HortScience 44(2):349–353. 2009.

Lignan and Nutrient Concentrations in American Mayapple (*Podophyllum peltatum* L.) in the Eastern United States

Valtcho D. Zheljazkov[1] and Andrew M. Jones
Mississippi State University, North Mississippi Research and Extension Center, 5421 Highway 145 South, Verona, MS 38879

Bharathi Avula
National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677

Victor Maddox
Mississippi State University, Geosystems Research Institute, P.O. Box 9627, Mississippi State, MS 39762

Dennis E. Rowe
Mississippi State University, Experimental Statistics, P.O. Box 9653, Mississippi State, MS 39762

Additional index words. mayapple, *Podophyllum*, podophyllotoxin, α-peltatin, β-peltatin

***Abstract*. Podophyllotoxin is an anticancer compound found in Indian mayapple (*Podophyllum emodii* Wall.), American mayapple (*Podophyllum peltatum* L.), and other species. Podophyllotoxin and its derivatives are used in several commercially available pharmaceutical products such as the anticancer drugs etoposide, teniposide, and etopophos. Currently, the commercial production of podophyllotoxin is based on Indian mayapple. The objective of this study was to estimate podophyllotoxin concentration in American mayapple across its natural habitats in the eastern United States and to identify high podophyllotoxin types that could be used for further selection and cultivar development. Analyses of American mayapple leaves collected from 37 mayapple colonies across 18 states indicated a significant variation in podophyllotoxin, α-peltatin, and β-peltatin content and the presence of chemotypes. Overall, the concentrations of podophyllotoxin, α-peltatin, and β-peltatin in the collected accessions ranged from below detectable levels to 45.1, 47.3, and 7.0 $mg \cdot g^{-1}$ dry weight, respectively. We classified American mayapple accessions into seven groups: 1) with very high concentration of podophyllotoxin (greater than 20 $mg \cdot g^{-1}$) and no α- or β-peltatin; 2) high podophyllotoxin (greater than 10 $mg \cdot g^{-1}$) and no α-peltatin but trace amounts of β–peltatin; 3) medium podophyllotoxin (1 to 10 $mg \cdot g^{-1}$) and no α- or β-peltatin; 4) low podophyllotoxin (0.05 to 1 $mg \cdot g^{-1}$) and high α-peltatin; 5) trace amounts of podophyllotoxin and high concentration of α-peltatin and α-peltatin; 6) high α-peltatin and trace amounts of podophyllotoxin or β–peltatin; and 7) high α–peltatin and no podophyllotoxin or β-peltatin. American mayapple was found to grow on various soil types with a range of pH (4.6 to 7.6) and dissimilar concentrations of phytoavailable soil nutrients. Tissue zinc concentration was positively correlated to podophyllotoxin, whereas soil and tissue phosphorus was positively correlated to the concentration of α-peltatin. The results from this study may contribute toward the development of high podophyllotoxin-containing varieties of American mayapple and the development of a new cash crop for American farmers.**

Podophyllotoxin is an anticancer compound found in high concentrations in Indian mayapple [*Podophyllum emodii* Wall. (syn. *P. hexandrum* Royle.)], American mayapple (*Podophyllum peltatum* L.), Eastern red cedar (*Juniperus virginiana* L.), and other species from different plant families (Arroo et al., 2002; Ayres and Loike, 1990; Bedir et al., 2002; Cushman et al., 2003; Gawde et al., 2009; Gordaliza et al., 2004; Ionkova, 2007; Moraes et al., 2000; Saraeva, 1952). Podophyllotoxin and its derivatives are used in the semisynthesis of several commercially available pharmaceutical products. These include the anticancer drugs etoposide, teniposide, and etopophos, which are used in the treatment of ailments such as small-cell lung cancer, lymphoblastic leukemia, testicular cancer, and brain tumors (Farkya et al., 2004; Holthuis, 1988; Stahelin and von Wartburg, 1991). In addition, podophyllotixin derivatives are being used for the treatment of psoriasis and malaria, and some are being tested for the treatment of rheumatoid arthritis. Furthermore, podophyllotoxin-containing preparations are commercially available for dermatological use.

Currently, the commercial production of podophyllotoxin is based on Indian mayapple (roots and rhizomes), an endangered species harvested from the wild in India, Pakistan, Nepal, and China. Mayapple (both *P. peltatum* and *P. emodii*) has been grown as a cash crop in Europe and Russia (Bogdanova and Sokolov, 1973; Kohlmunzer et al., 1971; Kuznetsova and Bogdanova, 1970; Saraeva, 1952). However, mayapple has never been introduced as a crop from another country and has not been domesticated in the United States, although the idea was suggested more than 3 decades ago (Meijer, 1974). Previous research demonstrated that American mayapple leaves contain podophyllotoxin (Bedir et al., 2002; Cushman et al., 2001, 2005a, 2005b; Moraes et al., 2001a, 2001b), raising the possibility for the development of American mayapple as a source of podophyllotoxin and a high-value crop for American growers.

