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* Present address: Arnar-Stone Laboratories, Mt. Prospect, IL 60056.
^{x} To whom inquiries should be directed.

Thebaine Content of Selections of *Papaver bracteatum* Lindl. at Different Ages

P. G. VINCENT^x, C. E. BARE, and W. A. GENTNER

Abstract □ Approximately 200 accessions of *Papaver* species were evaluated for identification as *P. bracteatum* Lindl. and for thebaine content. Fifteen authentic *P. bracteatum* accessions were selected on the basis of chromosome count. Statistical analyses of variation in alkaloid content were made for these accessions. Four of the 15 warranted further study based on the vigor of the plant and the total thebaine present in the tissue. Significant variability in thebaine content was found in wild strains of *P. bracteatum,* thus requiring genetic selection studies. The data showed that total yield potential of thebaine should be considered in selecting *P. bracteatum* strains for commercial growth rather than concentrating on strains that produce the highest thebaine concentrations. A method for the quantitative estimation of thebaine from *P. bracteatum* is presented.

Keyphrases □ Thebaine—GLC analysis, root and aboveground portions of various strains of *Papaver bracteatum* □ *Papaver bracteatum*—various strains, root and aboveground portions, GLC analysis of thebaine □ GLC—analysis, thebaine in root and aboveground portions of various strains of *Papaver bracteatum* □ Alkaloids—thebaine, GLC analysis in root and aboveground portions of various strains of *Papaver bracteatum* □ Narcotics—thebaine, GLC analysis in root and aboveground portions of various strains of *Papaver bracteatum*

Plants in the family Papaveraceae have been analyzed for alkaloids for more than a century. The genus *Papaver* and specifically *P. somniferum,* the opium poppy that produces thebaine, codeine, morphine, and other alkaloids, has received the most attention (1).

With the growing shortage of medically useful codeine, an alternative source has been sought. *P. bracteatum,* a potential source of thebaine, reportedly contains as much as 3.5% of this alkaloid in mature capsules at a purity of 95% or greater (2, 3). Treating thebaine with hydrogen bromide results in a 76% yield of codeinone. Codeinone is reduced to codeine by the Meerwein–Pondorff process or by sodium borohydride in commercial preparations (4).

Extraction from *P. bracteatum* of reliable supplies of thebaine and chemical transformation to codeine and related derivatives for legitimate medical needs might reduce dependency on morphine. Morphine from opium now supplies about 95% of the codeine used in the United States (5) as well as 100% of the heroin, the most abused illicit drug in the United States[1].

EXPERIMENTAL

Plant Source—The origin of collections and chromosome numbers of the 15 accessions of *P. bracteatum* used are shown in Table I. The initial genetic stock of the *Papaver* species consisted of about 200 accessions from the Middle East and were reduced to the 15 accessions on the basis of chromosome counts of root tip cells, floral characteristics, and alkaloid profile. The remaining accessions (~185) were found not to be *P. bracteatum.* Chromosome counts were made[2] on immature plants to separate three closely related species found in the same geographic area where the seeds were collected from stands with no apparent prior cultivation, *i.e., P. orientale* (2n = 28), *P. pseudo-orientale* (2n = 42), and *P. bracteatum* (2n = 14).

Plant Culture—Seeds from accessions were planted in 7.6-cm peat pots[3] in the greenhouse in composted potting soil with about 4.2% organic matter and 33% pit washed sand; the pH was 6.2–6.5. Greenhouse conditions were: illumination, ambient (September–November); temperature, 20–25°; fertilizer, none; and insecticides (applied once each month), (5-benzyl-3-furyl)methyl *cis-trans-*(+)-2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylate for white fly control and tricyclohexylhydroxystannane for spider mite control.

Seedlings were successively thinned to five, three, and one per pot. Plants were sampled for thebaine content at 5, 7, and 11 months of growth. In September, after 11 months, the plants were transferred to the field; field soil was Elkton silt loam (typic ochraquult), pH 5.8. Soil analysis showed: magnesium, 143 kg/hectare; phosphate, 280 kg/hectare; and potash, 77 kg/hectare. Fertilizer amendments were: limestone, 840 kg/hectare, ammonium nitrate (34% N), 84 kg/hectare; phosphate (46% P_2O_5), 90 kg/hectare; and potash (60% K_2O in KCl), 269 kg/hectare.

