PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

# On the Causes and Ecological Significance of Stomatal Frequency, with Special Reference to the Woodland Flora

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PHILOSOPHICAL TRANSACTIONS.

# I. *On the Causes and Ecological Significance of Stomatal Frequency, with Special Reference to the Woodland Flora.*

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Though numerous investigators have recorded observations on the number of stomata present in various species, comparatively little is known respecting the causes of their numerical variation. Studies on the "sun" and "shade" leaves of woodland plants brought to light the striking differences which the numerical frequency of stomata may exhibit in leaves of the same species when growing in different environments and even in different leaves of the same individual.

Various hypotheses have been put forward to explain the larger number of stomata in sun-leaves. These will be considered later, but we may note here the apparent discrepancy with the observations of Ziegeler that the leaves of the more xerophytic Carices possess fewer stomata than the leaves of those species characteristic of damper habitats. Spitzer obtained similar results from an examination of the Grasses and Adamson appears to have arrived at a similar conclusion with regard to the various species of *Veronica* studied by him ("Comparative anatomy of the leaves of certain species of *Veronica*," 'Linn. Soc. Jour., Bot.,' vol. XL, pp. 247–274, 1912).

In the less xerophyllous species such as *V. Cookiana*, the stomata are numerous, amounting to as many as 2,200 per sq. mm. below and 200 per sq. mm. above. In the more xerophyllous species, however, the number ranged from about 250 to 400 per sq. mm. On the other hand, Bonnier ("Recherches expérimentales sur l'adaptation des plantes au climat alpin," 'Ann. Sci. Nat.,' Ser. 7, T. XX, pp. 217–358, 1894) found that the stomatal frequency was greater in the alpine as compared with the lowland forms of the same species. Similarly Parkin and Pearson, investigating the leaf anatomy of plants from the Ceylon Patanas ('Jour. Linn. Soc.,' vol. XXXV, pp. 430–463, 1904), found that in 42 species of the wet Patanas characterised by an evenly distributed and copious rainfall the average frequency was 309 stomata per sq. mm. (both surfaces

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together), whilst in the dry Patanas, characterised by a low rainfall and high evaporation accentuated by the S.W. monsoon, the average frequency in 40 spp. was 356 stomata per sq. mm.

The woodland community offers obvious advantages for the investigation of this problem, which is important not merely from its bearing on the nature of a particular ecological assemblage, but also from its general significance. In the course of ecological studies on British Woodlands, a number of species have been grown under controlled conditions, and material of most of the woodland plants in the British Flora has been examined from this point of view, usually from a considerable diversity of localities and environments. The statistical terms and methods of calculation are those employed by YULE (*cf.*," Theory of Statistics," 6th Ed., London, 1922).

## I.—*Frequency in relation to leaf area.*

For the purpose of testing any possible correlation between the total area of a leaf and the number of stomata on a unit area, it is manifestly important that not only should the external environment be uniform, but that the influence of the plant itself (the internal environment) should be, as far as possible, the same on the contrasted members.

Plants with opposite leaves were, therefore, chosen, and the numerical frequency of the stomata determined for both members of each pair. The determinations were made on fresh material and the greatest care was exercised to ensure that the counts were made in comparable regions of the contrasted leaves. The data given represent the averages of six counts, each of an area of one sq. millimetre. Some years ago the writer showed that there was a certain degree of correlation between the area of a leaf and its vascular supply (" The determining factors in petiolar structure," 'New Phytologist,' vol. XII, pp. 281–289, 1913), and hence we may assume that, for leaves at the same level on the stem, the availability of water, as determined by the conducting tracts, although not equal is approximately proportional to their needs. URSPRUNG and BLUM have shown that the absorbing power of the cell increases with its distance from the conducting tissue, but determinations from comparable regions in each leaf eliminate errors due to this factor. It is probable, therefore, that no appreciable difference obtains between the internal factors affecting the two members of any pair, except those associated with leaf size, but as regards external conditions, a slight error is probably introduced by the fact that the two leaves at a node are not situated on the same side of the stem, otherwise differences due to relative maturity and position on the plant are eliminated by only comparing members of the same pair.

Consideration of the above data for *Mercurialis perennis* shows that, of all the fifty-six pairs of leaves examined, in no less than forty instances (71·4 per cent.) the average number of stomata per unit area was greater for the smaller than for the larger leaf of the pair.

TABLE I.—*Mercurialis perennis* L.