Several previous reports indicated variations in podophyllotoxin concentration among American mayapple sampled from different locations in the United States (Moraes et al., 2001a, 2001b, 2005). Like most species producing and accumulating secondary metabolites, American mayapple may include a number of chemotypes. A comprehensive study on the genetic resources of American mayapple colonies across the United States is lacking. We hypothesized that there might be great variation with respect to podophyllotoxin content within American mayapple across the eastern United States. Furthermore, soil type and the concentration of nutrients in plant tissue or in soil may affect the concentration of podophyllotoxin in American mayapple. The objective of this study was to study the effect of location, plant nutrient concentration, and phytoavailable nutrients in soil on podophyllotoxin concentration in American mayapple across its natural habitats in the eastern United States. This study is the largest of this kind in the world conducted so far.

Materials and Methods

In 2006, American mayapple colonies were sampled in the eastern part of the United States, from northern New England to Georgia, and west to Texas and Minnesota. Before initiation of this extensive sampling, a number of permits had to be obtained from

Received for publication 10 Dec. 2008. Accepted for publication 14 Jan. 2009.
This research was funded by ARS Specific Coop. Agreement 58-6402-4-026 with CRIS MIS-172050 and by the National Center for Natural Products Research grant.
Contribution of the Mississippi Agricultural and Forestry Experiment Station journal article J-11499 and the National Center for Natural Products Research.
We thank Mr. Thomas Horgan for his help with the collections.
[1]To whom reprint requests should be addressed; e-mail vj40@pss.msstate.edu.

National Park Services and National Forests. Thirty-seven mayapple colonies were sampled in 18 states: Mississippi, Alabama, Arkansas, Georgia, Kentucky, Louisiana, Maryland, Massachusetts, New York, New Hampshire, North Carolina, Ohio, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, and West Virginia.

Sampling was done in every state, using Global Positioning System (GPS) Garmin 276C (Garmin Ltd., Olathe, KS). Every location was assigned a GPS waypoint number in addition to the name of the county. Herbarium samples were assigned a voucher number that served as the accession number for each plant collected. A digital picture was also taken for a digital library. From every location, a composite plant tissue (leaves from five to 15 plants) and soil samples (four to five cores per site under the mayapple canopies at 0- to 15-cm soil depth) were collected. The collected plant tissue and soil samples were placed in brown paper bags, whereas samples for the herbarium were placed immediately in a botanical press for drying and preserving. The plant material designated for lignans and nutrient analyses were dried in an oven at 40 °C for 72 h, whereas the soil samples were dried at room temperature (at 24 ± 2 °C) for 7 d.

Soil and tissue nutrient analysis. The soil samples were analyzed for soil pH, phytoavailable (extractable) nutrients, total soluble salts, and organic matter at the Mississippi State University Extension Service, Soil Testing Laboratory, using the Lancaster soil test method (Cox, 2001). Determination of specific conductance (total soluble salts) was done using conductivity meter YSI (YSI, Yellow Springs, OH) Model 32 conductance meter (Willard et al., 1968). Plant tissue samples for nutrient analyses were ground to pass a 2-mm screen and decomposed using the dry ashing method (Jones and Steyn, 1973). The concentration of macro- and micronutrients in tissue and soil extracts was measured by an Inductively Coupled Argon Plasma Spectrometer Model 4300 Optima DV (Perkin Elmer Instruments, Norwalk, CT).

Plant tissue preparation, extraction, and analysis of podophyllotoxin, α-peltatin, and β-peltatin. The oven-dried plant tissue samples were weighed, ground with a coffee grinder, and used for extractions and measurements of podophyllotoxin, α-peltatin, and β-peltatin content using high-performance liquid chromatography (HPLC). The extraction and the measurements of podophyllotoxin, α-peltatin, and β-peltatin content was conducted as described by Canel et al. (2001). Briefly, 500 mg of the plant tissue was dissolved in 10.0 mL of phosphate buffer (at pH 7.0) and incubated on a rotary shaker for 30 min at 130 to 150 rpm. After that, 10.0 mL ethyl acetate was added, and the samples were incubated on the shaker for another 2 h. The ethyl acetate phase was separated using centrifugation, collected, dried under nitrogen, the residue was redissolved in methanol, sonicated for 20 min, filtered, and used for HPLC measurements. The HPLC instrument consisted of Waters Alliance 2695 (Waters Corp., Milford, MA) equipped with a 996 photodiode array detector (Waters Corp.) and a computerized data station equipped with Waters Empower-2 software. Separation was achieved on a Hypersil C18 column (Phenomenex, 150 × 4.6 mm i.d., 5-μm particle size; Phenomenex Inc., Torrance, CA) and operated at 30 °C. The column was equipped with a 2-cm LC-18 guard column (Supelco, Bellefonte, PA). The mobile phase consisted of water (A) and acetonitrile (B) both containing 0.025% trifluoroacetic acid, which were applied in the following gradient elution: 0 min, 72% A; 28% B for 15 min; and to 100% B in the next 5 min. Each run was followed by a 5-min wash with 100% acetonitrile and an equilibration period of 13 min. The flow rate was adjusted to 1.0 mL/min and detected at 220 nm. Using the reference standard of podophyllotoxin, α-peltatin, and β-peltatin (1 to 300 $\mu g \cdot mL^{-1}$), a standard curve was obtained, and the quantitative analysis was performed using the software generated calibration curves. All solvents were HPLC-grade from Fisher Scientific (Waltham, MA).

