VEINLET TERMINATION NUMBER A NEW CHARACTER FOR THE DIFFERENTIATION OF LEAVES

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THE venation of leaves and its numerical relationship to other leaf characters has been the object of study by a number of workers. Zalenski[1] has shown that the total length of veinlets per unit area of leaf surface is a specific character, and Shuster[2] has confirmed this. Benedict[3], however, has suggested that the average vein islet area has more direct physiological significance, while Ensign[4,5] has shown that it is not related to the age of the plant. This character was investigated further by Levin[6] and found to vary with the position on the leaf at which it was determined. He, therefore, made counts on 4 sq. mm. areas of leaf surface located in the centre of the lamina midway between midrib and margin, his results being expressed as the number of vein islets per sq. mm. (vein islet number). He found this to be a specific character independent of the total area of the leaf, and has suggested its use for the differentiation of whole and broken leaves.

The authors consider that, in the case of broken leaves, this is only possible where the particular portion of lamina upon which determinations are based can be identified. Forsdike[7] has shown that the appearance of leaf venation under a hand lens when viewed by transmitted and by reflected light may be used to distinguish medicinal leaves from their common adulterants, the main differences occurring in the shape and size of the vein islets and the visibility of the smaller veinlets. Of these three main methods by which venation has been used to identify and separate leaves, only that of the length of veinlets per unit area is adaptable to portions of lamina small enough to fall within the range of a coarse powder, and it would probably be too laborious to be of practical value.

In most leaves small veinlets, termed by Eames and MacDaniels[8] "bundle ends," project into the area of the vein islets and there terminate. They may be simple or branched. The xylem consists usually of spiral or reticulate elements only which become fewer in number towards the tip, which itself may consist of a single spiral or reticulate tracheid. This investigation has been conducted to ascertain whether: 1. the number of projecting veinlets per unit area of leaf surface is of value as a diagnostic character; 2. any relationship exists between this character and the number of vein islets per unit area; 3. any relationship exists between this character and leaf area; 4. the character is applicable to powdered leaves.

Veinlet Termination Number. The expression "veinlet termination"

