeca-8*Z*,11*E*,13*Z*-trien-2-one; **25B**, pentadeca-8*Z*,11*Z*,13*Z*-trien-2-one.

three alkamides reported in the roots, and they did not contain tetraenes (*3*).

Phytochemical profiles are availalbe for *E. atrorubens* var. *paradoxa*, *E. pallida* var. *tennesseensis*, and *E. pallida* var. *simulata* (*11*, *12*). The lipophilic profiles of the latter two were reportedly similar to those of *E. pallida* var. *angustifolia* (*11*, *12*). Alternatively, *E. atrorubens* var. *paradoxa* yielded polyynes/enes and their oxidized derivatives similar to those observed in *E. pallida* var. *pallida* (*12*). Among the reported phytochemical profiles, there were taxonomic errors between the two cultivated *E. pallida* varieties (*3*), as well as adulteration of *E. purpurea* with *Parthenium integrifolium* L., which were both detected and corrected in later publications (*12*, *13*). Overall, the phytochemical reports of the genus *Echinacea* are incomplete and inconsistent because of confusion in its taxonomic history (*1*).

Recently, selection of morphologically superior cultivated *E. purpurea* lines resulted in doubled average phytochemical content in each of the major chemical classes when compared to nonselected lines (*14*) and also demonstrated high genetic variability (*15*). Accurate phytochemical and taxonomic distinctions for wild *Echinacea* germplasm sources will have a positive impact on conservation and cultivation of this medicinal plant.

MATERIALS AND METHODS

Plant Materials. *Echinacea* plants and germplasm from 125 natural populations were sampled from throughout the range of each putative species and variety and given tentative taxonomic labels in the field, according to McGregor (*8*). One root per population was transplanted to a greenhouse at 25−30 °C in a medium of 2:1 soil/beach sand in large gallon pots, with 16 h of daylight, including natural and cool

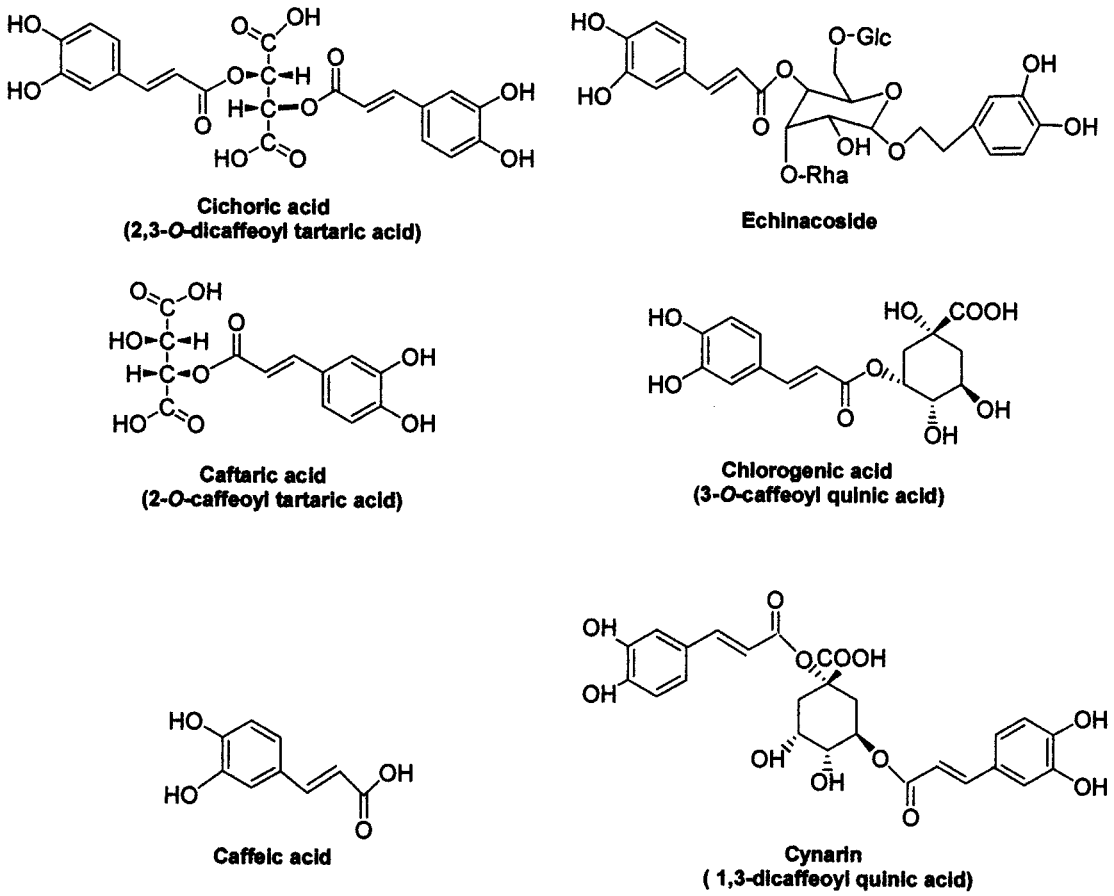

Figure 2. Phenolic constituents of the genus *Echinacea* used in this study.

white lighting (25 μM/m^2/s). Wild organ samples were coarsely chopped (or left whole) and placed in 95% ethanol in leakproof, inert plastic sample containers (Nalgene) in situ as follows: (1) root cutting from one individual plant, (2) bulk leaf sample from 10−20 random plants, and (3) randomly sampled, bulked inflorescences (3−10 capitula). Ripened achenes were collected when available, sterilized, and stratified at 4 °C (5 μM/m^2/s, incandescent) for 14 days. Germinated seedlings were grown in 5:5:2 Promix/vermiculite/sterile quartz sand (industrial grade 10, 4 mm particles). Developmental variation between accessions required that some plants be induced to flower with a cold treatment at 4 °C (20 μM/m^2/s, incandescent) for 14 days. All plants were fertilized with 20:20:20 weekly and watered equal amounts daily, according to age. Extracts from all greenhouse-cultivated *Echinacea* were made from the organs of individual plants.

Extraction. *Method A.* Plant material was blended to a homogeneous slurry in ethanol using an Osterizer blender. The biomass/solvent ratio was approximately 2 g of fresh weight/10 mL. Plant−solvent mixtures were mechanically agitated on a shaker (70 rpm) for 24 h. Solids were removed using a Büchner filter system (Whatman No. 1 filter paper), and the residues were dried and weighed to determine final extract concentrations (grams of dry weight, dwt/mL). Filtrate was rotoevaporated to dryness and redissolved into 60% ethanol to a final concentration of 0.1 g/mL and stored at 4 °C in amber glass containers. Other studies in this laboratory (*16*) determined that aqueous alcoholic extractions in 70% ethanol provided optimum recovery of *Echinacea* phytochemicals, such as cichoric acid, a hydrophilic marker compound, and dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides, a lipophilic standard. However, 95% ethanol was used with fresh samples to halt enzymatic degradation and ensure sterilization, as well as to account for the moisture content of the plants, but all *Echinacea* extracts were brought to final concentrations in 60% ethanol for storage and analyses.

Method B. A second blending method was developed to maximize the extraction of phytochemicals from very small quantities of young, single plants (<1 year old). Roots, leaves, and inflorescences were cut into 1 cm lengths using a razor blade and placed into 20 mL of 60% ethanol in plastic centrifuge tubes (50 mL, VWR, Toronto, Canada). Fresh biomass varied from approximately 2 to 10 g per tube. Samples were mixed into a slurry with a high-speed Polytron (Brinkmann Instruments, Westbury, NY) for 30 s (repeated three times). After 24 h on a shaker (70 rpm), samples were vortexed briefly and then centrifuged for 10 min. The supernatant was removed to a clean tube. Fresh 60% ethanol (20 mL) was added to the plant residue, vortexed, and then agitated on a shaker (70 rpm) for 24 h. The sample was again centrifuged for 10 min, and the supernatants were pooled. The entire process was repeated for a final 24 h extraction period in 10 mL of 60% ethanol. Residues were dried and weighed to determine the final extract concentrations. Extracts were adjusted to 0.1 g/mL by rotary evaporation or dilution.

The size of wild populations, rarity of each putative variety, and survival of achenes and live transplants all contributed to differences in samples sizes (3−157 individual organ extracts for each revised *Echinacea* variety).

HPLC. All extracts were filtered (0.2 μm, PTFE membrane) prior to HPLC separations using a validated method (*17*). Hydrophilic chromatography was achieved using a solvent system of acetonitrile/50 mM NaH_2PO_4, pH 2.95, at a flow rate of 1.5 mL/min following a linear gradient of 5−25% acetonitrile over 7 min. Lipophilic chromatography was achieved using a solvent system of acetonitrile/H_2O, at a flow rate of 1.0 mL/min following a linear gradient of 40−80% acetonitrile over 15 min. In both systems, 5 μL of sample was injected on a 7.5 cm × 4.6 mm reversed-phase C-18 LiChrospher column, 4 μm particle size (Merck, BDH, Toronto, Canada). Lipophilic compounds were detected at 210 and 260 nm and hydrophilic compounds at 326 nm.

Compounds were identified by comparison with reference standards; those that were isolated previously in our laboratory by column chromatography on silica gel and assessed for purity by ^{1}H and ^{13}C NMR spectral data (*17*) included undeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide (**1**), dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides (tetraenes) (**8** and **9**), pentadeca-2*E*,9*Z*-diene-12,14-diynoic acid

isobutylamide (**18**), 2,3-*O*-dicaffeoyltartaric acid (cichoric acid), and echinacoside. Dodeca-2*E*,4*E*-dienoic acid isobutylamide (**11**) was also determined by on-line UV spectra matching Bauer and Remiger (*5*) and cynarin by ^{1}H and ^{13}C NMR (*6*) in our laboratory (*18*). Standards of caffeic acid and chlorogenic acid (Sigma Aldrich, St. Louis, MO), as well as caftaric acid (Dalton Chemical Laboratories Inc., Toronto, Canada), were purchased. All other alkamide and polyene compounds were identified on the basis of relative retention time to the marker compounds (tetraenes) **8** + **9** and on-line photodiode array UV spectra (*5*). Each previously reported compound (micrograms per milliliter injected) was quantified using peak area multiplied by the response factor (calculated from the standard curve of tetraenes **8** + **9**). This figure was then divided by the original concentration of 0.5 g of extracted dried root/mL of sample and multiplied by 10^3 to reach milligrams per gram of dry weight. Compound identification by relative retention time and quantitation by relative response factor were acceptable for the purpose of overall profile comparisons within the present study.