Statistical analysis. Analysis of variance was conducted on the data collected to determine variations in the concentration of podophyllotoxin, α-peltatin, and β-peltatin, the concentration of soil phytoavailable nutrients, and tissue nutrients.

Results and Discussion

Analyses of American mayapple leaves collected from 37 mayapple colonies across 18 states indicated a significant variation in podophyllotoxin, α-peltatin, and β-peltatin content, confirming our hypothesis and supporting previous research about the presence of chemotypes (Moraes et al., 2001a). Overall, the concentrations of podophyllotoxin, α-peltatin, and β-peltatin in the collected accessions ranged from below detectable levels to 45.04, 47.30, and 7.01 $mg \cdot g^{-1}$ dry weight, respectively (Table 1). Our results suggest that mayapple accessions could be classified into seven groups or chemotypes: 1) very high concentration of podophyllotoxin (greater than 20 $mg \cdot g^{-1}$) and no α- or β-peltatin (e.g., accession 8); 2) high podophyllotoxin (greater than 10 $mg \cdot g^{-1}$) no α-peltatin but trace amounts of β-peltatin (i.e., accession 9); 3) medium podophyllotoxin (1 to 10 $mg \cdot g^{-1}$) and no α- or β-peltatin (accession 18); 4) low-podophyllotoxin (0.05 to 1 $mg \cdot g^{-1}$) and β-peltatin and high α-peltatin (accession 11); 5) trace amounts of podophyllotoxin and high concentration of α-peltatin and β-peltatin (accession 30); 6) high α-peltatin and trace amounts of podophyllotoxin and no β-peltatin (accession 13); and 7) high α-peltatin and no podophyllotoxin or β-peltatin (accession 5).

The soil sampled within the mayapple colonies had vastly dissimilar physical and chemical characteristics (Table 2). For example, soil organic matter (OM) varied from 1.6 to 8.3 and soil pH varied from 4.6 (acidic) to 7.6 (alkaline), demonstrating that mayapple may grow on a wide variety of soil alkalinity. The content of clay varied from 1.25% to 7.5%, the silt content was from 13.2% to 87%, and sand content varied from 8.0% to 85.5%, indicating mayapple could thrive on a wide variety of soil texture, ranging from sandy, sandy loams, to silty loams. Also, the concentration of phytoavailable nutrients in soil and accumulation of nutrients in plant tissue were different among the locations (Table 2). Our results suggest a wide environmental flexibility of American mayapple, which agree with previous research that demonstrated American mayapple natural habitats and colonies could be found from sea level to 1400 m above sea level (Sohn and Plicansky, 1977).

The results of this research supported the assumption that the level of soil phytoavailable or tissue nutrients may influence the concentration of lignans in mayapple. Zinc plant tissue concentration only, of all the measured nutrients in soil and plant tissue, was positively correlated ($r = 0.5$, $P = 0.02$) to podophyllotoxin concentration in mayapple leaves. Soil OM was positively correlated to the accumulation of phosphorus (P) ($r = 0.54$, $P = 0.0007$), potassium ($r = 0.85$, $P < 0.0001$) and calcium ($r = 0.54$, $P = 0.0007$) in mayapple leaves. Of soil physical properties, the silt content was positively ($r = 0.36$, $P = 0.035$), whereas sand content was negatively ($r = -0.37$, $P = 0.029$) correlated to the α-peltatin in leaves. Pearson correlations demonstrated a positive relationship ($r = 0.64$, $P < 0.001$) between soil-extractable P concentration and the concentration of β-peltatin and a highly significant correlation ($P < 0.0001$) of plant tissue P concentration and β-peltatin, indicating that elevated levels of P in soil or plant may increase the accumulation of this lignan in mayapple leaves. The concentration of podophyllotoxin in mayapple leaves was negatively correlated to the concentration of α-peltatin, whereas the concentration of β-peltatin was positively correlated to α-peltatin.