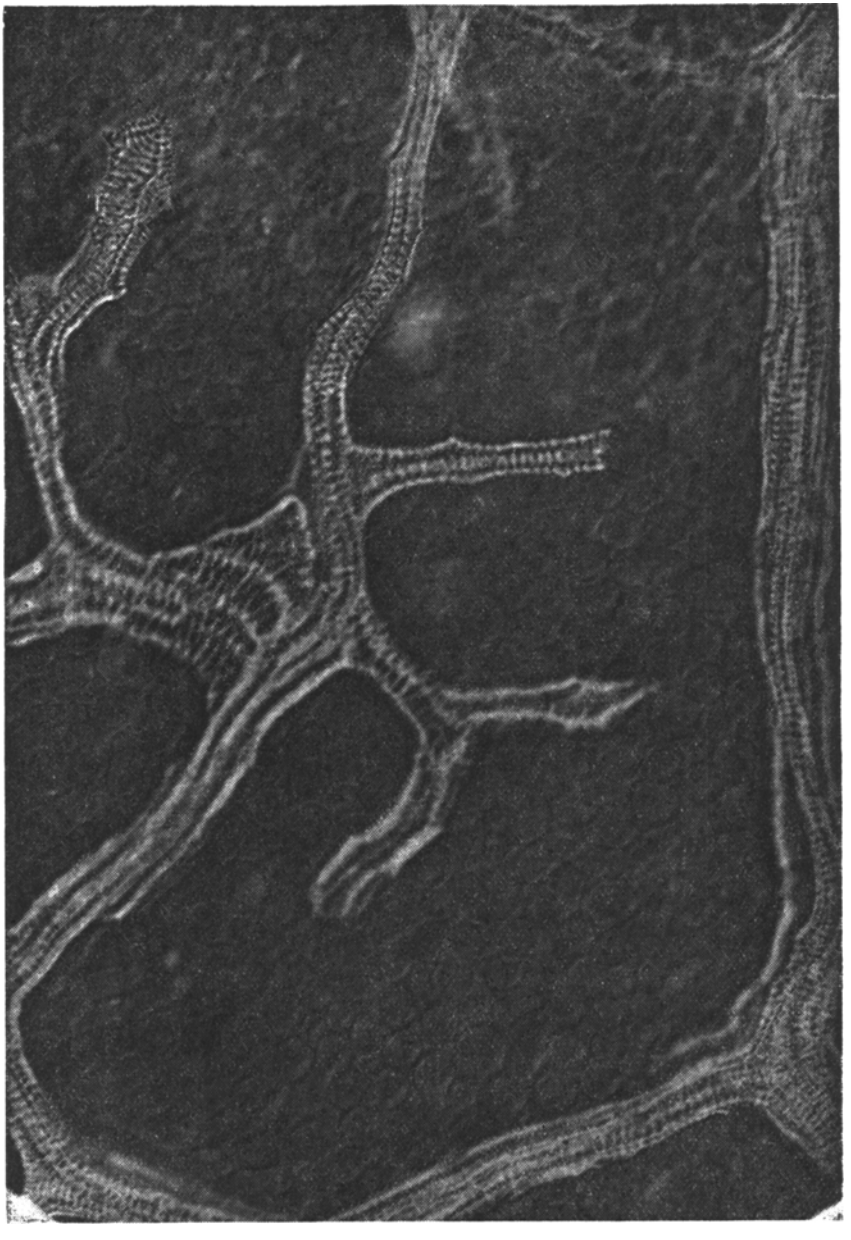

FIG. 1. *Cassia angustifolia*.—Part of the leaf venation showing 4 veinlet terminations (photograph ×455).

is introduced and defined as the ultimate free termination of a veinlet or branch of a veinlet. "Veinlet termination number" is the number of veinlet terminations per sq. mm. of leaf surface (see Figure 1).

Material. The following species were investigated: *Erythroxylum truxillense,* Rusby; *Erythroxylum coca,* Lamark; *Cassia acutifolia,* Delile; *Cassia angustifolia,* Vahl; *Barosma betulina,* B. and W.; *Barosma crenulata, Hooker,* variety *latifolia,* Berg. Each species contained leaves from authentic sources and from commercial samples.

Experimental

Preparation of leaves. Leaves containing calcium oxalate crystals were cleared in chloral hydrate solution (5 + 2), transferred to 10 per cent. v/v hydrochloric acid for 15 minutes, then re-cleared in chloral hydrate solution. Where a mucilaginous epidermis was present the leaves were first soaked in water overnight and the epidermis stripped. Other leaves were cleared in chloral hydrate solution.

The mounting medium used was prepared according to the following formula:—

High viscosity polyvinyl alcohol	4 g.
Acetone 70 per cent.	14 ml.
Glycerol	15 ml.
Lactic acid	15 ml.
Thymol	0·05 g.
Water	30 ml.

Method. All determinations on whole leaves were made by micro-projection through a 90° prism attached to the ocular, on to a screen at a distance of 45 cm., the whole apparatus being housed in a dark box. Two objectives, a 16 mm. and a 32 mm., were used in conjunction with a ×6 ocular so that a magnification of either 98·5 or 37 diameters could be obtained at will. The apparatus was calibrated using a stage micrometer, two concentric squares being drawn on the screen, the one with the lower power objective in position representing an area of 4 sq. mm., the other using the higher power, an area of 1 sq. mm. The procedure was as follows: The prepared slides were projected at the higher magnification and the veinlet terminations and vein islets in the square equivalent to 1 sq. mm. were counted. The nosepiece was then rotated, bringing the lower power objective into position and vein islets only counted in the square representing 4 sq. mm. Vein islets were determined by counting all within the square, including those intersected by two adjacent sides and excluding those intersected by the other two sides.

In preliminary experiments 10 positions were chosen on each leaf such that they lay evenly distributed along a line midway between mid-rib and margin, 5 on each half of the lamina. An analysis of variance showed that there was no significant difference between the figures determined on each half of the leaf, and for all subsequent experiments, therefore, 5 positions were used on one half of the leaf, position 1 being at the base and position 5 at the apex. Position 3, midway between the

apex and base, corresponded to the position used by Levin in the determination of vein islet number. 20 leaves were examined for each species. The figures obtained on *Erythroxylum truxillense* and *E. coca* are shown in Table I and the analysis of variance in Table II. Table III shows the ranges and means of the characters for all the species examined. In *E. coca* very occasional isolated groups of about 3 tracheids were noticed which had no apparent connection with the rest of the venation. These were ignored in making the veinlet termination counts.