Immature plants were analyzed for the thebaine content, fresh and dry weight yields of roots, and aboveground parts at time of harvest. The leaf, stem, capsule, and sometimes root tissue of mature plants were analyzed on a fresh and dry weight basis the following May, 2 weeks after petal fall. Approximately 80–90% of plants transferred to the field flowered. All plant harvesting was done at the same time of day to eliminate effects of diurnal variation on alkaloid concentrations.

Analytical—Analysis of variance was made of 11-month-old immature plants.

Thebaine yields from root, aboveground part, stem, and capsule tissues oven dried at 60° or freeze dried were compared. All tissues were ground to pass a 40-mesh sieve. Three 100-mg samples of each tissue dried by each method were taken. One sample was oven dried at 110° overnight for the determination of residual water content; the second was extracted in 50 ml of methanol–ammonium hydroxide (98:2 v/v) by rotation for 1 hr at 120 rpm; the third was extracted with 5% aqueous acetic acid, the reference extraction solvent, and purified as recommended by the United Nations (6). Routine analyses were from samples extracted in metha-

[1] Dr. Quentin Jones, Staff Scientist in charge of Narcotics Program, National Program Staff, Agricultural Research Service, U.S. Department of Agriculture, personal communication.

[2] M. L. Stiff, U.S. Department of Agriculture.

[3] Jiffy pots. (Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.)

Table I—Sources and Chromosome Numbers of *P. bracteatum*[a]

P.I.[b] or Other Designation	Seed Source	Location	SAT[c]	2n[d]
383211 (Arya II)	United Nations	Iran	2	14
383309 (Arya I)	United Nations	Iran	2	14
UNB-4	United Nations	Iran	2	14
378554	Abraham	Iran	1, 1	14, 21[e]
378555	Abraham	Iran	1	14
378556	Abraham	Iran	1	14
378581	Abraham	Iran	1	14
381600	Goldblatt	Mahabad, Iran	2	14
381601	Goldblatt	Pol-e-zanguleb, Iran	0	14
381602	Goldblatt	Damauand, Iran	2	14
381603	Goldblatt	Damauand, Iran	2	14
381604	Goldblatt	Marivan, Iran	1	14
381605	Goldblatt	51 Km. N. Marivan, Iran	2	14
381606	Goldblatt	81 Km. N. Marivan, Iran	1	14
381607	Goldblatt	Banch Road, Iran	0	14

[a] Data from M. L. Stiff. [b] Plant introduction number (U. S. Department of Agriculture). [c] Number of satellite chromosomes. [d] Diploid chromosome number. [e] Triploids and aneuploids not previously reported in these species.

nol–ammonium hydroxide (98:2 v/v). Other solvent systems and the stability of thebaine were evaluated and discussed elsewhere (7).

Thebaine was quantitated by GLC and by spectrophotometry at 285 nm. A dual-column chromatograph[4] was used. The U-shaped glass columns, 1.5 m × 4 mm i.d., previously treated with trimethylchlorosilane, were packed with 2% OV-17 on 100–120-mesh Gas Chrom Q. Packed columns were conditioned until stable at 300° in a forced stream of nitrogen. The hydrogen-flame detector was used with a sensitivity of 1×10^{-11} amp. The gases and pressures used were: air, 1.2 kg/cm^2; hydrogen, 2.5 kg/cm^2; and nitrogen, 1.2 kg/cm^2. Head pressure at the injection port was 2.5 kg/cm^2. Operating temperatures were: injection port, 280°; column, 270°; and detector, 300°.

Cholesterol acetate was the internal standard. Retention times, detector responses, and column efficiencies were calibrated with authentic thebaine, codeine, and morphine. Morphine was calibrated as its *N,O*-bis(trimethylsilyl)acetamide derivative because morphine partially adsorbs on the column. Data were reduced with a digital computing integrator[5]. The integrator, calibrated with different weights of alkaloids and a constant weight of cholesterol acetate in ethanol, was accurate to 0.02%. Standards used for daily calibration of the chromatograph and integrator were codeine (0.2 µg/µl), thebaine (0.2 µg/µl), isothebaine (0.2 µg/µl), morphine (0.4 µg/µl), and cholesterol acetate (1 µg/µl).