Cluster analysis for podophyllotoxin indicated that mayapple colonies in close proximity may exhibit a significant difference as clusters with respect to α-peltatin, β-peltatin, or podophyllotoxin (data not shown). Based on the cluster analysis, mayapple could be divided into three major groups with respect to podophyllotoxin concentration, with 16 and 34 (sampled at approximately the same longitude in Mississippi and Alabama, respectively) as a single group and the rest of the sites about evenly divided between the two other groups.

The results from this study suggest that: 1) there are great variations in lignan concentrations in mayapple colonies and there are chemotypes within mayapple natural habitats in the eastern United States; 2) neighboring mayapple colonies may not necessarily belong to the same chemotype group. Furthermore, it appears birds (and other animals) might have played a considerable role in the distribution of mayapple seeds resulting in

Table 1. Concentrations of podophyllotoxin, α-peltatin, and β-peltatin and plant macro- and micronutrients in mayapple leaves collected across 18 states in the eastern United States.

Location	α-peltatin	Podophyllotoxin	β-peltatin	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulfur	Iron	Manganese	Zinc	Copper	Boron
	($mg \cdot g^{-1}$)			(%)						($mg \cdot kg^{-1}$)				
1	0.0 ± 0[z]	10.7 ± 0.1	0.04 ± 0.002	2.5	0.13	2.9	1.0	0.19	0.07	71	161	10	2	—
2	1.0 ± 0	11.0 ± 0.08	0.03 ± 0	2.6	0.14	2.1	1.4	0.14	0.11	54	280	10	6	18
3	32.7 ± 0.1	0.0 ± 0	4.7 ± 0.4	2.2	0.10	2.1	1.0	0.31	0.11	363	437	13	6	20
4	45.4 ± 0.8	0.0 ± 0	3.3 ± 0.2	2.5	0.16	2.9	1.4	0.51	0.09	54	285	8	5	25
5	47.1 ± 0.4	0.0 ± 0	0.0	1.9	0.13	2.9	0.5	0.34	0.19	112	685	8	5	28
6	1.3 ± 0.3	17.9 ± 0	0.03 ± 0	2.1	0.23	3.3	0.9	0.30	0.25	175	572	7	8	27
7	0.6 ± 0.1	16.6 ± 0.1	0.0	2.3	0.12	2.5	0.8	0.33	0.14	93	440	12	5	41
8	0.0 ± 0	23.3 ± 0.1	0.0	3.1	0.14	1.8	1.4	0.46	0.14	74	538	9	6	24
9	0.0 ± 0	12.6 ± 0.2	0.1 ± 0.01	2.3	0.11	2.4	1.1	0.14	0.15	297	531	11	6	30
10	0.8 ± 0	12.6 ± 0.1	0.0	1.9	0.14	3.3	1.1	0.17	0.18	67	406	9	4	33
11	25.9 ± 0.1	0.5 ± 0.1	0.1 ± 0.002	2.5	0.20	2.8	1.1	0.29	0.12	63	698	13	9	48
12	28.5 ± 0.2	0.0 ± 0.01	0.0	2.3	0.16	2.7	0.9	0.28	0.12	109	425	12	6	34
13	22.9 ± 0.5	0.05 ± 0	0.0	1.7	0.14	2.3	1.4	0.13	0.18	55	226	15	3	27
14	1.3 ± 0.2	11.2 ± 0.01	0.1 ± 0.01	2.4	0.10	1.5	1.4	0.22	0.13	62	413	12	6	16
15	0.0 ± 0	3.3 ± 0.2	0.0	1.1	0.06	2.5	1.3	0.12	0.07	64	395	10	2	27
16	0.6 ± 0	27.8 ± 0.1	0.0	1.5	0.07	1.1	2.0	0.39	0.14	57	211	7	4	28
17	0.5 ± 0.01	1.0 ± 0.2	0.0	1.7	0.08	2.8	1.2	0.22	0.09	75	185	9	1	33
18	0.0 ± 0.01	4.1 ± 0.01	0.0	1.8	0.10	2.2	1.2	0.42	0.10	361	312	8	7	27
19	3.5 ± 0	1.2 ± 0.02	0.1 ± 0.01	1.7	0.18	2.5	0.9	0.19	0.08	116	519	17	3	21
20	0.2 ± 0.03	2.8 ± 0.02	0.0	1.5	0.07	2.6	0.9	0.36	0.13	58	433	10	2	28
21	0.5 ± 0.04	21.2 ± 0.4	0.0	2.1	0.08	2.3	0.9	0.17	0.18	94	408	15	5	28
22	0.1 ± 0.02	5.1 ± 0.3	0.1 ± 0.01	2.2	0.14	2.6	1.1	0.16	0.07	109	351	14	4	37
23	0.6 ± 0.01	5.7 ± 0.7	0.0	—	—	—	—	—	—	—	—	—	—	—
24	43.9 ± 0.08	0.0 ± 0	0.04 ± 0	1.9	0.09	1.9	0.9	0.24	0.07	59	634	20	6	22
25	47.3 ± 0.5	0.1 ± 0	1.1 ± 0.1	—	—	—	—	—	—	—	—	—	—	—
26	0.0 ± 0	8.1 ± 0.1	0.0	2.3	0.09	3.5	1.1	0.25±	0.09	138	59	6	1	26
27	3.0 ± 0.01	0.1 ± 0	0.0	2.0	0.13	3.2	1.1	0.16	0.20	46	382	8	2	27
28	0.7 ± 0.04	13.0 ± 0.3	0.1 ± 0	3.1	0.13	3.0	1.1	0.28	0.08	72	179	6	5	17
29	0.8 ± 0.1	5.1 ± 0.6	0.0	3.0	0.12	2.1	0.7	0.18	0.30	81	360	8	7	22
30	27.1 ± 0.6	0.1 ± 0	7.0	2.4	0.26	2.9	2.2	0.12	0.11	66	76	6	4	29
31	0.9 ± 0.01	17.5 ± 0.1	0.0	2.2	0.10	2.7	1.2	0.21	0.16	44	450	17	6	33
32	0.5 ± 0.01	13.6 ± 0.1	0.1 ± 0	2.0	0.12	1.6	1.9	0.39	0.18	78	103	6	5	29
33	1.4 ± 0.04	7.6 ± 0.1	0.2 ± 0.01	2.0	0.17	3.0	1.2	0.16	0.15	59	388	15	3	41
34	0.1 ± 0.01	45.1 ± 5.1	0.0	2.7	0.21	2.7	0.9	0.12	0.19	46	513	8	5	40
35	0.6 ± 0.01	0.1 ± 0.01	0.0	2.2	0.14	2.9	2.1	0.13	0.22	62	313	6	4	29
36	1.8 ± 0.02	0.2 ± 0.01	0.0	2.0	0.10	1.2	2.2	0.16	0.15	1,030	104	6	5	38
37	0.8 ± 0.02	0.4 ± 0.02	0.0	—	—	—	—	—	—	—	—	—	—	—