Statistical Analyses. Descriptive statistics (mean ± standard error of the mean, SEM) were calculated to compare all revised taxa by individual phytochemicals in 358 root extracts (single plants only) and 175 inflorescence extracts (approximately half were bulked wild population samples). Same-aged specimens grown under the same conditions were compared: (1) cultivated, ≤1 year old; (2) cultivated/transplanted, >1 year old; and (3) all wild-harvested roots and flowers.

Quantitative data collected from HPLC of 327 *Echinacea* root operational taxonomic units (OTUs) were arranged in a matrix as root concentrations (parts per million of root dry weight, dwt) for 26 phytochemical characters (all of which were previously reported alkamides, phenolics, and polyenes). In this matrix, OTUs were grouped according to revised species identity (*1*). Canonical discriminant analysis (CDA) was used to determine whether classes of OTUs (revised taxonomic groups in this case) were distinctly different from one another on the basis of a certain set of interrelated characters (26 quantitative phytochemicals). CDA of the phytochemical variation in four revised species and eight varieties of *Echinacea* was carried out with subsets of the data matrix, to accommodate the conditions under which the data were collected. Specifically, CDA was performed with SAS version 8.0 (SAS Institute Inc., Cary, NC, 2000) for (1) all roots regardless of age or growth conditions, (2) young, cultivated roots, (3) older, transplanted roots in cultivation, and (4) wild-harvested roots. Characters that did not vary within a taxonomic group were deleted from the analysis. A complete explanation of the CDA method can be found in Kshirsagar (*19*).

RESULTS AND DISCUSSION

Average phytochemical content of roots and inflorescences from each *Echinacea* species and variety can be found in **Tables 1**−**6**, identified according to the recent taxonomic revision (*1*). Chemotaxonomic differences are most evident from the lipophilic profiles, whereas fewer distinctions were made using phenolic variation. Typical average chromatographic profiles for roots of each wild species and variety of *Echinacea* are provided in **Figures 3** and **4**.

Root Tetraenes. The major alkamides in *Echinacea*, dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides **8** + **9**, henceforth called tetraenes, were recorded at the highest level in this study from wild *E. pallida* var. *sanguinea* at 1.9% root dwt (**Table 3**). Tetraenes were second highest in cultivated roots of *E. atrorubens* var. *atrorubens* (0.8−0.96% dwt) (all ages), although they were absent from *E. atrorubens* var. *paradoxa*. Tetraenes were present in the roots of all other varieties of *Echinacea*, with the highest mean quantity from older *E. pallida* var. *angustifolia*. Previously, tetraenes were reported as major constituents of *E. pallida* var. *angustifolia* and *E. purpurea* roots (*9*), buf, for the first time, tetraenes are here reported in roots of all four revised *Echinacea* species.

Echinacea roots generally accumulated more tetraenes with age, with the following exceptions: *E. pallida* var. *tennesseensis*, *E. laevigata* (insufficient data), and *E. atrorubens* var. *neglecta*, which had relatively small quantities overall but accumulated the most in younger cultivated roots. Commercially traded *E. pallida* var. *pallida* roots reportedly lack tetraenes; however, in the current study both wild and cultivated younger roots of *E. pallida* var. *pallida* (*1*) accumulated some tetraenes, along with their diagnostic ketoalkenes/ynes.

Flower Tetraenes. Tetraenes were the predominant lipophilic constituents in flowerheads of all *Echinacea* species and varieties, regardless of age or growth conditions. The highest amounts in this study were measured in seed-grown flowerheads of *E. pallida* var. *tennesseensis* at 1.04% dwt (**Table 2**). *E. atrorubens* var. *neglecta* and *E. pallida* var. *pallida* wild flowerheads both accumulated 0.55% dwt. Flowerheads cultivated from seed generally accumulated more tetraenes than wild plants of the same variety, except for *E. pallida* var. *sanguinea* and *E. pallida* var. *pallida*.

Other Diagnostic Alkamides. The diene alkamides **1**−**4** and **7** have two double bonds in conjugation with the carbonyl group and have been reported as diagnostic compounds for *E. purpurea*, which are generally lacking in *E. pallida* var. *angustifolia* and *E. pallida* var. *pallida* (*9*). In the current study, older, transplanted wild roots of *E. purpurea* accumulated the highest amounts of **1**, **2** (0.4% dwt), and **7**, whereas roots in native plant stands of the same species contained the highest amounts of **3** and **4**. However, as qualitative (presence/absence) diagnostic markers for *E. purpurea* roots, the dienes are not useful because they are found in all other species of *Echinacea*.

E. pallida var. *sanguinea* displayed all dienes at levels comparable to those found in *E. purpurea*, but with much smaller amounts of **2** and **4** in cultivation. *E. laevigata* contained the next highest amount of **3**, with medium to low levels of **1**, **2**, **4**, and **7**. The diene pattern in *E. atrorubens* mirrored that of *E. purpurea*, with lower quantities, except of **3**, which was high in younger roots of *E. atrorubens* var. *neglecta* (0.24% dwt) and in older roots of *E. atrorubens* var. *atrorubens* (**Tables 1**, **3**, and **4**). *E. pallida* vars. *angustifolia*, *tennesseensis*, and *simulata* all contained >0.1% dwt root dienes. The 2,4-diene moiety may be responsible for some biological activity, such as cyclooxygenase and 5-lipoxygenase inhibition (*20*).

E. purpurea roots can be identified by the lack of alkamide **18**, which is reported here for the first time in the highest amounts from the roots of *E. pallida* var. *sanguinea*. Alkamide **10** was identified in large quantities from young roots of *E. atrorubens* var. *atrorubens* (0.6% dwt), yet it was also present in *E. pallida* var. *angustifolia* roots and in small amounts in other species and varieties. Alkamide **11** was highest in older roots of *E. pallida* var. *angustifolia* (0.22% dwt) but was present in all species and varieties, sometimes very minimally, except *E. atrorubens* var. *paradoxa*. Possibly an indicator of hybridization, alkamide **11** was accumulated at the second highest amount in roots from hybrid populations. Alkamides **5** and **15** were found in highest amounts in *E. atrorubens* var. *atrorubens*, reported in this variety for the first time, followed by *E. pallida* var. *angustifolia*. They were also present in *E. purpurea* and *E. atrorubens* var. *neglecta*, along with some other varieties.

Quantitatively, *E. pallida* and *E. atrorubens* varieties were far superior in monoene−carbonyl conjugated alkamides **12**−**14** compared to *E. pallida* var. *angustifolia*, for which the pattern is reportedly diagnostic (*9*). Wild roots of *E. pallida* var. *sanguinea* had the highest amount of **14** in the genus (0.36% dwt) (**Table 3**), and young, cultivated *E. atrorubens* var. *atrorubens* roots had the highest level of **13** (3.7% dwt) (**Table 1**). Hybrid populations also typically revealed a monoene

Table 1. Phytochemical Content (Mean, Milligrams per Gram of Dry Weight; SEM Is Given below Each Mean) in *Echinacea* Root Germlings ≤1 Year

