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Lobeline Content of **LOBELIA INFLATA** : Structural, Environmental and Developmental Effects

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LOBELIA INFLATA L.

(Campanulaceae)

COMMON NAMES: Indian tobacco, asthma weed, bladder pod, bladder-podded lobelia, emetic herb, emetic weed, eyebright, field lobelia, gagroot, Indian tobacco lobelia, lobelia, obelia, pukeweed, tobacca lobelia, vomitwort, wild tobacco.

DESCRIPTION: A branching annual that grows to 3 feet in height. Leaves are 1 to 3 inches long. Produces small, violet-pinkish-white flowers situated in axils of alternate leaves, the bottom of which greatly inflate in fruiting stage.

FLOWERING PERIOD: July to September.

HABITAT: Weedy fields, roadsides, woods, and in partial shade.

HARVEST: Herb when in flower and forming seeds.

USES: The herb yields the alkaloid lobeline, which is used in anti-tobacco therapy. It is also used as a stimulant, antiasthmatic, and expectorant in cases of bronchitis. It is also used to measure circulation time (7).

From A GUIDE TO MEDICINAL PLANTS OF APPALACHIA (4)

A RESEARCH PROGRAM is being carried on to develop methods for germinating and cultivating *Lobelia inflata* L., a native forest plant that is the principal source of the alkaloid lobeline, which is used in an increasing number of anti-smoking preparations. The goal is to produce maximum lobeline yields.

To aid in estimating the quantities of plants required to meet the demand for lobeline, we have studied lobeline content as influenced by the age of the plant; the distribution of lobeline within different parts of the plant; lobeline content in relation to soil pH, phosphorous, potassium, and organic matter; and lobeline content as indicated by plant levels of nitrogen, phosphorus, and potassium.

BACKGROUND

The principal supply of lobeline comes from wild plants collected in the Appalachian Region of the eastern United States (*4*). But as social changes occur in Appalachia, a dwindling number of people are willing or able to collect plants in the forests. So the possibility of growing *L. inflata* as a cultivated plant in the forests merits careful study. The goal is maximum production of lobeline per acre rather than plants per acre.

A survey of anti-smoking preparations in tablet and pastille forms shows a range of content from 0.5 mg. to 2.0 mg. lobeline per tablet or pastille. The total quantity of lobeline used in the United States is not known. One company estimates that it purchases 50,000 to 75,000 pounds of *L. inflata* plant material

annually.[1] Another company estimates its purchases at to be about 30,000 pounds per year.[2] The authors estimate that about 300 to 400 pounds of lobeline is used per year for anti-smoking preparations.

MATERIALS AND METHODS

Field Study

Field studies were begun in the summer of 1967 to investigate the influence of soil pH, organic matter, potassium, and phosphorous on lobeline synthesis; to determine if plant analyses of nitrogen, phosporous, and potassium were suitable indicators of lobeline content; to study the distribution of lobeline within the plant; and to study lobeline content as related to stage of plant growth.

A very uniform, level, fallow cornfield with a high population of *L. inflata,* located in Madison County, Kentucky, was selected for a study site to minimize differences of drainage, slope, exposure, and other ecological factors. Some of the differences we were interested in related to soil fertility and we believed that soil variability would be enough within this limited area to show in soil analyses.

Fifty samples of above-ground portions of *L. inflata* were harvested from 0.8-meter square plots. A measuring circle was tossed at random and all *L. inflata* plants in the circle were harvested. If no plants were included, the device was thrown again. After drying in a forced hot-air drying box, the plant material was passed through the 40-mesh screen of a Wiley mill. The ground material was divided into two parts, one for lobeline analyses and the other for plant-nutrient analyses. Three soil samples, to a depth of 12 inches, were taken from each plot, composited, and dried in a laboratory oven.

[1] Wilcox Drug Company, Boone, North Carolina; personal communication, 1970.
[2] Coeburn Produce Company, Coeburn, Viriginia; personal communication, 1970.

For the studies of lobeline content in relation to plant age and plant organs, plants at the desired stage of maturity were harvested from the entire field and then were divided into 6 groups of 10 plants each. Studies of organs were based on 60 mature plants, divided into 6 groups of 10 plants each. Above-ground plant parts were used for both these studies.

Minerals and pH

Soil and plant mineral analyses were performed with standard methods. Total nitrogen was determined with regular Kjeldahl procedure and phosphorus and potassium with a Technicon Auto Analyzer.

Soil pH was determined at a 1:1 soil:water ratio. Organic matter was determined with a Walkley-Black heat-of-dilution method; phosphorus with the Bray No. 1 soil test, 1:10 ratio; and potassium with 0.15*N* H_2SO_4 extraction for 2 minutes and flame photometer.