[z]± SD.

Table 2. Concentrations of the phytoavailable nutrients in soil, soil pH, soil organic matter (OM), and percent clay, silt and sand in soil under each of the mayapple colonies sampled at 37 locations across 18 states in the eastern United States.

		Phosphorus	Potassium	Calcium	Magnesium	Zinc	Sulfur	Sodium	pH	Clay	Silt	Sand
Location	OM (%)	(lb/acre)										
1	3.3	66 ± 0.3[z]	314 ± 1.2	4,414 ± 17	410 ± 1.2	10.1 ± 0.1	207 ± 137	98 ± 0.9	6.4	1.2	68.8	30.0
2	2.4	25 ± 0.6	138 ± 1.5	2,031 ± 68	274 ± 1.7	3.7 ± 0.1	142 ± 98	85 ± 1.5	5.7	5.0	46.0	49.0
3	3.2	32 ± 0.9	100 ± 1.4	1,435 ± 64	351 ± 4.3	4.1 ± 0.2	143 ± 95	95 ± 3.1	5.3	5.0	71.0	24.0
4	1.6	17 ± 0.7	109 ± 0.9	680 ± 47	302 ± 2.6	2.2 ± 0.1	105 ± 63	93 ± 2.3	5.0	5.0	45.0	49.3
5	1.9	36 ± 1.2	152 ± 1.5	1,472 ± 64	487 ± 5.4	9.4 ± 0.3	126 ± 77	99 ± 1.5	5.1	5.0	74.0	20.3
6	1.6	85 ± 1.5	150 ± 1.0	631 ± 47	347 ± 4.7	5.1 ± 0.2	107 ± 60	98 ± 1.5	4.9	5.0	87.0	8.0
7	1.7	47 ± 1.0	108 ± 1.0	1,279 ± 59	332 ± 3.2	3.9 ± 0.1	111 ± 69	91 ± 0.9	5.4	2.5	54.3	43.3
8	1.7	35 ± 1.0	114 ± 0.9	1,304 ± 62	339 ± 4.7	6.2 ± 0.2	117 ± 65	116 ± 0.9	5.6	5.0	77.0	18.2
9	2.5	62 ± 1.2	143 ± 1.5	867 ± 54	269 ± 3.2	7.0 ± 0.2	181 ± 91	154 ± 0.7	5.0	5.0	71.0	24.0
10	2.2	40 ± 1.2	104 ± 1.0	515 ± 46	61 ± 1.9	1.2 ± 0.1	149 ± 86	96 ± 4.8	5.3	1.2	24.3	74.5
11	3.1	104 ± 2.1	149 ± 0.3	938 ± 48	106 ± 2.0	3.2 ± 0.1	205 ± 119	161 ± 8.3	4.8	2.5	55.0	42.25
12	5.8	233 ± 24.0	318 ± 3.5	2,306 ± 79	267 ± 4.2	7.2 ± 0.2	337 ± 248	162 ± 2.4	5.1	1.2	49.3	49.5
13	1.3	32 ± 0.9	62 ± 0.3	461 ± 46	77 ± 1.9	1.8 ± 0.1	92 ± 50	70 ± 0.6	4.7	2.5	20.75	76.75
14	1.5	20 ± 0.8	123 ± 1.5	1,592 ± 73	207 ± 3.1	2.7 ± 0.1	121 ± 49	80 ± 1.5	4.6	7.5	24.5	68.0
15	2.3	36 ± 0.9	99 ± 1.4	2,078 ± 81	202 ± 3.0	2.3 ± 0.1	143 ± 91	85 ± 3.1	4.9	6.2	43.5	50.3
16	2.9	50 ± 1.2	154 ± 1.8	1,787 ± 73	227 ± 3.7	4.6 ± 0.3	169 ± 127	93 ± 1.0	6.2	1.2	16.5	82.3
17	1.8	16 ± 0.7	65 ± 0.9	1,032 ± 59	139 ± 2.7	0.8 ± 0.1	108 ± 73	84 ± 0.6	5.4	2.5	23.0	74.5