	species										
	purpurea	*pallida angustifolia*	*pallida pallida*	*pallida sanguinea*	*pallida tennesseensis*	*pallida simulata*	*laevigata*	*atrorubens paradoxa*	*atrorubens neglecta*	*atrorubens atrorubens*	hybrids
N[a]	11	119	22	11	4		3		7	12	13
cic[b]	8.06	0.63	0.50	0.08	0.32		5.70		0.95	0.04	0.37
	1.24	0.10	0.10	0.05	0.08		0.66		0.29	0.01	0.17
ech	0.00	6.87	1.78	0.01	0.92		9.79		0.48	0.09	4.89
	0.00	0.54	0.82	0.00	0.32		0.76		0.22	0.01	1.18
chl	0.09	0.16	0.08	0.06	0.23		0.98		0.38	0.03	0.18
	0.03	0.02	0.02	0.02	0.03		0.98		0.21	0.01	0.06
ctar	2.39	0.26	0.12	0.00	1.00		2.59		0.02	0.00	0.08
	0.22	0.05	0.04	0.00	0.40		0.48		0.01	0.00	0.01
cyn	0.00	5.00	0.00	0.00	4.23		0.00		0.00	0.04	5.21
	0.00	0.68	0.00	0.00	0.22		0.00		0.00	0.01	0.98
1	0.92	0.09	0.04	0.91	0.25		0.39		0.67	0.95	0.10
	0.17	0.01	0.01	0.26	0.05		0.05		0.54	0.12	0.03
2	3.16	0.09	0.58	0.25	0.82		1.03		1.28	0.35	0.12
	0.54	0.01	0.09	0.03	0.12		0.01		0.46	0.10	0.08
3	1.99	0.15	0.09	1.52	0.34		3.08		2.38	0.89	0.30
	0.15	0.02	0.03	0.30	0.04		0.40		1.26	0.17	0.19
4	0.23	0.05	0.02	0.00	0.39		0.00		0.24	0.04	0.13
	0.04	0.01	0.01	0.00	0.14		0.00		0.21	0.01	0.05
5 + **15**	0.47	1.23	0.01	0.21	0.11		0.18		0.71	1.87	0.18
	0.12	0.71	0.00	0.07	0.04		0.01		0.59	0.22	0.07
7	1.99	0.05	0.14	0.00	0.61		0.71		1.84	0.00	0.11
	0.46	0.01	0.03	0.00	0.17		0.06		0.59	0.00	0.04
8 + **9**	2.79	5.23	0.00	14.36	6.78		1.30		1.62	8.00	4.87
	0.64	0.40	0.00	0.86	1.57		0.14		1.62	1.12	0.91
10	0.30	0.46	0.00	0.42	0.58		0.08		0.00	6.23	0.45
	0.03	0.06	0.00	0.06	0.30		0.02		0.00	3.84	0.08
11	0.19	0.76	0.00	0.26	0.36		0.13		0.00	0.33	0.67
	0.04	0.08	0.00	0.05	0.08		0.02		0.00	0.05	0.14
12	0.00	0.64	0.00	1.87	4.69		1.42		0.10	2.30	0.57
	0.00	0.10	0.00	0.18	1.60		0.28		0.10	0.28	0.13
13	0.00	0.62	0.00	1.30	9.14		0.30		0.12	36.50	0.43
	0.00	0.07	0.00	0.17	4.16		0.02		0.12	24.55	0.08
14	0.00	0.59	0.03	1.69	1.49		0.68		0.00	1.41	0.36
	0.00	0.18	0.02	0.21	0.64		0.19		0.00	0.17	0.05
6	0.00	0.07	0.00	0.30	0.31		0.11		0.22	0.04	0.09
	0.00	0.01	0.00	0.06	0.04		0.01		0.22	0.03	0.04
16	0.40	0.16	0.02	0.31	1.13		0.00		0.03	0.34	0.10
	0.11	0.02	0.01	0.07	0.74		0.00		0.03	0.04	0.04
17	0.41	0.08	0.00	1.05	0.35		0.04		0.15	0.11	0.07
	0.16	0.01	0.00	0.34	0.15		0.04		0.15	0.01	0.01
18	0.02	0.77	0.38	29.00	1.28		0.26		0.49	1.40	0.84
	0.01	0.07	0.07	18.26	0.43		0.01		0.49	0.32	0.20
19	0.00	0.05	0.00	0.00	0.00		0.00		0.04	0.00	0.08
	0.00	0.01	0.00	0.00	0.00		0.00		0.03	0.00	0.02
22	0.00	0.02	0.66	0.00	0.00		0.00		0.80	0.00	0.00
	0.00	0.01	0.07	0.00	0.00		0.00		0.21	0.00	0.00
24	0.00	0.02	1.12	0.00	0.04		0.05		3.21	0.00	0.02
	0.00	0.01	0.08	0.00	0.03		0.03		0.62	0.00	0.01
25	0.00	0.04	1.56	0.00	0.00		0.00		0.26	0.00	0.14
	0.00	0.03	0.29	0.00	0.00		0.00		0.08	0.00	0.14

[a] *N* denotes sample sizes for each of the revised species and varieties. [b] Phytochemical abbreviations: cic, cichoric acid; ech, echinacoside; chl, chlorogenic acid; caf, caffeic acid; ctar, caftaric acid; cyn, cynarin; numbers correspond to lipophilic alkamides **1**–**19** and ketoalkynes/enes **20**–**25**.

alkamide pattern rather than a diene pattern, which is to be expected because most hybrids likely occurred between *E. pallida* and *E. atrorubens* (*1*, *8*). The use of the monoene-type alkamides **12**−**14** as industry markers to identify *E. pallida* var. *angustifolia* and *E. pallida* var. *tennesseensis* from the other species and varieties (*9*, *20*) is therefore contradicted by the current study. Both the monoene and diene patterns that diagnose *Echinacea* species roots are not present in appreciable quantities from the flowerheads for identification of market botanicals.

Alkamide **18**, a longer chain monoene type, was found in the highest amount from the roots of *E. pallida* var. *sanguinea* (2.9% young root dwt), and it was also present in every other variety of *Echinacea* except *E. purpurea*. Alkamide **19** was rare, especially in young roots, but was found at up to 0.05% dwt in the wild roots of *E. atrorubens* var. *neglecta*.

Root Ketoalkenes/ynes. Ketoalkenes/ynes with a carbonyl group in the 2-position, such as **22**, **24**, and **25**, were reportedly predominant in the roots of *E. pallida* var. *pallida* (*5*, *7*, *9*). They have been used to identify *E. pallida* var. *pallida* root powders on the market since it was discovered that most "*E. angustifolia*" cultivated in Europe contained these compounds, especially **22** (*3*). In this study, wild and older root transplants of *E. pallida* var. *simulata* contained twice the amount of **22** found in *E. pallida* var. *pallida*, to which it is morphologically

Table 2. Phytochemicals (Mean, Milligrams per Gram of Dry Weight; SEM Is Given below Each Mean) in Inflorescences of *Echinacea* Germlings ≤1 Year

	species										
	purpurea	*pallida angustifolia*	*pallida pallida*	*pallida sanguinea*	*pallida tennesseensis*	*pallida simulata*	*laevigata*	*atrorubens paradoxa*	*atrorubens neglecta*	*atrorubens atrorubens*	hybrids
N[a]	4	7	9	6	5	2	2	0	0	4	0
cic[b]	4.17	0.45	1.18	3.30	0.03	0.49	1.78			0.01	
	2.40	0.12	0.46	1.12	0.01	0.25	1.56			0.00	
ech	0.01	0.11	0.56	0.00	0.00	0.17	0.00			0.06	
	0.01	0.03	0.39	0.00	0.00	0.08	0.00			0.06	
chl	0.83	1.33	0.46	0.12	0.00	0.52	0.14			0.06	
	0.69	0.68	0.15	0.03	0.00	0.05	0.13			0.01	
caf					0.09						
					0.05						
ctar	1.67	0.08	0.23	0.30	0.04	0.12	0.73			0.03	
	1.06	0.04	0.14	0.07	0.01	0.11	0.68			0.01	
cyn	0.00	0.18	0.00	0.32	0.02	0.08	0.00			0.08	
	0.00	0.06	0.00	0.05	0.01	0.08	0.00			0.08	
1	0.48	1.16	0.30	0.06	0.10	1.04	0.50			1.51	
	0.40	0.83	0.16	0.02	0.02	0.85	0.14			0.49	
2	0.00	1.46	0.01	0.00	0.03	0.51	0.01			0.01	
	0.00	0.83	0.01	0.00	0.01	0.13	0.00			0.01	
3	0.03	0.01	0.02	0.01	0.02	0.04	0.03			0.06	
	0.02	0.00	0.01	0.01	0.01	0.04	0.01			0.03	
4	0.05	0.03	0.02	0.00	0.01	0.10	0.01			0.14	
	0.04	0.01	0.01	0.00	0.00	0.07	0.00			0.03	
5 + 15	0.05	0.06	0.08	0.04	0.02	0.18	0.11			0.10	
	0.05	0.03	0.05	0.02	0.01	0.18	0.02			0.03	
7	0.01	0.02	0.01	0.00	0.07	0.07	0.00			0.06	
	0.01	0.01	0.00	0.00	0.02	0.00	0.00			0.02	
8 + 9	3.45	4.53	1.59	1.33	10.35	4.96	1.80			2.89	
	2.45	2.81	0.48	0.40	2.03	3.60	0.39			0.88	
10	0.03	0.18	0.07	0.02	0.22	0.25	0.08			0.07	
	0.01	0.10	0.03	0.01	0.07	0.12	0.01			0.03	
11	0.06	0.11	0.09	0.01	0.15	0.29	0.05			0.06	
	0.04	0.07	0.05	0.00	0.05	0.18	0.01			0.03	
12	0.68	0.09	0.04	0.02	1.56	0.10	0.28			0.03	
	0.40	0.07	0.01	0.01	0.38	0.04	0.16			0.02	
13	0.00	0.00	0.03	0.00	0.04	0.00	0.00			0.09	
	0.00	0.00	0.02	0.00	0.02	0.00	0.00			0.07	
14	0.00	0.01	0.01	0.03	0.04	0.01	0.00			0.00	
	0.00	0.01	0.00	0.01	0.01	0.01	0.00			0.00	
6	0.08	0.14	0.04	0.00	1.33	0.18	0.00			0.12	
	0.07	0.05	0.02	0.00	0.19	0.15	0.00			0.05	
16	0.24	0.33	0.08	0.07	0.13	0.06	0.49			0.17	
	0.16	0.28	0.03	0.03	0.04	0.06	0.17			0.08	
17	0.05	0.02	0.03	0.00	0.02	0.06	0.00			0.04	
	0.03	0.01	0.01	0.00	0.01	0.03	0.00			0.02	
18	0.39	0.20	0.52	0.36	0.05	0.19	0.00			1.41	
	0.27	0.08	0.20	0.16	0.02	0.07	0.00			0.45	
19	0.03	0.00	0.02	0.02	0.00	0.01	0.00			0.09	
	0.03	0.00	0.01	0.01	0.00	0.01	0.00			0.03	
22	0.00	0.00	0.00	0.07	0.00	0.03	0.00			0.01	
	0.00	0.00	0.00	0.07	0.00	0.03	0.00			0.01	
24	0.00	0.01	0.07	0.11	0.38	0.05	0.00			0.00	
	0.00	0.00	0.05	0.04	0.06	0.02	0.00			0.00	
25	0.00	0.00	0.02	0.01	0.41	0.03	0.00			0.02	
	0.00	0.00	0.01	0.01	0.19	0.03	0.00			0.01	

[a] See footnote *a* of **Table 1**. [b] See footnote *b* of **Table 1**.

the most similar (**Tables 3** and **4**, respectively). Furthermore, young and wild roots of *E. atrorubens* vars. *neglecta* and *paradoxa* contained more **22** than both *E. pallida* vars. *pallida* and *simulata*. Older roots of *E. laevigata* also contained **22** (see **Table 4**). Ketoalkenes **24** and **25** were major components of *E. pallida* var. *pallida* roots and *E. pallida* var. *simulata*, as previously reported (*7*, *9*, *21*). However, these compounds were also accumulated to a large extent in *E. atrorubens* var. *neglecta* (0.3% dwt in young roots to 0.5% dwt in wild roots) and *E. atrorubens* var. *paradoxa* (1% dwt in wild roots). Clearly, the presence of **22** does not serve to identify only *E. pallida* var. *pallida*, although in a single species sample, its presence would eliminate the possibility of *E. purpurea*.