One milliliter of absolute ethanol containing cholesterol acetate (1 µg/µl) was added to the dried plant extracts prior to analyses. Sample size was about 1.5 µl. Standards and extractants were analyzed in triplicate. Stock solutions of thebaine standards were monitored by spectrophotometry at 285 nm in accordance with the recommendations proposed by the United Nations Working Group on *P. bracteatum* (6).

TLC of extracted alkaloids of plants corroborated the GLC data. Silica gel C was used as the adsorbent on 20 × 20-cm glass plates. The solvent system was toluene–acetone–ethanol–6 *N* ammonium hydroxide (20:20:3:1 v/v) (8, 9). Plates were activated at 105° for 2 hr and allowed to develop beyond 10 cm. For visualization of alkaloids, the developed plates were sprayed with Dragendorff's reagent (10) and potassium iodoplatinate reagent (11, 12).

Data in Table II show the range, mean $R_f \times 100$, R_x, and standard deviation values for nine *Papaver* alkaloids.

RESULTS AND DISCUSSION

In all tissues examined, 13–17% less thebaine was extracted from 60° oven-dried tissues than from freeze-dried tissues. Residual water content of freeze-dried tissue was ~5% after atmospheric equilibration compared to 110° oven-dried tissue.

Table III shows means, standard deviations, ranges, and coefficients of variation for 11-month-old plants. All accessions were tested since

[4] Searle-Analytic.
[5] Spectra-Physics.

Table II—TLC Values for Opium Alkaloids[a]

Alkaloid	Range ($R_f \times 100$)	Mean[b] ($R_f \times 100$)	R_x[c]
Narceine	0	0	0
Morphine	8–18	11 ± 3	0.3
Codeine	13–19	15 ± 2	0.3
Oripavine	30–40	35 ± 2	0.8
Thebaine	39–50	45 ± 3	1.0
Orientalidine	49–60	55 ± 3	1.2
Isothebaine	52–63	57 ± 3	1.3
Papaverine	53–65	59 ± 5	1.3
Alpinigenine	60–75	67 ± 4	1.5

[a] Results of no less than 60 TLC runs. The solvent system was toluene–acetone–ethanol–ammonium hydroxide (20:20:3:1). [b] Mean of 60 determinations ± *SD*. [c] Retention relative to thebaine.

differentiation or identification of *P. bracteatum* beyond the species level was based on the area of geographic origin of seed. The total thebaine in root tissue varied from 75 to 22,015 µg, a difference between values of 99.7% of the highest value. For all variables evaluated, the coefficient of variation ranged from 22% (thebaine yield from roots per 100 mg of dry weight) to 55% (thebaine yield from shoots per total dry weight). These data show that the characteristics of *P. bracteatum* vary among accessions.

Table IV shows the mean thebaine in micrograms per 100 mg of dry weight of *P. bracteatum* after 11 months of growth. The highest thebaine concentration (micrograms per 100 mg of plant dry weight) was found in roots of Arya II plants. Although this concentration was not significantly higher than that of Arya I plants, it was significantly higher than that of all other accessions. However, thebaine content per plant (aboveground, roots, and total) was highest for Arya I (Table V), but it was not significantly higher than the content in P.I. 381607, for aboveground parts. Thus, when dry weights were evaluated (Tables IV and V), it was apparent that although accession Arya II may have slightly more thebaine on a per unit weight basis (although not significantly so) than Arya I, accession Arya I does have significantly more thebaine yield per plant (dry weight).

The magnitude of the difference by which Arya I exceeded Arya II in thebaine per plant could be attributed to differences in vigor of plants grown at Beltsville but, nevertheless, indicates the need for determining thebaine content in plants on a total dry weight basis rather than by concentration (*i.e.*, percent) when evaluating new germ plasm sources for alkaloid content. Most reports of thebaine content in *P. bracteatum* have been made as a percent of unit weight. Among mature field-grown plants, total dry weight was greater for Arya I than for Arya II.

Characteristics of two promising high thebaine-yielding accessions of *P. bracteatum*, Arya I and Arya II, appear in Table VI. The total thebaine variation in dried plants of Arya I and Arya II was 52 and 45%, respectively. The difference between the high and low values for total thebaine was 98% of the highest value for Arya I and 82% of the highest value for Arya II, which suggested that Arya II plants vary less than do Arya I plants. For all variables, the coefficient of variation was lower for Arya II than for Arya I.