Stomatal number in relation to leaf area (Nodes numbered from below upwards).

| Plant. | Node. | Area of leaf. | Range of stomatal frequency. | Average frequency. | Average for node. |
|---|---|---|---|---|---|
| | | sq. cms. | | | |
| A | 1 | 2·4 | 85–131 | 104·6 | |
| | 1 | 3·4 | 90–101 | 94·6 | 99·6 |
| | 2 | 6·0 | 82–108 | 93 | |
| | 2 | 6·8 | 96–103 | 98·7 | 95·0 |
| | 3 | 7·8 | 94–107 | 101 | |
| | 3 | 8·1 | 75–88 | 81·6 | 91·3 |
| | 4 | 8·0 | 90–111 | 97·3 | |
| | 4 | 8·5 | 89–101 | 93·6 | 95·5 |
| | 5 | 6·8 | 79–93 | 87·3 | |
| | 5 | 6·9 | 82–96 | 86·6 | 87·0 |
| | 6 | 0·5 | One leaf only | 160 | |
| B | 1 | 4·3 | 74–83 | 78·0 | |
| | 1 | 4·6 | 75–107 | 88·0 | 83·0 |
| | 2 | 8·8 | 76–85 | 81·6 | |
| | 2 | 9·8 | 85–86 | 85·5 | 83·2 |
| | 3 | 12·7 | 84–103 | 96·3 | |
| | 3 | 13·3 | 75–83 | 80·0 | 88·1 |
| | 4 | 12·4 | 83–90 | 86·5 | |
| | 4 | 14·1 | 82–88 | 85·0 | 85·6 |
| | 5 | 10·9 | 76–94 | 86·0 | |
| | 5 | 11·1 | 75–85 | 80·0 | 83·5 |
| | 6 | 9·9 | 79–103 | 91·0 | |
| | 6 | 10·5 | 75–87 | 79·8 | 84·9 |
| | 7 | 2·5 | 97–109 | 103·0 | |
| | 7 | 4·0 | 80–92 | 86·6 | 93·2 |
| C | 1 | 3·7 | 85–90 | 88·0 | |
| | 1 | 4·3 | 80–89 | 84·5 | 86·2 |
| | 2 | 9·0 | 86–90 | 88·3 | |
| | 2 | 9·2 | 84–91 | 87·5 | 88·0 |
| | 3 | 9·3 | 70–83 | 76·5 | |
| | 3 | 10·1 | 70–101 | 85·5 | 81·0 |
| | 4 | 8·7 | | ? 85 | |
| | 4 | 8·8 | | ? 99 | 92·0 |
| | 5 | 6·5 | | ? 80 | |
| | 5 | 9·0 | 64–82 | 73 | 75·0 |
| D | 1 | 4·8 | 55–82 | 68·5 | |
| | 1 | 6·6 | 69–85 | 77·0 | 73·0 |
| | 2 | 7·8 | 75–93 | 84·0 | |
| | 2 | 9·5 | 53–93 | 76·0 | 79·2 |
| | 3 | 10·1 | 57–81 | 72·0 | |
| | 3 | 11·1 | 72–87 | 79·5 | 74·5 |
| | 4 | 10·1 | 82–87 | 85·2 | |
| | 4 | 11·4 | 69–90 | 78·8 | 81·6 |
| | 5 | 10·5 | 61–90 | 77·6 | |
| | 5 | 10·7 | 75–82 | 79·6 | 78·3 |
| | 6 | 7·0 | 76–90 | 82·8 | |
| | 6 | 7·9 | 83–87 | 85·6 | 83·8 |
| E | 1 | 59·3 | 71–102 | 83·7 | |
| | 1 | 59·6 | 64–103 | 77·6 | 80·6 |
| | 2 | 52·5 | 74–87 | 78·3 | |
| | 2 | 27·2 | 103–117 | 110·0 | 94·1 |

Table I (continued).