Flowerhead Ketoalkenes/ynes. Ketoalkene **24** was measured in appreciable quantities from the wild flowerheads of *E. pallida* var. *pallida*, *E. pallida* var. *sanguinea*, *E. pallida* var. *angustifolia*, and *E. atrorubens* var. *paradoxa*. The only cultivated variety that accumulated **24** in flowerheads at ~0.4% dwt was *E. pallida* var. *tennesseensis*.

Root Phenolics. The highest root concentrations of cichoric acid in all *Echinacea* collections were found in cultivated *E. purpurea* at 0.8% dry weight of young roots (**Table 1**). This

Table 3. Phytochemical Concentrations (Mean, Milligrams per Gram of Dry Weight; SEM Is Given below Each Mean) in Wild-Harvested *Echinacea* Root

	species										
	purpurea	*pallida angustifolia*	*pallida pallida*	*pallida sanguinea*	*pallida tennesseensis*	*pallida simulata*	*laevigata*	*atrorubens paradoxa*	*atrorubens neglecta*	*atrorubens atrorubens*	hybrids
N[a]	4	20	14	6	4	2	1	4	5	5	11
cic[b]	5.88	0.61	1.19	0.12	0.35	0.46		1.75	0.23	0.03	1.20
	3.23	0.70	0.93	0.03	0.08	0.23		0.81	0.06	0.01	0.71
ech	0.10	2.03	1.13	0.38	0.38	1.57		32.99	8.41	0.18	2.62
	0.10	0.49	0.68	0.13	0.18	0.75		9.07	4.28	0.16	1.04
chl	1.92	0.06	0.14	0.45	0.08	0.17		0.30	0.17	0.05	0.29
	0.63	0.19	0.07	0.17	0.03	0.10		0.07	0.06	0.03	0.19
ctar	0.00	0.04				0.04			0.10	0.04	0.08
		0.01				0.00				0.02	0.04
cyn	0.07	0.42	0.00						0.00	0.00	0.21
	0.06	0.10	0.00							0.00	0.11
1	0.25	0.06	0.08	0.52	0.13	0.06		0.06	0.68	0.43	0.46
	0.12	0.04	0.05	0.25	0.01	0.03		0.01	0.64	0.21	0.12
2	2.13	0.65	0.12	1.26	0.16	0.24		0.01	0.11	0.06	0.25
	1.08	0.58	0.07	0.77	0.08	0.13		0.01	0.06	0.03	0.15
3	3.88	0.14	0.62	2.71	0.00	0.32		0.13	0.25	0.03	0.82
	1.49	0.40	0.30	1.42	0.00	0.09		0.06	0.07	0.01	0.48
4	1.94	0.11	0.31	0.36	0.03	0.15		0.02	0.26	0.34	0.46
	1.52	0.30	0.11	0.21	0.03	0.06		0.02	0.26	0.17	0.25
5 + 15	0.10	0.13	0.05	0.07	0.11	0.00		0.00	0.00	0.00	0.11
	0.10	0.06	0.03	0.05	0.07	0.00		0.00	0.00	0.00	0.06
7	1.56	0.07	0.21	0.42	0.12	0.52		0.10	0.87	0.60	0.48
	0.67	0.17	0.08	0.09	0.03	0.31		0.01	0.62	0.34	0.13
8 + 9	3.12	4.74	4.83	19.00	0.17	0.55		0.00	0.85	1.98	9.23
	0.87	0.87	2.11	7.02	0.14	0.25		0.00	0.81	0.78	2.23
10	0.08	0.32	0.50	0.94	0.03	0.03		0.00	0.09	0.17	0.80
	0.03	0.07	0.21	0.33	0.03	0.02		0.00	0.09	0.09	0.21
11	0.63	0.58	0.70	0.34	0.03	0.02		0.00	0.31	0.11	0.84
	0.58	0.19	0.35	0.09	0.02	0.01		0.00	0.30	0.06	0.24
12	0.02	0.55	0.10	1.57	1.36	0.05		0.00	0.64	0.17	1.21
	0.02	0.13	0.06	0.78	0.24	0.03		0.00	0.63	0.07	0.29
13	0.00	1.38	0.15	1.66	8.42	5.37		0.12	0.10	0.41	1.04
	0.00	0.44	0.08	0.47	1.16	5.34		0.03	0.05	0.41	0.24
14	0.00	0.87	0.09	3.61	0.58	0.02		0.03	0.03	0.03	1.23
	0.00	0.31	0.04	1.66	0.29	0.01		0.01	0.02	0.03	0.46
6	0.10	0.15	0.05	0.44	0.42	0.04		0.00	0.01	0.00	0.08
	0.08	0.04	0.03	0.35	0.31	0.01		0.00	0.01	0.00	0.03
16	0.35	0.29	0.15	0.31	2.23	0.07		0.02	0.07	0.12	0.20
	0.21	0.13	0.06	0.09	0.64	0.02		0.00	0.05	0.06	0.05
17	0.00	0.28	0.02	1.62	0.20	0.02		0.00	0.17	0.11	0.34
	0.00	0.07	0.01	0.39	0.06	0.02		0.00	0.10	0.10	0.12
18	0.04	0.33	0.57	1.04	0.19	0.31		0.22	0.52	0.31	0.62
	0.02	0.10	0.15	0.34	0.04	0.11		0.08	0.22	0.09	0.14
19	0.02	0.02	0.04	0.08	0.02	0.04		0.11	0.47	0.03	0.07
	0.02	0.01	0.01	0.04	0.02	0.04		0.03	0.38	0.02	0.02
22	0.02	0.04	0.20	0.00	0.06	0.49		0.60	0.99	0.26	0.01
	0.02	0.02	0.08	0.00	0.06	0.18		0.30	0.43	0.20	0.01
24	0.13	0.02	0.39	0.19	0.18	0.88		10.08	5.29	0.58	0.18
	0.09	0.02	0.16	0.05	0.18	0.28		2.23	2.82	0.50	0.07
25	0.02	0.07	0.43	0.20	0.07	0.26		0.49	0.41	0.01	0.06
	0.01	0.06	0.15	0.12	0.05	0.08		0.23	0.20	0.01	0.02

[a] See footnote *a* of **Table 1**. [b] See footnote *b* of **Table 1**.

level was >10 times higher than that in *E. pallida* var. *angustifolia* roots of the same age and development, 0.063% dwt (**Table 1**). Conversely, the quinic acid derivative cynarin was highest in young, cultivated *E. pallida* var. *angustifolia* (0.5% root dwt, **Table 1**) and absent from all other *Echinacea* varieties of the same age, except *E. pallida* var. *tennesseensis* roots (0.4%) and hybrid populations (0.5%). Both cichoric acid and cynarin in the roots decreased with age, with the exception of *E. atrorubens* var. a*trorubens* (**Tables 1**, **3**, and **4**).

Current industry practice emphasizes the phenolic glycoside echinacoside as a presence marker for *E. pallida* var. *angustifolia* vs. *E. purpurea* (absent). Reportedly, this compound is not responsible for the immunostimulant clinical activity of *Echinacea* phytomedicines and has only minor antimicrobial activity (*9*). In this study, echinacoside was present in the roots of three *Echinacea* species (seven of eight varieties, see **Tables 1**−**6**), and this is the first report of the phenolic from *E. atrorubens* and *E. laevigata*. Previously, echinacoside was reported only from the roots of both *E. pallida* var. *pallida* (0.4−1.7%) and *E. pallida* var. *angustifolia* (0.3−1.3%) (*5*). Comparable levels of echinacoside were noted in the current study for those two market varieties, but the highest average amount was determined from wild *E. atrorubens* var. *paradoxa* roots (3.3% dwt, **Table 3**). Usually the standard industrial marker for *E. pallida* var. *angustifolia*, echinacoside, was present in young, cultivated roots at an average of 0.69% dwt (**Table 1**), but it decreased with

Table 4. Phytochemical Concentrations (Mean, Milligrams per Gram of Dry Weight; SEM Is Given below Each Mean) in Roots of Wild *Echinacea* Transplants