The mean dry weight of roots of Arya II was 64% that of Arya I. The mean fresh weight of Arya II was 68% that of Arya I. Although Arya II plants contained a higher concentration of thebaine (micrograms per 100 mg of plant dry weight) in the roots (2% more than Arya I plants), the total thebaine yield was higher for Arya I roots, shoots, and whole plants by 55, 85, and 60%, respectively.

Differences in thebaine content in root tissue per unit weight among 14 of the 15 accessions at 5, 7, and 11 months were significant. The combined values for the thebaine content of 14 accessions declined about 20% between 7 and 11 months, indicating that maximum thebaine synthesis occurs prior to 7 months in immature plants.

Data showed that thebaine content varied among and within accessions. In root tissue from 20 plants of Arya I (data not shown), thebaine content ranged from 502 to 22,016 µg and averaged 11,153 µg with a standard deviation of 5955. The extent of variation in thebaine content on comparison of one germ plasm source to another may be seen by calculating the difference in total thebaine dry weight per plant needed between adjacent values in the array for significance with plants for each accession (the least significant difference). Thus, if 20 plants are evaluated for thebaine content, the thebaine content must exceed 2525 µg between plants to be significant.

The Third Working Group for study of *P. bracteatum*, sponsored by the United Nations, at Beltsville, Md., (5) reported "... that Arya II

Table III—Average Values for a *P. bracteatum* Plant[a]

Variable	Mean	SD	Low	High	CV, %
Dry weight of root, g	1.00	0.38	0.21	3.97	38.0
Fresh weight of root, g	5.21	1.95	0.47	19.91	37.4
Thebaine concentration in root, μg per 100 mg of dry weight	595.04	131.67	61.24	1,615.98	22.1
Dry weight of shoots[b], g	0.57	0.15	0.10	1.80	26.3
Fresh weight of shoots, g	3.57	1.08	0.91	12.48	30.3
Thebaine concentration in shoots, μg per 100 mg of dry weight	293.93	108.23	5.43	947.75	36.8
Total thebaine yield from root, μg	5950.40	2,337.98	74.85	22,015.89	39.3
Total thebaine yield from shoots, μg	1367.60	740.26	32.00	11,278.23	54.1
Total thebaine yield from plant, μg	7318.00	2,909.56	174.90	26,077.01	39.8

[a] Average of 300 observations; 20 values each of 15 accessions for 11-month-old plants. [b] Aboveground parts.

Table IV—Mean Thebaine in Micrograms per 100 mg of Dry Weight of Different Accessions of *P. bracteatum* after 11 Months of Growth in a Greenhouse[a]

Accession[b]	Aboveground Parts	Roots
383211 (Ayra II)	298.5 b–d[c]	839.7 a
383309 (Arya I)	331.2 bc	825.9 ab
UNB-4	305.7 b–d	673.9 cd
378554	197.9 f	533.1 d–g
378555	206.6 ef	451.3 fg
378556	409.4 a	697.8 bc
378581	82.5 g	497.6 e–g
381600	279.8 c–e	603.0 c–e
381601	98.5 g	399.4 g
381602	92.2 g	581.8 c–f
381603	83.2 g	453.4 fg
381604	287.9 g	521.8 e–g
381605	309.2 b–d	680.1 c
381606	248.1 d–f	522.7 e–g
381607	368.0 ab	643.8 c–e

[a] Means of 20 immature plants of each accession. [b] Where appropriate, numbers refer to U.S. Department of Agriculture plant introduction numbers. [c] Means within columns followed by a common letter are not significantly different at the 5% level by the new Duncan's multiple range test.

material gave consistently higher and more uniform values for the thebaine content in the various plants parts." Arya I and other plants had previously been evaluated by the Working Group by using concentrations of thebaine content as the criteria for selecting germ plasm.

The thebaine content was determined in this laboratory for mature plants of Arya I (four plants) and Arya II (five plants) 2 weeks after petal fall; root, leaf, stem, and capsule tissues were evaluated (Table VII). The mean thebaine content for all tissues was 161,150 μg of total dry weight for Arya I and 97,540 μg for Arya II, a difference of 63,610 μg. Means of the four plants of Arya I showed that thebaine was distributed as follows: root, 60%; leaf, 13%; stem, 12%; and capsule, 15%. For the five plants of Arya II, the values were: root, 47%; leaf, 14%; stem, 13%; and capsule, 26%.