| Plant. | Node. | Area of leaf. | Range of stomatal frequency. | Average frequency. | Average for node. |
|---|---|---|---|---|---|
| | | sq. cms. | | | |
| F | 1 | 14·5 | 61–64 | 62·5 | |
| | 1 | 16·4 | 60–63 | 61·5 | 62·0 |
| | 2 | 21·7 | 70–73 | 71·5 | |
| | 2 | 24·1 | 56–64 | 60·0 | 65·7 |
| | 3 | 22·3 | 76–80 | 78·0 | |
| | 3 | 23·1 | 70–73 | 71·5 | 74·7 |
| | 4 | 17·3 | 76–80 | 78·0 | |
| | 4 | 18·6 | 68–92 | 80·0 | 80·2 |
| | 5 | 9·1 | 96–146 | 125·5 | |
| | 5 | 10·9 | 100–123 | 111·5 | 120·0 |
| G | 1 | 16·4 | 90–93 | 91·5 | |
| | 2 | 24·8 | 62–64 | 63·0 | |
| | 2 | 25·2 | 69–70 | 69·5 | 66·2 |
| | 3 | 24·0 | 82–86 | 84·0 | |
| | 3 | 29·8 | 77–82 | 79·5 | 81·7 |
| H | 1 | 30·7 | 60–61 | 60·5 | |
| | 1 | 32·0 | 64–69 | 66·5 | 63·5 |
| | 2 | 32·0 | 64–76 | 70·0 | |
| | 2 | 35·0 | 65–68 | 66·5 | 68·6 |
| | 4 | 6·9 | 77–88 | 83·3 | |
| | 4 | 10·9 | 80–82 | 81·0 | 82·4 |
| J | 1 | 6·1 | 74–78 | 76·0 | |
| | 1 | 7·6 | | 53·0 | 64·5 |
| | 3 | 25·6 | 69–75 | 72·0 | |
| | 3 | 29·6 | 59–60 | 59·5 | 65·5 |
| K | 1 | 3·7 | | 90·0 | |
| | 1 | 4·3 | | 85·0 | 87·5 |
| | 2 | 9·0 | | 88·0 | |
| | 2 | 9·2 | | 87·0 | 87·5 |
| | 3 | 9·3 | | 76·0 | |
| | 3 | 10·1 | | 85·0 | 80·5 |
| | 4 | 8·7 | | 85·0 | |
| | 4 | 8·8 | | 99·0 | 92·0 |

| Plant. | Node. | Area of leaf. | Average stomatal frequency. | Average for node. |
|---|---|---|---|---|
| | | sq. cms. | | |
| K | 5 | 6·5 | 80·0 | |
| | 5 | 9·0 | 73·0 | 76·5 |
| L | 1 | 14·5 | 62·5 | |
| | 1 | 16·4 | 61·5 | 62·0 |
| | 2 | 21·7 | 71·0 | |
| | 2 | 24·1 | 60·0 | 65·5 |
| | 3 | 22·3 | 78·0 | |
| | 3 | 23·1 | 71·5 | 74·7 |
| | 4 | 17·3 | 78·0 | |
| | 4 | 18·6 | 81·0 | 79·5 |
| | 5 | 10·9 | 112·0 | |
| | 5 | 9·1 | 125·0 | 118·5 |
| | 1 | 16·4 | 91·5 | |
| | 2 | 24·8 | 63·0 | |

Table I (continued).

| Plant. | Node. | Area of leaf. | Average stomatal frequency. | Average for node. |
|---|---|---|---|---|
| L | 2 | 25·2 | 69·5 | 66·0 |
| | 3 | 24·0 | 84·0 | |
| | 3 | 29·8 | 80·0 | 82·0 |
| | 1 | 33·7 | 66·0 | |
| | 1 | 28·4 | 61·0 | 63·5 |
| | 2 | 40·7 | 70·0 | |
| | 2 | 43·9 | 66·5 | 68·2 |
| | 3 | 20·9 | 74·0 | |
| | 3 | 22·4 | 71·0 | 72·5 |
| | 4 | 5·3 | 83·0 | |
| | 4 | 8·7 | 81·0 | 82·0 |
| M | 1 | 8·7 | 91·0 | |
| | 1 | 6·4 | 104·0 | 97·0 |
| | 2 | 14·7 | 77·0 | |
| | 2 | 15·9 | 70·6 | 74·0 |
| | 3 | 17·7 | 88·0 | |
| | 3 | 16·5 | 78·0 | 83·0 |
| | 4 | 14·9 | 70·0 | |
| | 4 | 13·0 | 72·0 | 71·0 |

Mean leaf area = 15·4 sq. cms. $\sigma = 10{\cdot}9$. Mean stomatal frequency 81·6 $\sigma = 14{\cdot}7$.