Lobeline

Five-g. portions of coarsely powdered dried plant material were extracted continuously for 16 hours in a Soxhlet extractor, using 120 ml. of chloroform. When larger samples were available, 10-g. portions were extracted in a similar manner except that two consecutive extractions of 10 hours each were made, using 120 ml. of fresh chloroform for the second extraction.

The total chloroform extract from each sample was concentrated in a flash evaporator at a temperature below 20°C.; and the resulting residue was weighed, then redissolved in chloroform to exactly 10 ml. in a volumetric flask. Very small samples were diluted to 5 ml. with chloroform.

Of each of the resulting solutions, 0.2 ml. was steamed on a Silicagel-G preparative plate 0.5 mm. thick. These plates were developed with a cyclohexane : chloroform : diethylamine (50 : 40 : 10) solvent system (*13*).

The developed plates were dried, then were examined under long- and short-wave ultraviolet light and exposed to various alkaloid reagent sprays such as Dragendorff's reagent and iodine

vapors. Lobeline was identified by chromatography by comparing it with an authenic sample. By comparison of spotting on a plate, resulting from a known lobeline sample, and by comparison of R_f values, the band corresponding to lobeline was identified.

This material was removed from the plate and eluted with chloroform. The infrared and ultraviolet spectra of the dried eluted material compared favorably with the spectra of known lobeline and were different from the spectra of lobelanine and lobelanidine. The band identified as lobeline with an R_f value of 0.63 was removed from the plate of each sample, eluted with chloroform in a microcolumn, and diluted to known volume; and the concentration of the resulting solution was determined with a Beckman DB-G spectrophotometer at a wave length of 250 mu. (*10*). The lobeline content of the dried plant was then calculated.

Spots indicating the possible presence of as many as five alkaloids (*11*) were obtained from some of the samples.

RESULTS

Development and Lobeline Content

Collectors of *L. inflata* harvest the above-ground parts of mature plants along with flowers and seeds, although pharmacopoeias (*8, 13*) refer to *lobelia* as the dried aerial parts or tops, omitting reference to flowers. In Great Britain (*12*) a minimum alkaloid quantity, calculated as lobeline, is specified at 0.3 percent.

Juvenile, adolescent, and mature plants (table 1 and figs. 1, 2, 3) were analyzed for lobeline. Lobeline concentration decreased with maturity, but total plant lobeline content actually increased with maturity (table 1).

Mature flowering plants were found to average 0.76 percent lobeline; adolescent plants 1.46 percent, and juvenile plants in the rosette stage 1.95 percent (table 1).

Tests of difference were based on Tukey's method (*9*) applied to log-transformed data:

	Mean % lobeline
Juvenile	1.95
Adolescent	1.46
Mature	0.76

The only means that were not significantly different between maturity classes at the 5-percent level of significance were the means for percent lobeline for the juvenile and adolescent groups. This is indicated by the brace in the above tabulation.

Table 1.—Percentage of lobeline occurring in *Lobelia inflata* at three stages of growth

[Dry-weight basis]

Growth stage	Lot	Lobeline
	No.	*Percent*
Juvenile [1]	100	1.41
	101	1.81
	102	.89
	103	4.28
	104	2.02
	105	1.27
Mean	—	1.95
Adolescent [2]	200	1.81
	201	1.47
	202	2.82
	203	.46
	204	.87
	205	1.33
Mean	—	1.46
Mature [3]	400	0.98
	401	.67
	402	.72
	403	.80
	404	.69
	405	.69
Mean	—	0.76

[1] Juvenile—basal rosettes, not yet elongated into a stem.

[2] Adolescent—plants with elongated stems but without flowers or seed capsules.

[3] Mature—branched, with inflated capsules and flowers.

Figure 1.—Laboratory-grown Lobelia inflata plants.

Figure 2.—A young rosette of Lobelia inflata, compared in size with a half dollar (white disk).