	species										
	purpurea	*pallida angustifolia*	*pallida pallida*	*pallida sanguinea*	*pallida tennesseensis*	*pallida simulata*	*laevigata*	*atrorubens paradoxa*	*atrorubens neglecta*	*atrorubens atrorubens*	hybrids
N[a]	5	8	9	7	17	1	3	0	2	6	0
cic[b]	4.81	0.08	0.05	0.06	0.03		0.53			0.07	
	2.66	0.01	0.02	0.03	0.01		0.27			0.02	
ech	0.54	1.21	0.18	0.25	0.01		0.00			0.48	
	0.53	0.54	0.04	0.11	0.01		0.00			0.31	
chl	0.16	0.04	0.26	0.09	0.02		0.07			0.03	
	0.10	0.02	0.13	0.05	0.01		0.06			0.01	
caf	0.07				0.01		0.14				
	0.01				0.00		0.04				
ctar	0.83	0.06	0.04	0.06	0.00		0.00			0.04	
	0.64	0.01	0.01	0.03	0.00		0.00			0.02	
cyn	0.08	0.56	0.00	0.00	0.14		0.03			0.00	
	0.04	0.36	0.00	0.00	0.03		0.03			0.00	
1	1.25	0.38	0.08	0.28	0.14		0.17			0.65	
	0.57	0.12	0.03	0.20	0.02		0.07			0.15	
2	4.04	0.53	0.61	0.16	0.44		0.34			0.74	
	1.59	0.19	0.17	0.07	0.14		0.16			0.34	
3	2.83	0.93	0.16	1.10	0.37		1.33			1.24	
	1.02	0.36	0.08	0.74	0.07		0.63			0.62	
4	0.30	0.02	0.02	0.02	0.27		0.02			0.01	
	0.15	0.01	0.01	0.01	0.08		0.01			0.01	
5 + **15**	0.53	0.71	0.06	0.11	0.24		0.08			1.70	
	0.25	0.17	0.03	0.06	0.04		0.08			1.03	
7	2.30	0.13	0.17	0.13	0.33		0.23			1.22	
	0.80	0.06	0.06	0.07	0.09		0.11			1.03	
8 + **9**	4.61	9.94	0.39	6.64	1.62		0.06			9.50	
	1.81	1.82	0.21	4.15	0.24		0.03			2.61	
10	0.39	1.43	0.03	0.32	0.20		0.01			0.97	
	0.14	0.33	0.02	0.21	0.03		0.01			0.44	
11	0.20	2.27	0.04	0.18	0.11		0.00			0.68	
	0.07	0.58	0.02	0.13	0.02		0.00			0.24	
12	0.06	2.19	0.03	1.30	2.83		0.48			1.78	
	0.06	0.43	0.02	0.84	0.34		0.25			0.49	
13	0.00	2.27	0.02	0.87	6.93		0.20			0.49	
	0.00	0.48	0.02	0.45	0.46		0.09			0.46	
14	0.02	1.22	0.08	1.57	0.66		0.28			1.68	
	0.02	0.28	0.04	1.06	0.08		0.14			0.63	
6	0.00	0.18	0.07	0.17	0.24		0.09			0.17	
	0.00	0.06	0.03	0.09	0.03		0.04			0.09	
16	0.74	0.22	0.03	0.09	0.83		0.04			0.25	
	0.19	0.04	0.02	0.04	0.13		0.04			0.09	
17	0.06	0.25	0.03	0.45	0.57		0.11			0.07	
	0.03	0.05	0.02	0.29	0.05		0.06			0.06	
18	0.08	1.33	0.42	0.80	0.43		0.01			1.28	
	0.02	0.31	0.13	0.48	0.09		0.01			0.47	
19	0.00	0.14	0.02	0.12	0.00		0.01			0.13	
	0.00	0.03	0.01	0.08	0.00		0.01			0.05	
22	0.00	0.04	0.40	0.14	0.00		0.44			0.01	
	0.00	0.04	0.14	0.10	0.00		0.23			0.01	
24	0.02	0.07	0.74	0.22	0.04		0.04			0.13	
	0.02	0.02	0.19	0.10	0.01		0.04			0.04	
25	0.00	0.04	0.71	0.08	0.00		0.00			0.03	
	0.00	0.01	0.23	0.05	0.00		0.00			0.02	

[a] See footnote *a* of **Table 1**. [b] See footnote *b* of **Table 1**.

age to 0.12−0.2% dwt (**Tables 3** and **4**), similar to the other phenolics mentioned above. Other varieties of *E. pallida* also demonstrated this decreasing trend with age, such as roots of *E. pallida* var. *pallida* and *E. pallida* var. *tennesseensis*, as well as *E. laevigata* and hybrids (**Tables 1**, **3**, and **4**). However, *E. pallida* var. *sanguinea* and *E. atrorubens* vars. *atrorubens* and *neglecta* showed increased levels of echinacoside with root age. A consistent lack of echinacoside denoted *E. purpurea* root phenolic profiles.

Caftaric acid in *Echinacea* roots was present in the same species and varieties that contained cichoric acid. The age trend was the same (decreasing with age), with the same exception; *E. atrorubens* vars. *atrorubens* and *neglecta* showed increased levels of caftaric acid with age (**Tables 1**, **3**, and **4**). Finally, chlorogenic acid, which is widely distributed throughout the plant kingdom, was highest in wild roots of *E. purpurea* (0.1% dwt) and young cultivated roots of *E. laevigata* (0.1% dwt). The next highest levels were found in the rare varieties: *E. pallida* var. *sanguinea* wild roots (0.05% dwt) and *E. atrorubens* var. *paradoxa* wild roots (0.03% dwt) (**Table 3**). Chlorogenic acid was present in low levels in all other varieties of the genus; it showed an increasing trend with age in *E. pallida* var. *pallida*

Table 5. Phytochemicals (Mean, Milligrams per Gram of Dry Weight; SEM Is Given below Each Mean) in Inflorescences of Wild *Echinacea* Transplants

	species										
	purpurea	*pallida angustifolia*	*pallida pallida*	*pallida sanguinea*	*pallida tennesseensis*	*pallida simulata*	*laevigata*	*atrorubens paradoxa*	*atrorubens neglecta*	*atrorubens atrorubens*	hybrids
N[a]	46	3	2	4	0	2	0	0	1	2	0
cic[b]	8.89	0.17	3.24	10.13		2.38				0.04	
	0.92	0.13	3.24	4.62		2.30				0.04	
ech	0.00	0.18	0.34	1.05		0.05				0.03	
	0.00	0.16	0.06	0.76		0.05				0.03	
chl		0.33	1.31	0.63		0.02				2.66	
	.	0.19	0.98	0.38	.	0.01	.	.	.	2.64	.
ctar	.	0.13	1.23	1.61	.	0.02	.	.	.	.	.
		0.05	1.01	0.25		0.01					
cyn		0.31	0.00			0.00					
		0.29	0.00			0.00					
1		0.24	0.54	0.42						1.70	
		0.18	0.48	0.28						1.61	
2		0.71	0.09	0.00						0.05	
		0.62	0.09	0.00						0.05	
3		0.02	0.06	0.14						0.07	
		0.01	0.06	0.14						0.06	
4		0.02	0.03	0.01						0.16	
		0.01	0.03	0.01						0.15	
5 + **15**		0.03	0.03	0.03		0.00				0.28	
		0.01	0.03	0.02		0.00					
7		0.01	0.14	0.00		0.38				0.12	
		0.01	0.08	0.00		0.32				0.11	
8 + **9**	3.13	2.07	1.76	2.17		1.84				0.32	
	0.44	0.81	1.43	0.94		.				0.24	
10		0.02	0.27	0.01		0.03				0.00	
		0.01	0.13	0.01		0.03				0.00	
11		0.08	0.22	0.00		0.03				0.00	
		0.03	0.14	0.00		0.01				0.00	
12		0.05	0.05	0.00		0.01				0.00	
		0.02	0.03	0.00		0.01				0.00	
13		0.00	0.00	0.00		0.00				0.00	
		0.00	0.00	0.00		0.00				0.00	
14		0.00	0.00	0.00		0.00				0.00	
		0.00	0.00	0.00		0.00				0.00	
6		0.02	0.00	0.00		0.07				0.00	
		0.02	0.00	0.00		0.07				0.00	
16		0.05	0.52	0.02		0.13				0.32	
		0.04	0.00	0.01		0.10				0.31	
17		0.01	0.02	0.01		0.00				0.00	
		0.01	0.02	0.01		0.00				0.00	
18		0.26	0.00	0.20		1.12				0.10	
		0.03	0.00	0.10		1.06				0.03	
19		0.00	0.00	0.00		0.04				0.03	
		0.00	0.00	0.00		0.04				0.03	
22		0.00	0.00	0.47						0.00	
		0.00	0.00	0.41						0.00	
24		0.02	0.03	0.00		0.00				0.00	
		0.02	0.03	0.00		0.00				0.00	
25		0.00	0.00	0.00		0.00				0.05	
		0.00	0.00	0.00		0.00				0.05	

[a] See footnote *a* of **Table 1**. [b] See footnote *b* of **Table 1**.

and *E. pallida* var. *sanguinea* and a decreasing trend with age in *E. pallida* var. *angustifolia* and *E. pallida* var. *tennesseensis* (**Tables 1**, **3**, and **4**).

Flowerhead Phenolics. Cynarin and cichoric acid, both major phenolics in *Echinacea* species from different precursors, showed differential distribution temporally and spatially. At the time of first flowering, cynarin was concentrated in roots and cichoric acid was concentrated in flowerheads, for example, cultivated *E. pallida* var. *tennesseensis* (**Tables 1** and **2**). Significantly higher levels of cichoric acid in old, wild flowerheads accompanied decreased root levels, especially in *E. pallida* var. *sanguinea*, which indicated either developmental translocation of this phenolic from roots to vegetative tissues or spatiotemporal shifts in biosynthetic pathways.