18	1.8	20 ± 0.9	59 ± 0.6	579 ± 48	88 ± 2.0	1.3 ± 0.1	111 ± 73	75 ± 0.3	5.7	1.2	18.0	80.8
19	3.7	49 ± 1.2	92 ± 0.9	4,077 ± 104	544 ± 5.3	10.2 ± 0.4	205 ± 163	79 ± 0.6	7.3	1.3	31.0	67.7
20	4.6	220 ± 1.0	281 ± 0.6	1,643 ± 1.3	256 ± 1.0	7.3 ± 0.1	262 ± 203	89 ± 0.3	5.1	1.25	41.2	57.6
21	2.6	52 ± 0.7	187 ± 0.3	1,160 ± 3.0	319 ± 1.1	7.8 ± 0.1	161 ± 107	102 ± 0.1	5.3	2.5	47.3	50.2
22	5.8	93 ± 0.3	210 ± 0.6	1,049 ± 5.2	172 ± 1.0	9.6 ± 0.1	338 ± 250	77 ± 0.3	4.9	2.5	43.8	53.7
23	4.8	79 ± 0.6	224 ± 1.7	2,434 ± 12	223 ± 1.5	4.1 ± 0.1	281 ± 206	99 ± 0.3	5.6	2.5	68.5	29.0
24	—[y]	—	—	—	—	—	—	—	—	—	—	—
25	3.5	54 ± 0.3	211 ± 0.3	744 ± 3.8	165 ± 0.9	6.0 ± 0.1	214 ± 143	98 ± 0.3	5.4	2.5	66.5	31.0
26	—	—	—	—	—	—	—	—	—	—	—	—
27	4.7	34 ± 0.3	298 ± 1.5	6,835 ± 6.2	791 ± 4.7	4.3 ± 0.1	259 ± 206	78 ± 0.0	7.0	1.25	22.0	76.75
28	3.2	49 ± 0.7	247 ± 0.7	645 ± 3.1	120 ± 1.0	6.2 ± 0.1	203 ± 127	87 ± 0.7	5.2	5.0	30.5	64.5
29	4.8	50 ± 0.6	370 ± 1.5	1,853 ± 4.8	306 ± 34	6.5 ± 0.1	277 ± 209	83 ± 0.3	5.6	2.5	25.25	72.25
30	2.8	68 ± 0.6	153 ± 0.3	863 ± 6.1	98 ± 1.1	6.7 ± 0.1	194 ± 106	87 ± 0.9	5.3	3.75	45.75	50.5
31	6.4	769 ± 71	333 ± 2.3	5,637 ± 6.7	245 ± 1.5	5.1 ± 0.1	370 ± 275	144 ± 0.6	7.4	1.25	47.5	51.25
32	8.0	187 ± 0.5	446 ± 3.5	2,628 ± 7.2	390 ± 1.3	25.9 ± 0.1	476 ± 340	113 ± 0.6	4.6	3.75	44.25	52.0
33	4.5	57 ± 0.6	183 ± 0.9	6,151 ± 18	824 ± 3.6	17.7 ± 0.2	258 ± 193	92 ± 0.7	6.8	2.5	37.0	60.5
34	3.6	72 ± 0.6	185 ± 0.6	1,513 ± 4.2	145 ± 1.2	11.6 ± 0.2	219 ± 152	128 ± 0.3	4.9	1.25	13.25	85.5
35	4.8	224 ± 0.7	272 ± 1.9	815 ± 2.0	129 ± 1.5	32.7 ± 0.3	322 ± 186	92 ± 0.6	5.5	1.25	26.25	72.5
36	5.3	65 ± 0.3	434 ± 3.0	4,331 ± 20	393 ± 2.3	6.5 ± 0.1	298 ± 229	122 ± 0.6	6.1	2.5	55.25	2.25
37	5.1	79 ± 0.7	332 ± 3.5	6,040 ± 15	554 ± 3.2	4.5 ± 0.1	297 ± 219	92 ± 0.9	7.6	2.5	43.25	54.25
38	4.8	37 ± 0.3	273 ± 2.1	4,373 ± 14	549 ± 1.3	4.2 ± 0.1	261 ± 213	48 ± 0.3	6.4	1.25	50.0	48.75