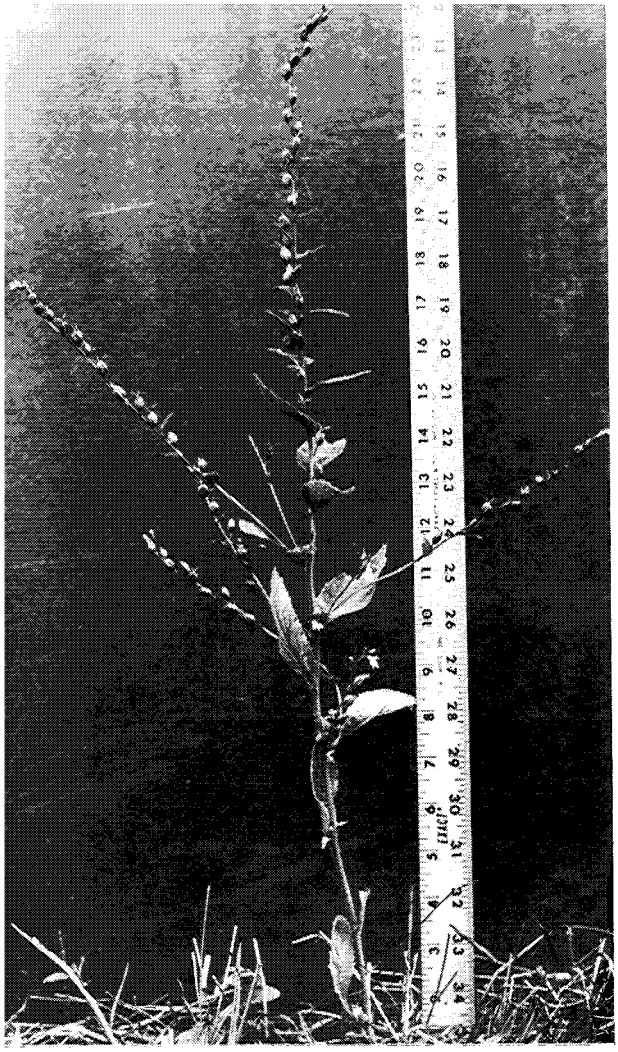

Figure 3.—A mature plant of Lobelia inflata, showing seed capsules and some flowers.

Structure and Lobeline Content

Leaves, stems, and flowers from mature plants were analyzed separately to determine levels of lobeline occurring in the different above-ground parts of the plant.

Structural differences in lobeline concentration were noted. Leaves analyzed 0.38 percent and stems 0.58 percent (table 2). The greatest concentration of the alkaloid was found in the flowers, which yielded 3.03 percent, eight times more lobeline than the leaves and over five times more than the stems.

Table 2.—Lobeline content of different plant parts

[Dry-weight basis]

Part	Lot	Lobeline content
	No.	
Leaves	300	0.35
	301	.36
	302	.33
	303	.69
	304	.33
	305	.20
Mean	—	0.38
Stems	300	0.41
	301	.38
	302	.57
	303	.80
	304	.40
	305	.90
Mean	—	0.58
Flowers	300	3.22
	301	2.34
	302	2.04
	303	5.95
	304	3.31
	305	1.34
Mean	—	3.03

An analysis of variance on the log-transformed data showed no effect due to lot, and showed that the lobeline concentration in leaves and stems is not significantly different at the 5-percent level of significance.

In Italy (8) lower lobeline yields were reported, flowers containing an average of 1.0 percent, leaves 0.4 percent, and entire plants 0.55 percent. These differences may reflect different analytical methods, different ecological conditions, experimental variability, or a combination of these factors.

Environment and Lobeline Synthesis

Differences in alkaloid content of *L. inflata* from different soils have been reported (*2*), suggesting that differences may be attributed to the state of plant nutrition, analytical techniques, and soil variation.

Hoffman (*3*) reported high levels of soil nitrogen and P_2O_5 to be associated with high alkaloid content. An increase in alkaloid content with increase in soil K_2O has also been reported (*1*).

On the other hand, studies of the effects of different forms of nitrogen, phosphorus, and potassium on lobeline formation have led to the conclusion that fresh weight can be increased by the use of fertilizers, but that alkaloid content (or percent concentration) decreased under such trials by as much as 2.5 times (*6*).

A response surface was used up to second-order values of soil pH in fitting the 50 data points. Optimal conditions for lobeline production could not be determined. In fact, the observed pH, organic matter, and phosphorus and potassium levels of the soil as related to lobeline content could be attributed to the random fluctuations observed in this experiment ($F_{11,19} = .794$); that is, statistical analysis of the data in table 3 revealed no relation between the measurements of these soil properties and lobeline content.

In a detailed study of the relation between plant growth and lobeline production, it was noted that growth responses to the addition of fertiliizers were not tied to a proportionate increase in lobeline production (*5*). The greatest increase in vegetative material came from the use of phosphorus in combination with nitrogen from sulfate of ammonia.