Similar to the root cichoric acid pattern, *E. pallida* var. *angustifolia* flowerheads had only 0.017% dwt (**Table 5**), but *E. purpurea* flowerheads from 2-year-old cultivated transplants contained about the same amount of cichoric acid as the young roots of that species (0.9% dwt, **Table 5**). Wild flowerheads contained far more cichoric acid than cultivated flowerheads or roots. The highest measured value was 3% dwt cichoric acid in *E. pallida* var. *sanguinea* (**Table 6**), the species that also yielded the most cynarin in flowerheads (0.03% dwt, **Table 2**), not *E. pallida* var. *angustifolia* as would be inferred from root

Table 6. Phytochemical Concentrations (Mean, Milligrams per Gram of Dry Weight; SEM Is Given below Each Mean) in Wild *Echinacea* Inflorescences

	species										
	purpurea	*pallida angustifolia*	*pallida pallida*	*pallida sanguinea*	*pallida tennesseensis*	*pallida simulata*	*laevigata*	*atrorubens paradoxa*	*atrorubens neglecta*	*atrorubens atrorubens*	hybrids
N[a]	1	16	14	9	5	5	1	4	5	6	10
cic[b]		3.40	12.73	29.75	13.88	6.69		0.39	0.54	0.12	3.12
		2.12	2.89	5.39	3.37	2.12		0.27	0.16	0.05	1.94
ech		0.48	0.67	1.56	1.70	0.21		6.33	18.20	0.07	4.34
		0.11	0.20	0.48	0.56	0.07		1.88	5.32	0.03	3.85
chl		2.17	2.10	2.69	1.72	2.58		3.36	3.80	3.62	3.24
		0.52	0.39	0.98	0.53	0.93		0.70	0.97	1.62	0.76
1		0.33	0.04	0.04	0.07	0.21		0.05	0.28	0.40	0.27
		0.05	0.03	0.03	0.01	0.10		0.02	0.10	0.08	0.15
2		0.25	0.01	0.00	0.01	0.14		0.75	0.69	0.02	0.02
		0.08	0.00	0.00	0.01	0.11		0.41	0.14	0.01	0.01
3		0.02	0.01	0.00	0.01	0.03		0.04	0.03	0.02	0.05
		0.01	0.00	0.00	0.00	0.02		0.03	0.00	0.01	0.03
4		0.03	0.00	0.00	0.00	0.08		0.02	0.07	0.01	0.02
		0.02	0.00	0.00	0.00	0.03		0.01	0.03	0.01	0.01
5 + 15		0.03	0.00	0.00	0.03	0.02		0.63	0.19	0.01	0.00
		0.01	0.00	0.00	0.02	0.01		0.03	0.03	0.01	0.00
7		0.05	0.01	0.00	0.04	0.03		0.12	0.05	0.05	0.15
		0.01	0.00	0.00	0.02	0.01		0.12	0.03	0.05	0.14
8 + 9		3.01	5.55	2.60	4.47	2.46		4.77	5.64	2.54	3.94
		0.41	4.15	0.74	1.74	0.81		0.95	0.52	0.96	1.52
10		0.10	0.09	0.33	0.06	0.03		0.02	0.00	0.04	0.18
		0.02	0.03	0.21	0.03	0.01		0.02	0.00	0.03	0.13
11		0.13	0.04	0.14	0.04	0.00		0.00	0.03	0.01	0.14
		0.02	0.02	0.05	0.01	0.00		0.00	0.03	0.01	0.10
12		0.02	0.02	0.00	0.81	0.05		0.19	0.01	0.09	0.51
		0.01	0.01	0.00	0.21	0.02		0.11	0.01	0.04	0.37
13		0.02	0.01	0.03	0.05	0.02		0.01	0.01	0.03	0.02
		0.01	0.01	0.03	0.03	0.01		0.01	0.01	0.03	0.01
14		0.02	0.03	0.00	0.00	0.02		0.04	0.02	0.01	0.08
		0.01	0.01	0.00	0.00	0.02		0.02	0.01	0.01	0.05
6		0.01	0.01	0.01	0.34	0.04		0.08	0.02	0.03	0.05
		0.00	0.00	0.01	0.26	0.02		0.01	0.02	0.02	0.04
16		0.10	0.03	0.04	0.80	0.23		0.02	0.16	0.04	0.09
		0.02	0.01	0.03	0.43	0.07		0.02	0.04	0.03	0.05
17		0.08	0.17	0.00	0.07	0.02		1.07	0.00	0.01	0.64
		0.07	0.07	0.00	0.02	0.01		0.65	0.00	0.01	0.48
18		0.21	0.21	0.37	0.14	0.23		0.05	0.12	0.68	0.47
		0.05	0.05	0.11	0.06	0.05		0.02	0.04	0.22	0.14
19		0.01	0.01	0.08	0.05	0.00		0.00	0.00	0.02	0.03
		0.01	0.01	0.04	0.02	0.00		0.00	0.00	0.01	0.02
22		0.05	0.00	0.00	0.00	0.29		0.08	0.00	0.04	0.01
		0.05	0.00	0.00	0.00	0.29		0.08	0.00	0.04	0.01
24		0.27	0.67	1.33	0.06	0.04		0.36	0.06	0.02	0.12
		0.17	0.29	0.39	0.05	0.04		0.33	0.05	0.01	0.04
25		0.03	0.06	0.01	0.04	0.01		0.06	0.14	0.04	0.06
		0.01	0.03	0.01	0.03	0.01		0.03	0.06	0.04	0.03

[a] See footnote *a* of **Table 1**. [b] See footnote *b* of **Table 1**.

content. In addition, young flowerheads of *E. pallida* var. t*ennesseensis*, *E. purpurea*, *E. atrorubens* var. *atrorubens*, and *E. pallida* var. *simulata* also contained minute quantities of cynarin (**Table 2**).

Echinacoside measured in inflorescences was highest from the wild-collected *E. atrorubens* var. *neglecta* (1.82% dwt, **Table 6**) as well as *E. atrorubens* var. *paradoxa* (0.63% dwt, **Table 6**). *E. purpurea* and *E. laevigata* lacked echinacoside in flowerheads at all ages and growth conditions, but all five varieties of *E. pallida* revealed a large range in wild-harvested material (0.17−0.27%, **Table 6**), whereas almost nothing in cultivated flowerheads (**Tables 2** and **5**). There were insufficient data to firmly assess echinacoside in wild flowerheads of *E. purpurea* and *E. laevigata*. Overall, three of the four *Echinacea* species contained echinacoside, making this compound not a useful "species identification marker" in cultivated aerial parts on the market.

Chlorogenic acid is a widespread quinic acid derivative both within and outside the genus *Echinacea*, so it is not a diagnostic marker compound, nor is it thought to be medicinally "active" (*9*, *13*, *21*). The levels of chlorogenic acid in roots and flowerheads of the present study were fairly equal across all of the different varieties of *Echinacea*, with the highest levels of each variety occurring in wild flowerheads preserved in situ (0.17−0.38% dwt, **Table 6**).

Chemically similar to cichoric acid, caftaric acid was highest in flowerheads from seed-grown *E. purpurea* at 0.17% dwt (**Table 2**). Flowerheads from transplanted varieties of *E. pallida* also produced close to that amount of caftaric acid (0.013−0.16%, **Table 5**). For example, cultivated flowerheads from *E. pallida* var. *sanguinea* transplants accumulated caftaric acid at

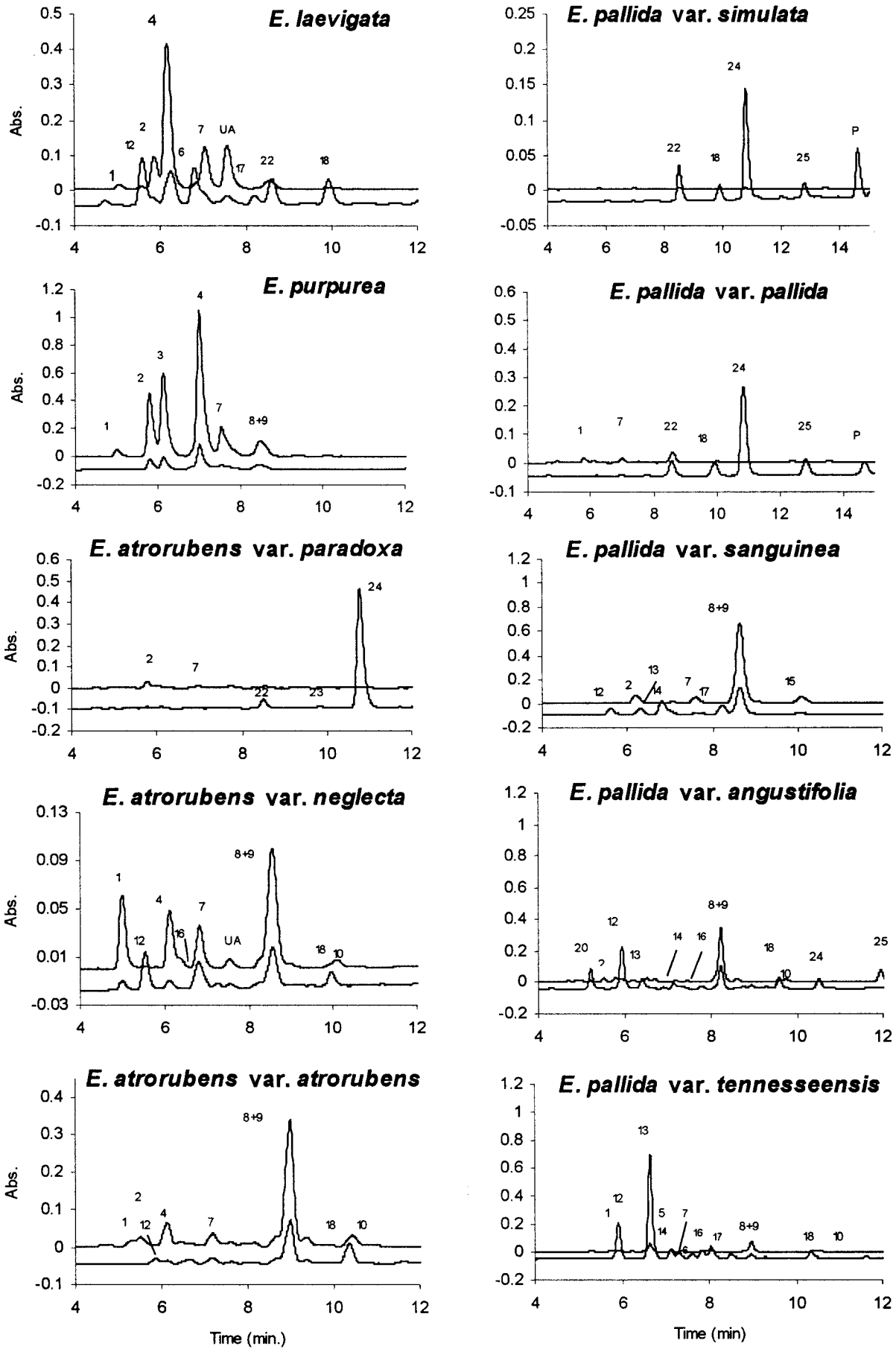

Figure 3. HPLC chromatograms representing typical root profiles of lipophilic phytochemicals in each *Echinacea* taxon. Numbered peaks refer to structures in **Figure 1**: (P) unreported polyene that resembles **22** by UV scan; (UA) unreported alkamide that is usually diene-like by UV scan.