Thebaine was also determined in Arya I (11 plants) and Arya II (12 plants) for leaf, stem, capsule, and total but not for root tissue (Table VII). Plants were harvested 5 cm from ground level so that roots could remain for future studies. For plants grown at Beltsville, these data showed that Arya I had nearly twice the thebaine content of Arya II and followed the trend for immature 11-month-old plants shown in Table V for thebaine content in root and aboveground tissue. Distributions of total thebaine yield in Arya I were: leaves, 16%; stems, 30%; and capsules, 54%. In Arya II, the distributions were: leaves, 32%; stem, 28%; and capsules, 40% (Table VII).

In the study in which roots were analyzed, the mean dry weights (data not shown) for Arya I root, leaf, stem, capsule, and total tissues were 13.78, 9.35, 6.03, 2.79, and 31.95 g, respectively. The corresponding values for Arya II were 7.11, 7.38, 3.76, 1.54, and 19.79 g. When the mean total micrograms of thebaine for each accession was divided by its total mean dry weight, the thebaine per unit weight for Arya I and Arya II was 5044 and 4929 μg, respectively. The capsules of Arya I and II plants contained about equal amounts of thebaine (~25,000 μg), and plants sampled from each accession had one capsule per plant. The percent distribution of thebaine in the aboveground parts for Arya I and II capsules was 39 and 49%, respectively.

In the second study in which roots were not harvested, the capsules of Arya I and II plants contained ~77,000 and 32,000 μg of total thebaine, respectively; some plants sampled from Arya I plots had multiple capsules per plant, whereas all plants sampled from Arya II plots had one capsule per plant. The percent distribution of thebaine in the aboveground parts for Arya I and II capsules was 54 and 40%, respectively, in the second study. A projected yield for 100,000 plants, assuming harvest of all aboveground parts 2 weeks after petal fall, would be 14.2 kg of thebaine for Arya I and 7.9 kg of thebaine for Arya II. A similar projection based on harvest of capsules alone would be 5.1 kg of thebaine for Arya I and 2.8 kg for Arya II.

Thebaine content varies significantly among the accessions of *P. bracteatum*, and yield potential should be considered in selecting accessions for cultivation. *P. bracteatum*, although a wild and variable species, contains thebaine at relatively high concentrations (2.5–3.5%) when compared to closely related species containing this alkaloid such as *P. somniferum*, *P. pseudo-orientale*, and *P. orientale*. Another advantage in cultivating *P. bracteatum* is the purity in which thebaine occurs; 95% of the total alkaloid content occurs as thebaine and thus may permit commercial extraction with minimal purification. As a source of thebaine and, hence, codeine, *P. bracteatum* cultivation offers a significant potential answer to the world shortage of legitimate narcotics and may reduce dependence on the cultivation of *P. somniferum*.

Table V—Mean Thebaine in Micrograms per Plant (Total Dry Weight) of 15 Accessions of *P. bracteatum* Grown in a Greenhouse for 11 Months[a]

Accession[b]	Aboveground Parts	Roots	Total
383211 (Ayra II)	1,418.5 cd[c]	7,194.0 b–d	8,612.5 bc
383309 (Arya I)	2,695.4 a	11,152.0 a	13,847.4 a
UNB-4	1,881.7 bc	5,687.6 d–f	7,569.3 b
378554	1,451.7 cd	9,177.0 ab	10,628.7 b
378555	1,546.1 cd	6,778.3 c–e	8,324.4 bc
378556	2,330.2 ab	8,030.8 bc	10,361.0 b
378581	625.1 ef	4,472.3 eh	5,097.4 d–f
381600	963.3 d–f	5,351.2 d–g	6,314.5 c–e
381601	502.6 ef	4,545.7 e–h	5,048.3 d–f
381602	352.5 f	5,215.3 d–h	5,567.8 d–f
381603	501.1 ef	2,894.4 h	3,395.5 f
381604	1,482.0 cd	3,725.5 f–h	5,207.5 d–f
381605	997.9 d–f	3,541.5 f–h	4,539.4 ef
381606	1,085.0 de	3,269.5 gh	4,354.5 ef
381607	2,465.6 ab	6,482.9 c–e	8,948.5 bc

[a] Twenty immature plants of each accession were used in analysis. [b] Where appropriate, numbers refer to U.S. Department of Agriculture plant introduction numbers. [c] Means within columns followed by a common letter are not significantly different at the 5% level by the new Duncan's multiple range test.