Plant Nutrient Levels

Since analyses for nitrogen, phosphorus, and potassium are easier and less expensive than those for lobeline, it was interesting to determine whether levels of nitrogen, phosphorus, and potassium in plant tissue could be related to those of lobeline. Our statistical analysis did not establish any such relationship. The effects of N, K, and P could not be distinguished from the

Table 3.—Mineral analyses of plants and soil from fallow corn-field in Madison County, Berea, Kentucky

Sample No.	Plant			Soil				Lobeline, percent of dry weight
	N	P	K	pH	Organic matter	K	P	
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Lbs./ acre*	*Lbs./ acre*	*Pct.*
1	1.92	0.26	1.53	4.5	—	57	22	0.67
2	1.35	.17	1.56	4.5	—	92	23	1.34
3	1.32	.16	1.43	4.7	—	53	13	.60
4	1.28	.14	1.22	4.6	—	62	11	2.38
5	1.21	.15	1.18	5.3	—	62	11	.62
6	1.40	.15	.85	4.5	—	53	20	1.23
7	1.34	.17	1.45	4.6	—	48	10	.44
8	1.33	.15	.76	4.6	—	47	13	.46
9	1.13	.15	.15	4.8	—	54	12	.65
10	1.33	.14	.89	4.8	—	50	11	.23
11	1.28	.17	1.40	4.3	—	55	9	.78
12	1.01	.16	1.62	4.4	—	58	19	.87
13	1.41	.15	1.07	4.7	—	57	10	.93
14	1.27	.13	1.15	5.8	—	71	12	.96
15	1.45	.17	1.69	5.2	—	72	9	1.04
16	1.31	.16	1.54	4.6	—	68	13	.46
17	1.43	.13	1.29	5.0	—	70	12	.35
18	1.22	.13	1.02	4.7	—	64	18	.41
19	1.06	.14	1.44	4.6	—	62	11	.54
20	1.10	.12	1.50	4.2	2.54	90	9	.70
21	1.72	.13	1.35	4.5	3.96	71	16	.63
22	1.13	.15	1.19	4.5	3.57	67	16	.53

23	1.13	.14	1.32	4.5	2.83	70	21	.52
24	1.41	.14	1.08	5.0	3.39	81	14	.61
25	1.57	.15	1.44	4.9	3.14	72	10	.65
26	1.41	.14	1.36	4.6	2.09	69	17	.60
27	1.22	.14	1.58	4.6	1.82	70	20	1.31
28	1.38	.12	1.17	4.3	3.27	72	25	1.22
29	1.34	.15	1.45	4.6	3.63	70	32	.52
30	1.39	.16	1.40	4.4	3.13	69	24	.56
31	.99	.19	1.83	4.3	3.07	90	36	.28
32	1.66	.13	1.09	4.6	4.02	74	32	.34
33	1.49	.15	1.32	4.5	3.33	75	36	.67
34	1.08	.15	1.42	4.3	2.70	95	40	.36
35	1.31	.12	1.12	4.2	2.35	83	35	.19
36	1.32	.14	1.04	4.3	3.73	63	42	.53
37	1.04	.09	.96	4.3	2.35	65	26	.19
38	1.13	.15	1.37	4.1	2.70	70	39	.50
39	.83	.14	1.14	4.1	4.03	68	44	.40
40	.81	.14	1.47	4.1	3.67	70	38	.25
41	.96	.12	1.22	4.2	3.48	72	42	.53
42	1.00	.14	1.11	4.4	3.24	70	37	.41
43	1.40	.20	1.88	4.5	3.12	73	48	.51
44	1.25	.17	1.48	4.4	3.19	63	41	.85
45	1.21	.15	1.53	4.5	3.31	71	23	.60
46	1.16	.14	1.62	4.5	4.30	80	36	.60
47	1.54	.15	1.57	4.4	4.10	83	29	.45
48	.93	.14	1.69	4.4	4.15	79	30	.20
49	1.25	.15	1.75	4.4	3.96	66	26	.50
50	1.21	.18	1.60	4.2	4.41	60	43	.55
Range	0.81+	0.09+	0.76+	4.1+	1.82+	47+	9+	0.19+
	—1.92	—0.26	—1.88	—5.8	—4.41	—75	—48	—2.38

random fluctuations in the measured levels of lobeline ($F_{9,40} = .798$).

SUMMARY

We found that lobeline concentrations decrease from juvenile to mature plants, but that total amounts of lobeline per plant increase from juvenile to mature plants.

A comparison of lobeline concentrations in plant parts showed a small difference between leaves and stems, but a markedly higher concentration in flowers.

Within the range of conditions encountered in this study, we found no relationship between lobeline content and plant levels of nitrogen, phosphorus, and potassium, and no relationship between lobeline content and soil pH, organic matter, phosphorus, or potassium.

Conclusion

Because no relationship could be found between soil organic matter, phosphorus, potassium, pH, and lobeline content in the plants we analyzed, we conclude that: (1) these factors were at a suitable level for lobeline synthesis; (2) the plants' ability to synthesize lobeline was not sensitive to the differences in the levels of these minerals encountered in this experiment; (3) the minerals studied, phosphorus and potassium, soil organic matter, and pH were too uniform to be reflected in any differences in lobeline synthesis in *L. inflata.*

Mature flowering plants provide a maximum yield of lobeline because of the high lobeline content of the flowers and the weight of the mature plants.

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