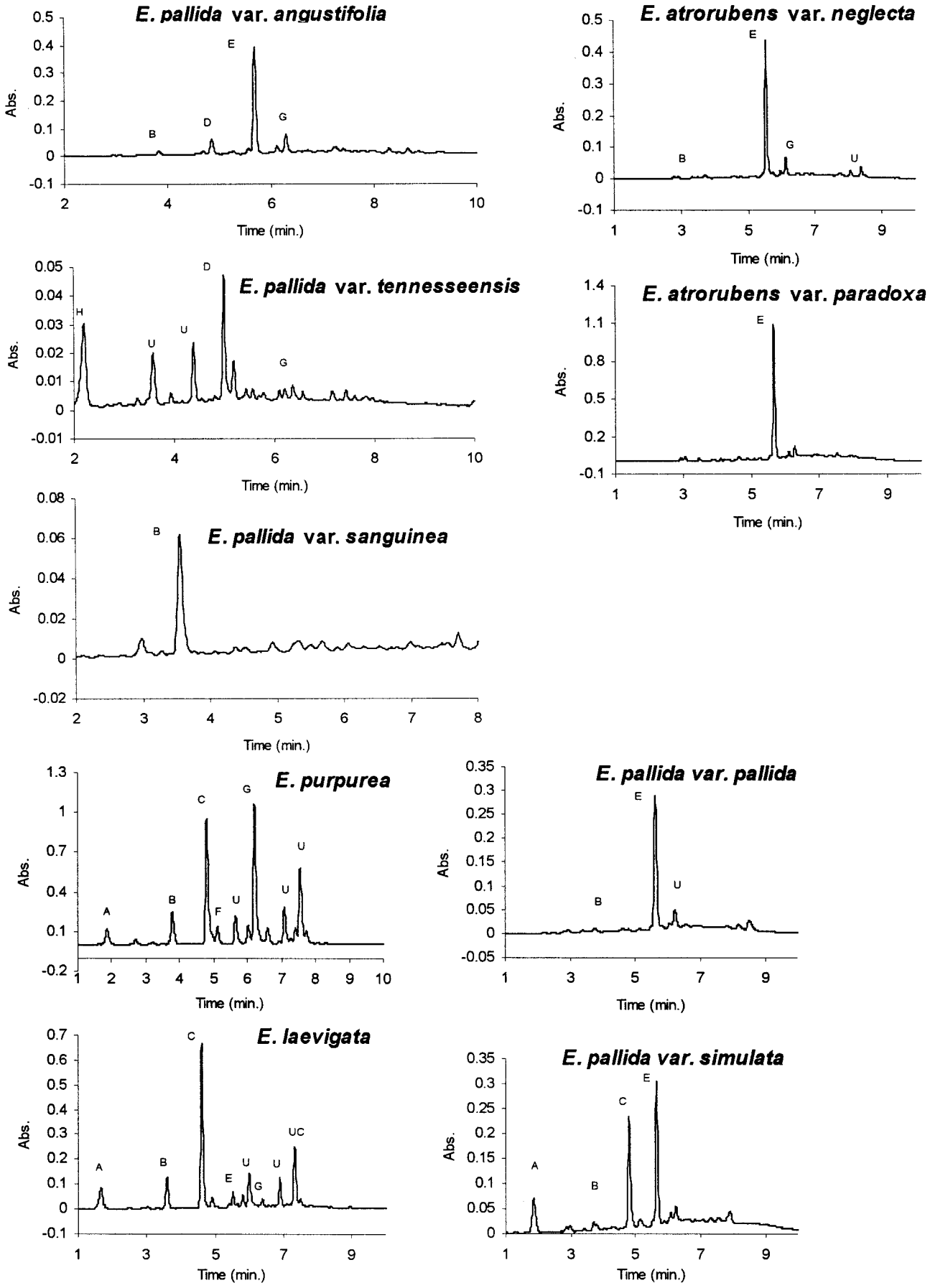

Figure 4. HPLC chromatograms representing typical root profiles of hydrophilic phytochemicals in each *Echinacea* taxon. Peaks refer to structures in **Figure 2**: (A) caftaric acid; (B) chlorogenic acid; (C) cichoric acid; (D) cynarin; (E) echinacoside; (F) cichoric acid methyl ester; (G) rutin; (H) caffeic acid; (U) UV scan resembles chlorogenic acid (unconfirmed); (UC) UV scan resembles cichoric acid (unconfirmed). Absorbance was detected at 326 nm.

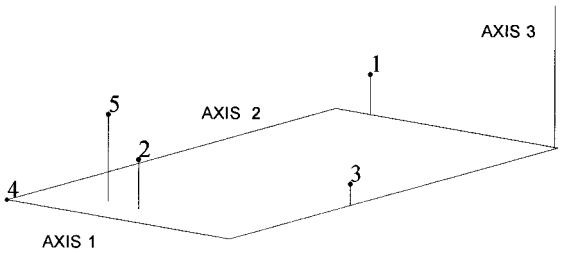

Figure 5. Squared Mahalanobis distances between *Echinacea* species, as determined by CDA using variations in quantitative phytochemistry and represented as canonical coordinates of group centroids in three-space: (**1**) *E. purpurea*; (**2**) *E. pallida*; (**3**) *E. laevigata*; (**4**) *E. atrorubens*; (**5**) hybrids (*E. pallida* × *E. atrorubens*).

0.16% dwt, compared to 0.03% in seed-grown flowerheads (**Tables 2** and **5**). Taxonomic identifications made with phenolics should be reinforced with lipophilic identification markers.

Most hydrophilic *Echinacea* phytochemicals are caffeic acid derivatives, which are known to break down enzymatically in certain preparations (*22*). This activity can be reduced by adding alcohol and reducing agents; caffeic and caftaric acids were formed from the breakdown of cichoric acid in *Echinacea* preparations with <30% ethanol (*22*). In the current study, the *Echinacea* species and varieties containing the most free caffeic acid molecules were the same ones that contained the quinic acid derivative cynarin (results not shown), despite some "missing data" in **Tables 1**−**6** for the cis/trans isomers of caffeic acid. Free caffeic acid may have been higher in these plants as a result of less of the conjugated forms of caffeic acid, such as cichoric acid and caftaric acid.

Species-Level Canonical Discriminant Analysis (CDA) of Roots. CDA allowed differentiation among and between *Echinacea* species and varieties by the overall combination of root phytochemicals instead of single compounds. Lienert et al. (*23*) successfully discriminated three commercial *Echinacea* species using a few accesssions of each in a CDA of phytochemicals measured with GC-MS. In the current study, all four revised *Echinacea* species were distinguished by root CDA, with some exceptions based on different growth conditions and age factors.

Overall phytochemical variation between species is represented as distances between group centroids in three-space on the first three canonical axes (**Figure 5**). The squared Mahalanobis distance between all group centroids was statistically significant ($p < 0.0001$) except between *E. pallida* and the "hybrids" group ($p = 0.6546$). All assumptions of CDA were satisfied using 21 of 26 measured phytochemical characters and 327 of 342 OTUs. The first canonical axis explained 60% of the total variation, whereas the second axis included another 32%. Those compounds responsible for the greatest amount of variation in the CDA, according to the *F* statistic and pooled within canonical structure, were (in order) **2**, cichoric acid, **7**, **3**, **24**, and **1** (**Figure 6**). Furthermore, for the purpose of species identification, CDA showed that all ages of *E. purpurea* roots were lacking cynarin, echinacoside, **6**, **12**−**14**, **19**, **22**, **24** and **25** and that *E. laevigata* roots were lacking cynarin, **4**, **16**, **19**, **22**, and **25**.

CDA of *Echinacea* species was also performed separately for different treatments in the present study (age and growth conditions) to reduce the effect of phenotypic variation on root phytochemical content. Discrimination between species groups was significant in all three root treatments (except for *E. laevigata* and *E. purpurea* older cultivated roots, see below).

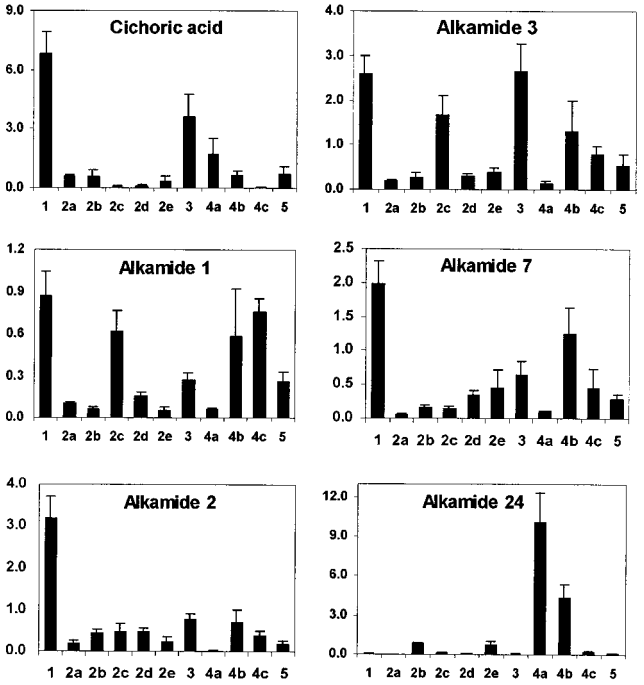

Figure 6. Phytochemicals from *Echinacea* roots of all ages (mean ± SEM in mg/g of dry weight): cichoric acid, alkamide **1**, alkamide **2**, alkamide **3**, alkamide **7** and ketoalkene/yne **24** were important for discrimination by CDA between the following taxa: 1, *E. purpurea*; 2, *E. pallida* varieties *angustifolia* (2a), *pallida* (2b), *sanguinea* (2c), *tennesseensis* (2d), and *simulata* (2e); 3, *E. laevigata*; 4, *E. atrorubens* varieties *paradoxa* (4a), *neglecta* (4b), and *atrorubens* (4c); 5, hybrids (*E. pallida* × ? *E. atrorubens*) (overall $n = 337$).