Application to powdered leaves. Powders of commercial samples of *E. coca, E. truxillense, C. acutifolia* and *C. angustifolia* were examined to determine the applicability of veinlet termination number to powdered leaves. In all cases other than coarse powder the mesophyll and veins

TABLE I

Species	Leaf	Veinlet terminations 1 sq. mm.					Vein islets 1 sq. mm.					Vein islets 4 sq. mm.				
		Position					Position					Position				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Erythroxylum truxillense	1	22	23	28	23	25	11	16	21	21	32	54	53	78	71	88
	2	25	24	26	29	28	17	17	20	21	16	57	68	60	63	58
	3	34	30	34	26	35	15	22	20	26	24	61	65	83	86	108
	4	28	20	26	24	21	12	15	13	17	20	55	42	53	54	56
	5	32	29	37	36	33	26	26	23	28	22	59	99	92	102	101
	6	29	30	26	27	33	16	16	17	17	18	77	63	70	66	66
	7	31	27	31	31	39	20	18	17	20	21	72	58	73	64	81
	8	31	33	31	29	40	16	20	14	17	20	61	58	60	64	69
	9	22	28	28	24	26	13	19	15	18	15	44	50	54	68	57
	10	20	21	24	22	21	20	14	16	15	13	56	52	51	46	49
	11	20	28	32	27	31	14	16	18	18	13	60	49	65	67	55
	12	30	31	29	34	28	14	15	12	16	15	51	53	58	53	62
	13	19	24	29	23	23	9	13	11	12	15	34	48	45	46	65
	14	22	27	20	18	24	14	10	15	19	16	49	51	56	57	61
	15	24	31	31	27	34	26	26	16	28	21	90	77	95	81	97
	16	23	35	32	31	23	22	17	16	12	18	57	61	58	46	70
	17	27	32	28	22	21	18	13	18	16	16	40	51	58	55	55
	18	24	31	28	24	24	16	18	24	21	26	55	56	85	72	68
	19	20	26	23	26	21	16	13	18	13	17	50	57	60	62	62
	20	22	33	31	19	28	17	19	16	15	21	64	73	79	64	68
Erythroxylum coca	1	24	18	23	37	36	16	17	20	18	24	53	63	39	69	70
	2	16	23	23	27	26	11	11	11	12	18	34	36	49	41	64
	3	18	27	20	32	27	17	13	16	15	14	49	50	50	52	51
	4	19	20	22	21	26	9	10	10	12	24	50	40	46	56	67
	5	15	21	19	21	19	14	17	17	15	16	49	45	46	50	52
	6	23	19	16	16	17	12	16	9	17	20	40	45	42	45	62
	7	13	15	15	16	17	14	15	13	24	25	57	49	48	54	75
	8	13	19	21	17	15	14	9	9	11	18	40	32	36	40	60
	9	14	14	10	12	15	11	9	12	9	12	37	29	34	36	39
	10	12	16	14	16	15	11	10	11	10	17	40	34	43	45	72
	11	14	13	20	19	14	7	9	10	7	15	32	30	33	37	40
	12	14	11	18	17	23	8	7	6	8	13	35	32	34	41	51
	13	17	16	14	19	22	11	10	13	13	15	40	39	45	42	54
	14	22	21	26	23	17	11	11	11	14	25	50	49	42	46	58
	15	9	14	19	18	20	12	9	6	10	13	38	42	33	39	57
	16	17	23	20	24	22	16	11	19	17	25	55	46	75	64	90
	17	16	15	14	16	13	7	7	9	14	17	39	27	34	43	61
	18	16	20	9	15	13	14	10	17	18	23	43	32	47	61	62
	19	16	18	19	18	18	19	15	16	15	25	47	53	57	54	80
	20	25	25	25	32	28	15	17	15	17	21	48	58	52	64	79

were somewhat disintegrated, and attempts to make counts were unsuccessful. Determinations were made, therefore, on coarse powders only. In order to make the method as practical as possible the projection

apparatus was dispensed with and counts were made using the microscope with a 16 mm. objective and a ×15 ocular which gave a field of view of 0·6943 sq. mm.