Table VI—Average Value for *P. bracteatum* Accessions Arya I (P.I. 383309) (I) and Arya II (P.I. 383211) (II) Plants at 11 Months of Age[a]

Variable	Arya	Mean	*SD*	Low	High	*CV*, %
Dry weight of root, g	I	1.32	0.53	0.58	2.48	40.2
	II	0.84	0.28	0.41	1.39	33.3
Fresh weight of root, g	I	7.07	2.45	3.40	13.65	34.7
	II	4.80	1.18	2.71	7.19	24.6
Thebaine concentration in	I	825.91	349.64	61.24	1,615.98	42.3
roots, μg per 100 mg of dry weight	II	839.70	255.10	399.59	1,325.03	30.4
Dry weight of shoots[b], g	I	0.80	0.27	0.41	1.37	33.8
	II	0.48	0.16	0.21	0.85	33.3
Fresh weight of shoots, g	I	5.19	1.81	2.71	10.29	34.9
	II	3.02	0.95	1.61	4.84	31.5
Thebaine concentration in	I	331.19	215.16	5.43	947.75	65.0
shoots, μg per 100 mg of dry weight	II	298.49	139.95	100.09	635.14	46.9
Total thebaine yield from	I	10,902.01	5954.88	502.17	22,015.89	54.6
root, μg	II	7,053.48	3296.58	2277.66	13,825.44	46.7
Total thebaine yield from	I	2,649.52	2324.42	32.04	11,278.23	87.7
shoots, μg	II	1,432.75	907.24	500.45	3,857.98	63.3
Total thebaine yield from	I	13,551.53	7177.42	534.11	26,077.01	53.0
plant, μg	II	8,486.23	3860.69	2846.04	15,720.95	45.5

[a] Each item represents the mean of 20 plants. [b] Aboveground parts.

Table VII—Distribution of Thebaine in Various Mature Plant Tissues of Accessions Arya I (P.I. 383309) and Arya II (P.I. 383211)

Accession	Thebaine Yield, μg				
	Root	Leaf	Stem	Capsule	Total
Arya I	94,810[a]	4,193	9,583	16,896	125,482
	131,063	44,935	35,449	32,900	244,347
	102,476	18,974	20,748	32,616	174,814
	56,739	15,590	9,387	18,242	99,958
$\bar{x}$	96,272	20,923	18,792	25,164	$\Delta\bar{x}_1$ 161,150
	—[b]	3,671	9,070	15,934	28,675
	—	62,575	69,785	86,840	219,200
	—	1,288	24,390	45,881	71,559
	—	8,340	30,917	34,482	73,739
	—	69,563	77,920	150,062	297,545
	—	29,450	47,376	59,530	136,356
	—	18,834	64,956	88,697	172,487
	—	19,492	47,297	122,028	188,817
	—	5,873	29,466	99,775	135,114
	—	15,008	24,248	29,675	68,931
	—	13,787	48,200	111,111	173,098
	$\bar{x}$	22,535	43,057	76,729	$\Delta\bar{x}_2$ 142,320
Arya II	57,483	17,818	12,512	27,593	115,406
	63,142	4,693	23,565	31,974	123,374
	28,796	5,726	10,067	8,239	52,828
	37,044	36,692	8,169	48,010	129,915
	44,320	2,366	9,697	9,796	66,179
$\bar{x}$	46,157	13,459	12,802	25,122	$\Delta\bar{x}_3$ 97,540
	—	44,636	9,937	21,339	75,912
	—	13,120	20,921	25,839	59,880
	—	20,280	28,963	41,367	90,610
	—	6,749	16,865	12,242	35,856
	—	49,117	28,165	31,059	108,341
	—	22,866	50,382	29,948	103,196
	—	6,332	6,621	12,003	24,956
	—	43,572	19,531	37,298	100,401
	—	47,114	30,204	43,609	120,927
	—	9,052	16,365	23,742	49,159
	—	23,841	22,288	90,400	136,529
	—	10,402	15,295	11,618	37,315
	$\bar{x}$	24,757	22,128	31,705	$\Delta\bar{x}_4$ 78,590

[a] Each entry represents an individual plant. All values are rounded to whole numbers. [b] Plant root tissue not taken.

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[x] To whom inquiries should be directed.