[z]± SD.
[y]Missing data.

wide spacial distribution of American mayapple genotypes from the same chemotype (which one may assume originated from a single colony); 3) American mayapple thrives on different soil types with various concentration of phytoavailable nutrients; and 4) tissue zinc concentration was positively correlated to podophyllotoxin, whereas soil and tissue P were positively correlated to the concentration of α-peltatin.

This study demonstrated that the mayapple colonies available in the eastern part of the United States can be used for the development of high podophyllotoxin cultivars, which could subsequently provide the base for commercial production of podophyllotoxin in the United States. Podophyllotoxin concentration in American mayapple found in this study was similar to podophyllotoxin concentration in Indian mayapple (*P. hexandrum*) leaves, which was recently reported to vary between 2.19% and 5.97% on a dry matter basis (Sultan et al., 2008). Although previous research addressed some of the mayapple cultural techniques and requirements to the environment (Cushman and Maqbool, 2005; Cushman et al., 2001, 2005a, 2005b), further research is needed to develop a production protocol for mayapple. In addition, one of the most important questions to be addressed is the overall economics; would American mayapple really be a sustainable source for podophyllotoxin? Research has shown that podophyllotoxin is found in a number of other plant species, some from unrelated families (Ayres and Loike, 1990; Bedir et al., 2002; Eyberger et al., 2006; Gordaliza et al., 2004). For example, the Eastern red cedar (*Juniperus virginiana* L.), another native plant found across the United States, also contains podophyllotoxin (Bedir et al., 2002; Cushman et al., 2003; Gawde et al., 2009; Maqbool et al., 2004). Although the concentration of podophyllotoxin in mayapple leaves may be greater than in Eastern red cedar leaves, potential advantages of Eastern red cedar over mayapple might be its abundance, high biomass production, and year-round availability. Nevertheless, the results from this study may contribute toward the development of high podophyllotoxin-containing varieties of American mayapple and the development of a new cash crop for farmers in the United States as previously suggested (Meijer, 1974).

Literature Cited

Arroo, R.R.J., A.W. Alfermann, M. Medarde, M. Petersen, N. Pras, and J.G. Woolley. 2002. Plant cell factories as a source for anticancer lignans. Phytochem. Rev. 1:27–35.

Ayres, D.C. and J.D. Loike. 1990. Lignans. Chemical, biological, and clinical properties. Cambridge University Press, Cambridge, UK.

Bedir, E., I. Khan, and R.M. Moraes. 2002. Bioprospecting for podophyllotoxin, p. 545–549. In: Janick, J. and A. Whipkey (eds.). Trends in new crops and new uses. ASHS Press, Alexandria, VA.

Bogdanova, V.P. and V.S. Sokolov. 1973. Developmental features of *Podophyllum peltatum* L. and *Podophyllum emodii* Wall. In cultivation conditions in the Lvov province. Herba Polonica. 19:379–384.

Canel, C., F.E. Dayan, M. Ganzera, A. Rimando, C. Burandt, I. Khan, and R.M. Moraes. 2001. Increased yield of podophyllotoxin from leaves of *Podophyllum peltatum* by in situ conversion of podophyllotoxin 4-O-b-D glucopyranoside. Planta Med. 67:97–99.

Cox, M.S. 2001. The Lancaster soil test method as an alternative to the Mehlich 3 soil test method. Soil Sci. 166:484–489.

Cushman, K.E. and M. Maqbool. 2005. Propagule type and planting time affect subsequent mayapple growth. HortScience 40:640–644.

Cushman, K.E., M. Maqbool, and P.D. Gerard. 2005a. Mulch type, mulch depth, and rhizome planting depth for field grown American mayapple. HortScience 40:635–639.

Cushman, K.E., M. Maqbool, H. Lata, E. Bedir, I.A. Khan, and R.M. Moraes. 2005b. Podophyllotoxin content and yield of American mayapple leaves in sun and shade. HortScience 40:60–63.

Cushman, K.E., M. Maqbool, P.D. Gerard, E. Bedir, H. Lata, and R.M. Moraes. 2003. Variation of prodophyllotoxin in leaves of Eastern red cedar (*Juniperus virginiana*). Planta Med. 69:477–478.