Method. The powders were prepared for examination using the same method as for whole leaves except that stripping the epidermis was unnecessary. All fragments which completely filled the field of view were counted until a total of 50 had been made for each species. The means and standard deviations were calculated and referred to 1 sq. mm. to give the veinlet termination number. The results are given in

TABLE II

Erythroxylum truxillense AND *E. coca*
ANALYSIS OF VARIANCE IN TABLE I

Character	Species	Source of Variance	Degress of freedom	Sum of squares	Mean square	Variance ratio	V.R P=0·01
Veinlet termination number	*E. truxillense*	Leaves	19	1225·16	64·48	4·53	2·3
		Positions	4	173·06	43·27	3·04	3·65
		Error	76	1082·94	14·25		
	E. coca	Leaves	19	1726·51	90·87	7·94	2·3
		Positions	4	215·66	53·92	4·71	3·65
		Error	76	869·14	11·44		
		Species	1	3486·13	3486·13	130·42	6·64
		Error	198	5292·47	26·73		
Vein islets 1 sq. mm.	*E. truxillense*	Leaves	19	979·84	51·57	5·04	2·3
		Positions	4	83·74	20·94	2·05	3·65
		Error	76	777·46	10·23		
	E. coca	Leaves	19	966·16	50·85	7·76	2·3
		Positions	4	702·86	175·72	26·82	3·65
		Error	76	497·54	6·55		
		Species	1	706·88	706·88	34·93	6·64
		Error	198	4007·60	20·24		
Vein islets 4 sq. mm.	*E. truxillense*	Leaves	19	13750·44	723·71	10·66	2·3
		Positions	4	2145·14	536·29	7·90	3·65
		Error	76	5159·26	67·89		
	E. coca	Leaves	19	6960·35	366·33	9·22	2·3
		Positions	4	5514·70	1378·68	34·70	3·65
		Error	76	3019·70	39·73		
		Species	1	11719·81	11719·81	63·49	6·64
		Error	198	36549·59	184·59		

Table IV. The following ranges of the means of 20 counts (P = 0·01) were calculated from the data obtained on coarse powders, using the expression $R = M \pm 2{\cdot}576 \frac{\sigma}{\sqrt{n}}$: *E. truxillense* 23·1 to 32·3; *E. coca* 16·8 to 21·0; *C. acutifolia* 32·7 to 40·2; *C. angustifolia* 25·9 to 32·8.

TABLE III

RANGES AND MEANS OF CHARACTERS

Character	Species	Range	Mean	Standard deviation
Veinlet termination number	*E. truxillense*	18 — 22·21 — 32·23 — 40	27·22	5·01
	E. coca	9 — 13·54 — 24·20 — 37	18·87	5·33
	C. acutifolia	22 — 30·59 — 42·29 — 49	36·44	5·85
	C. angustifolia	21 — 24·80 — 32·72 — 39	28·76	3·96
	B. betulina	8 — 10·04 — 15·54 — 20	12·79	2·75
	B. crenulata	6 — 10·24 — 16·76 — 24	13·50	3·26
Vein islets 1 sq. mm.	*E. truxillense*	9 — 13·33 — 21·95 — 32	17·64	4·31
	E. coca	7 — 9·20 — 18·56 — 25	13·88	4·68
	C. acutifolia	18 — 27·39 — 41·45 — 49	34·42	7·03
	C. angustifolia	14 — 18·09 — 28·53 — 37	23·31	5·22
	B. betulina	5 — 8·47 — 19·83 — 29	14·15	5·68
	B. crenulata	5 — 8·00 — 17·64 — 26	12·82	4·82
Vein islets (4 sq. mm.) 1 sq. mm. •	*E. truxillense*	9 — 12·22 — 19·51 — 27	15·87	3·65
	E. coca	7 — 8·91 — 15·17 — 23	12·04	3·13
	C. acutifolia	23 — 28·53 — 38·50 — 44	33·51	4·98
	C. angustifolia	15 — 18·48 — 25·19 — 29	21·83	3·35
	B. betulina	5 — 8·76 — 15·11 — 20	11·94	3·17
	B. crenulata	4 — 8·21 — 14·10 — 19	11·15	2·94

• Vein islets determined on 4 sq. mm. and expressed as vein islets per 1 sq. mm.