Those phytochemical variables that were shown to contribute significantly to all of the CDA analyses were determined to be the most robust for quantitative phytochemical species identification markers (**Figure 6**).

Each revised *Echinacea* species was distinctly different according to CDA of the young, cultivated roots ($n = 203$) (squared Mahalanobis distances between centroids were all $p < 0.0001$). The hybrids were not significantly different from *E. pallida* ($p = 0.997$). Eighty percent of the variation was explained in the first canonical axis and 15% in the second, for a total of 95% variation explained in the first two axes. In order of importance, the phytochemicals from young, cultivated roots with the most weight in discriminating between *Echinacea* species were cichoric acid, **2**, **7**, **3**, and **1**.

CDA of older, cultivated roots ($n = 54$) indicated the following differentiation by phytochemical variation between revised taxa. *E. atrorubens* and *E. laevigata* were significantly different ($p = 0.01$), *E. pallida* and *E. atrorubens* were significantly different ($p = 0.03$), and *E. pallida* and *E. laevigata* were significantly different ($p = 0.04$). *E. purpurea* and *E. laevigata* were not signifcantly different ($p = 0.06$), which supported the morphological similarity of these two species (*1*). Those compounds with the most weight in this analysis were **2**, cichoric acid, and **7**, in order of importance by *F* statistics. The first canonical axis represents 53% of the variation, whereas the second axis adds another 33% for a total of 86% in the first two axes.

CDA of wild (old) roots ($n = 75$) distinguished each revised *Echinacea* taxon by phytochemical variation ($p < 0.0001$ for *F* statistics of the squared Mahalanobis distances). However, the hybrids were not significantly distinct from *E. pallida* or from *E. atrorubens*. The first two canonical axes explained 83%

of the variation between the remaining taxa. In this wild root analysis, the most important chemotaxonomic compounds were (in order) chlorogenic acid, **2**, cichoric acid, **7**, and echinacoside. This particular analysis was inconclusive due to insufficient root phytochemical data for *E. purpurea* and *E. laevigata*.

Variety Level CDA. A CDA of 11 taxonomic classes of *Echinacea* (*1*) was performed with 342 OTUs and 13 phytochemical root characters. The probability that squared Mahalanobis distances between the centroids for each taxon were significantly different was $p < 0.0001$, except for the following centroid pairs: *E. pallida* var. *pallida* and *E. pallida* var. *simulata* ($p = 0.9841$), *E. pallida* var. *simulata* and "hybrids" ($p = 0.2105$), and *E. pallida* var. *angustifolia* and "hybrids" ($p = 0.2220$). These findings explain why relatively few key morphological characters were found to distinguish between the above overlapping taxonomic classes in the morphometric CDA (*1*). The CDA between all *Echinacea* varieties, regardless of growth conditions and age, explained 42% of the variation in the first canonical axix and up to 83% in the first three canonical axes. The most important root phytochemicals for this discrimination were (in order) **24**, cichoric acid, echinacoside, **7**, **12**, **2**, and **3**.

Hybrids/introgressants were not distinguishable here from *E. pallida* var. *simulata* or *E. pallida* var. *angustifolia* by root phytochemistry at the species or the variety level. Similarly, hybrids were not distinct in morphometric CDA analyses (*1*). Finally, despite significant distances, the variety-level CDA was a poor ordination compared to the species-level CDA, only 42% compared to 66% in the first axis.

CDA confirmed phytochemical marker compounds for taxonomic identification of *Echinacea* root materials. For species delimitation, they were cichoric acid, **1**−**3**, **7**, and **24**. For variety delimitation, they were the same, with the addition of echinacoside and **12**. Chemotaxonomy at the variety level may be facilitated by the average content of the above compounds (**Tables 1**−**6**) ($n = 358$ roots). Lack of echinacoside and the presence of many diene-type alkamides **1**−**3** and **7** were previously suggested to be diagnostic for *E. purpurea* roots (*9*).

Spatial and Temporal Phytochemical Variation. The extensive alkamide variation within and between *Echinacea* varieties with respect to age and distribution in the plant may be explained by biosynthesis of numerous closely related molecules from a pool of precursors, often termed "phytochemical redundancy". Also, alkamides may serve as precursors for the ketoalkenes, which would explain the lack of alkamides in varieties that accumulate high levels of **22**, **24**, and **25**. Alternatively, a reduction in carbon-based secondary metabolites such as the phenolic caffeic acid derivatives in cultivated *Echinacea* may result from a shift in the carbon/nutrient balance toward growth with greater nutrient availability (*24*). This theory is supported by the results of this study, especially with respect to the cichoric acid content of flowerheads from seed-grown compared to wild-grown *Echinacea* (**Tables 2** and **5**). Fertilization with nitrogen increased the biomass of *E. purpurea* aerial parts, demonstrating the relationship between nutrient availability and growth (*25*). As well, the same group of researchers determined that alkamide content of *E. purpurea* L. roots increased with age and reached a maximum at seed set, whereas the content in vegetative tissues decreased with age (*26*). Therefore, alkamides in *E. purpurea* are spatiotemporally distributed and/or manufactured, much like cynarin in *E. pallida* var. *angustifolia*.

In the present study, tetraenes and alkamides in all roots increased with age with some exceptions and flowerheads cultivated from seed generally accumulated more tetraenes than their wild counterparts, also reported in the literature (*26*). Our results suggested that more mature aerial tissues (all wild populations were >1 year old) translocated their alkamide defenses to the roots or slowed alkamide production over time. Furthermore, the alkamides were predominantly spatially oriented to the roots, whereas phenolics were predominantly located in flowerheads. Two mature plant varieties accumulated higher mean levels of flowerhead tetraenes: *E. pallida* var. *sanguinea* and *E. pallida* var. *pallida*. Biosynthetic changes in response to environmental cues may account for these exceptions; they were growing in dense stands with higher competition and herbivory than what is found in most other *Echinacea* habitats. There have been many reports of such phenotypic plasticity to buffer the effects of variation in resource availability, and species with relatively high growth rates may be more plastic than those of slower growth (*27*). In *Echinacea*, the secondary constituent classes polyynes/enes, alkamides, and caffeic acid derivatives all contain many closely related compounds the relative concentrations of which vary throughout the growth of the plants.

Phytochemical Support of Taxonomic Relationships. Polyploidy is known to promote novel adaptations, such as phytochemical defenses, which may lead to stable introgressants and/or speciation in plants (*28*). In *Echinacea*, one tetraploid is known throughout its range, *E. pallida* var. *pallida* with chromosome number $2n = 44$ (*8*). All other *Echinacea* taxa were determined to be diploid $2n = 22$, except *E. pallida* var. *simulata* (sometimes $2n = 33$ triploidy and sometimes $2n = 22$) and triploid hybrids or stable introgressants (*8*). There are reports of diploidy, triploidy, and, in the southern ranges, tetraploidy for the group of stable introgressants, which McGregor called *E. pallida* var. *strigosa* McGregor.

The ketoalkynes **24** and **25** were previously reported from the roots and flowerheads of tetraploids *E. pallida* var. *pallida* (*5*) and roots of *E. atrorubens* var. *paradoxa* and *E. pallida* var. *simulata* (*12*), yet they have never been correlated to ploidy or taxonomic inferences. From current results, the ability to accumulate ketoalkenes/ynes may be associated with polyploidy. We detected high quantities of ketoalkenes **24** and **25** in the roots of *E. pallida* var. *simulata*, which were sometimes identified as triploids in the past (*8*). Sympatric and possibly hybridizing populations of the putative diploids *E. atrorubens* var. *paradoxa* and *E. atrorubens* var. *neglecta* were also found here to contain **24** and **25** in their roots, indicating the possibility of triploids and/or tetraploids among them, although ploidy was not confirmed in the current study. We also detected **24** in small amounts in the wild flowerheads of *E. pallida* var. *sanguinea* and *E. atrorubens* var. *paradoxa*, both diploids according to McGregor (*8*).

On the basis of ploidy and phytochemistry, there may be a chemotaxonomic relationship between the putative allotetraploids *E. pallida* var. *pallida* and the varieties of *E. atrorubens* (vars. *paradoxa* and *neglecta*), as well as the other varieties of *E. pallida* (vars. *sanguinea* and *simulata*). In addition, McGregor (*8*) suggested that *E. pallida* var. *pallida* (which he treated as a species) arose from hybridization between *E. pallida* var. *simulata* and *E. pallida* var. *sanguinea*. Alloploidy from multiple or recurrent origins exists in plants, for example, in *Asplenium* (*29*), and it may explain the range of phytochemical variation observations in *E. pallida* var. *pallida* tetraploids. Allopolyploidy may also support the apparent prevalence of hybridization events along intermediate geographic zones in the current study as well as others (*1*, *8*). A more thorough examination of ploidy and

its relationship to ketoalkyne/ene production is suggested in view of the current results.

The phytochemical variation reported in this study from *Echinacea* species and varieties was useful from a chemotaxonomic perspective in combination with the recent morphometric taxonomic revision (*1*). Accurate phytochemical profiles and taxonomic distinctions described herein for current wild *Echinacea* germplasm sources will prove to be invaluable for conservation, cultivation, germplasm improvement, and quality control of *Echinacea* phytomedicines.

Supporting Information Available: HPLC chromatograms representing typical flowerhead profiles of lipophilic phytochemicals and root profiles of hydrophilic phytochemicals in each *Echinacea* taxon. This material is available free of charge via the Internet at http://pubs.acs.org.

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