Cushman, K.E., M. Maqbool, and R. Moraes. 2001. Type of propagule and time of planting affect first year of field-established Mayapple (*Podophyllum peltatum*), p. 533–536. In: Janick, J. (ed.). Trends in new crops and new uses. ASHS Press, Alexandria, VA.

Eyberger, A.L., R. Dondapati, and J.R. Porter. 2006. Endophyte fungal isolates from *Podophyllum peltatum* produce podophyllotoxin. J. Nat. Prod. 69:1121–1124.

Farkya, S., V.S. Bisaria, and A.K. Srivastava. 2004. Biotechnological aspects of the production of the anticancer drug podophyllotoxin. Appl. Microbiol. Biotechnol. 65:504–519.

Gawde, A.J., V.D. Zheljazkov, V. Maddox, and C.L. Cantrell. 2009. Bioprospection of Eastern red cedar from nine physiographic regions in Mississippi. Ind. Crops Prod. (in press).

Gordaliza, M., P.A. Garcia, J.M. Miguel-Corral, M.A. Castro, and M.A. Comez-Zurita. 2004. Podophyllotoxin: Distribution, sources, applications and new cytotoxic derivatives. Toxicon 44:441–459.

Holthuis, J.J.M. 1988. Etoposide and teniposide: Bioanalysis, metabolism and clinical pharmacokinetics. Pharm. Weekbl. 10:101–116.

Ionkova, I. 2007. Biotechnological approaches for the production of lignans. Pharmacol. Rev. 1:57–68.

Jones, J.B. and W.J.A. Steyn. 1973. Sampling, handling, and analyzing plant tissue samples, p. 249–270. In: Walsh, L.M. and J.D. Beaton (eds.). Soil testing and plant analysis. Soil Sci. Soc. Amer. Inc., Madison, WI.

Kohlmunzer, S., A. Kosch, and H. Danek. 1971. Lignan compounds of *Podophyllum peltatum* L. cultivated in Poland. Poznan Inst. Przemyslu Zielarkiego Biul. 17:110–117.

Kuznetsova, G.A. and V.P. Bogdanova. 1970. The dynamics of the accumulation of podophyllin and the change in its qualitative composition in *Podophyllum peltatum* L. cultivated in the Leningrad region. Rastitel. Resursy. 6:240–243.

Maqbool, M., K.E. Cushman, P.D. Gerard, E. Bedir, H. Lata, and R. Moraes. 2004. Podophyllotoxin content in leaves of Eastern red cedar (*Juniperus virginiana*). Acta Hort. 629:87–92.

Meijer, W. 1974. *Podophyllum peltatum*—Mayapple: A potential new cash crop plant of eastern North America. Econ. Bot. 28:68–72.

Moraes, R.M., C. Burandt, Jr., M. Ganzera, X. Li, I. Khan, and C. Canel. 2000. The American mayapple revisited—*Podophyllum peltatum*—Still a potential cash crop? Econ. Bot. 54:471–476.

Moraes, R.M., H. Lata, E. Bedir, M. Maqbool, and K. Cushman. 2001a. The American mayapple and its potential for podophyllotoxin production, p. 527–532. In: Janick, J. (ed.). Trends in new crops and new uses. ASHS Press, Alexandria, VA.

Moraes, R.M., E. Bedir, H. Barrett, C. Burandt, Jr., C. Canel, and I. Khan. 2001b. Evaluation of *Podophyllum peltatum* L. accessions for podophyllotoxin production. Planta Med. 68:341–344.

Moraes, R.M., H.G. Momm, B. Silva, V. Maddox, G.L. Easson, H. Lata, and D. Ferreira. 2005. Geographic information system method for assessing chemo-diversity in medicinal plants. Planta Med. 71:1–8.

Saraeva, P.I. 1952. Mayapple, p. 273–274. In: Saraeva, P.I. (ed.). Medicinal plants and their cultivation. Medgiz, Moscow, Russia.

Sohn, J.J. and D. Plicansky. 1977. The costs of reproduction in the mayapple *Podophyllum peltatum* (Berbericadeae). Ecology 58:1366–1374.

Stahelin, H.F. and A.V. von Wartburg. 1991. The chemical and biological route from podophyllotoxin glucoside to etoposide. Cancer Res. 51:5–15.

Sultan, P., A.S. Shawl, P.W. Ramteke, A. Kour, and P.H. Qazi. 2008. Assessment of diversity of Podophyllum hexandrum by genetic and phytochemical markers. Sci. Hort. 115:398–408.

Willard, H.H., L.L. Merritt, and J.A. Dean. 1968. Instrumental methods of analysis. 4th Ed. Van Norstrand Co., Inc., Princeton, NJ.