TABLE IV

COARSE POWDERS—RANGES AND MEANS OF VEINLET-TERMINATION NUMBER

Species	Range	Mean	Standard deviation
E. truxillense	14·40 — 19·66 — 35·70 — 53·28	27·68	8·02
E. coca	12·96 — 15·23 — 22·51 — 27·36	18·87	3·64
C. acutifolia	27·36 — 29·88 — 43·04 — 50·41	36·46	6·58
C. augustifolia	18·72 — 23·41 — 35·29 — 44·65	29·35	5·94

DISCUSSION

The analysis of variance in Table II for the two species of coca indicates that, for all three characters, the variance due to leaves and to position is greater in *E. coca* than in *E. truxillense*. For veinlet termination number, positional variance in both species is less significant than that due to differences between leaves, hence the position on the leaf at which the character is determined is comparatively unimportant. This is not so in the case of vein islets for *E. coca*, for which species positional variance is significantly greater than that due to differences between leaves. When all three characters are considered together, it is seen that the variance due to differences between species is more significant for veinlet termination number than for either of the other two. The veinlet termination number would, therefore, appear to be

the more desirable character, and since it can be determined on either whole, broken or coarsely powdered leaves, it is also better suited to practical application. Similar results were obtained for the other species under examination except that, in the case of *B. betulina* and *B. crenulata*, the closeness of the means for all three characters rendered the differentiation of the two species impracticable. Consideration of the figures for veinlet termination number and vein islets per sq. mm. in Table I does not suggest that any relationship exists between the two characters, and this was confirmed by calculation of the correlation coefficients which were not significant. During the course of this investigation the areas of the leaves of *B. betulina*, *B. crenulata* and *E. coca* were measured with a planimeter after projection. Neither veinlet termination number nor the number of vein islets per sq. mm. was found to bear any relationship to the area of leaf. The ranges for single counts of veinlet termination number on coarse powders in Table IV approximate to those for the whole leaves given in Table III and the means are practically identical, thus confirming that this character can be accurately determined on coarse powders.

SUMMARY

1. Veinlet termination number is defined as the number of veinlet terminations per sq. mm. of leaf surface. A veinlet termination is the ultimate free termination of a veinlet or branch of a veinlet.

2. Veinlet termination numbers, the number of vein islets per 1 sq. mm. and per 4 sq. mm. of leaf surface, have been determined on whole leaves and their relative values as specific differential characters discussed.

3. Veinlet termination number and the number of vein islets per 1 sq. mm. of leaf surface show no significant correlation. Veinlet termination number is independent of the area of the leaf and is not significantly dependent on the position at which it is determined.

4. Veinlet termination number may be used to differentiate coarse powders of certain leaves belonging to co-generic species. The ranges of the means of 20 counts ($P = 0{\cdot}01$) are: *E. truxillense* 23·1 to 32·3; *E. coca* 16·8 to 21·0; *C. acutifolia* 32·7 to 40·2; *C. angustifolia* 25·9 to 32·8.

The authors thank Dr. J. M. Rowson, Curator of the Pharmaceutical Society's Museum, and Mr. J. E. Roger, late Assistant Keeper of the Manchester Museum, for the supply of certain specimens of leaves used in this investigation.

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DISCUSSION

The paper was presented by MR. J. P. HALL.

DR. J. W. FAIRBAIRN (London) expressed surprise at the lack of correlation between the number of veinlet terminations and the number of vein islets.

DR. J. M. ROWSON (London), in a written contribution, said that he welcomed the application of the method to coarse powders of leaves and the fact that it was statistically more reliable than determination of the vein islet number which had left much to be desired. He offered the following two suggested emendations: (a) Materials: The number of leaves examined for each species should be stated in Table III and in both Tables I and III the number of different samples of each species examined should also be stated. He understood that 20 leaves were drawn from several distinct parcels of authenticated material for each species and that made the figures of much greater significance than if representing one parcel only of each drug. To confirm that fact an analysis of variances between parcels within a sample could well be included in Table II. (b) The second decimal place in values Tables I, III, IV was of no significance and should be omitted.

MR. HALL, in reply, said that he had expected to find a correlation between vein islet number and veinlet termination number on physiological grounds, but in fact no such correlation had been found. They had since separated 2 species of belladonna by the method and work was continuing on its further applications. The samples of 20 leaves were made up from 2 to 5 different samples and every endeavour was made to pick a full range of leaf size. As to the second decimal place recorded in Tables I, II, III and IV, he was of the opinion that it was significant.