In most plants the effect of position on the stem, dealt with in the next section, masks to a greater or less degree the relation between leaf size and frequency of stomata as between leaves at different nodes. The following data regarding *Daphne laureola* show a general relation of this kind, but it will be noted that the two lowest leaves, despite their small size, have the lowest stomatal frequencies.

TABLE II.—Stomatal frequency in *Daphne laureola*.
(The numbering of the nodes is from below upwards.)

| Area of leaf. | Observed range. | Average frequency. | Number of node. |
|---|---|---|---|
| sq. cms. | | | |
| 1·9 | 63–69 | 63·6 | 2 |
| 2·4 | 115–125 | 120·0 | 15 |
| 3·5 | 123–138 | 130·0 | 14 |
| 3·8 | 43–74 | 57·6 | 3 |
| 4·4 | 96–111 | 101·3 | 13 |
| 5·1 | 103–127 | 112·0 | 12 |
| 6·0 | 76–91 | 79·5 | 4 |
| 6·7 | 103–112 | 107·3 | 11 |
| 7·4 | 85–103 | 96·3 | 10 |
| 8·9 | 96–114 | 106·6 | 9 |
| 9·8 | 67–76 | 70·6 | 5 |
| 10·7 | 91–98 | 93·6 | 8 |
| 11·1 | 82–94 | 90·7 | 6 |
| 11·2 | 80–94 | 87·0 | 7 |

Even in those species whose leaves are borne at approximately the same level comparison cannot usually be made of a number from the same plant, owing to their crowding and the consequent differences in the environment which surrounds the different leaves. Nevertheless, a marked negative correlation between leaf size and stomatal frequency is shown by the narrow-leaved form of *Statice binervosa* when growing in conditions of extreme insolation (*cf.* fig. 1).

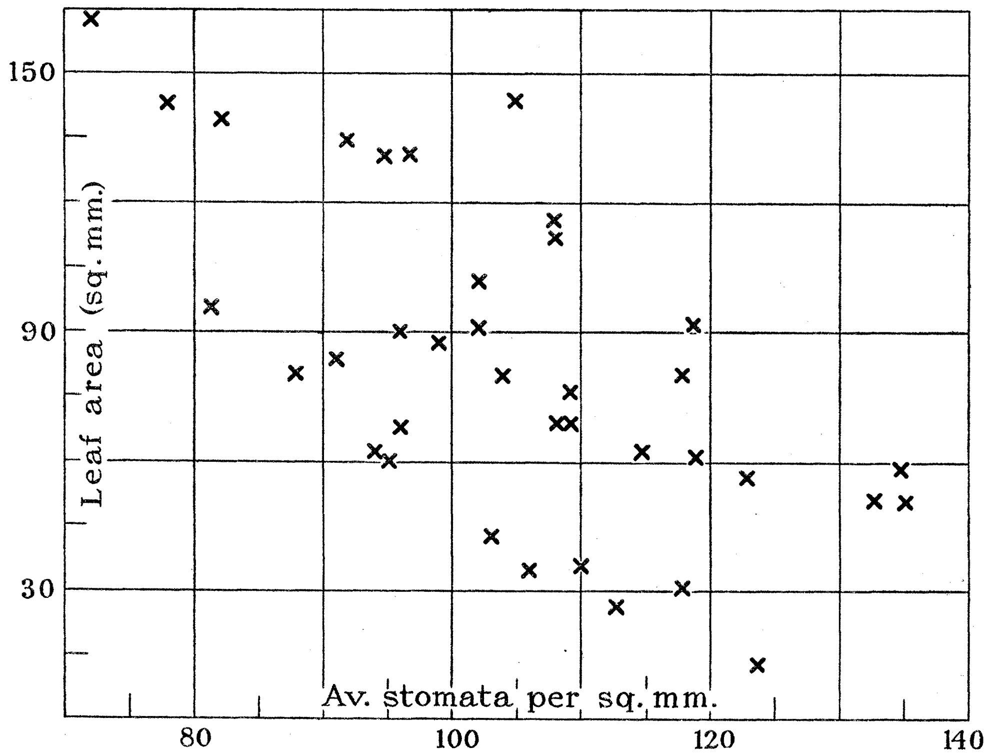


FIG. 1.—*Statice binervosa.*

Examination of different leaves, as nearly comparable as possible except as regards size, showed the same general relation to hold in most cases, and it would seem justifiable, therefore, to regard the negative correlation between leaf area and stomatal frequency as of widespread occurrence. Even if we ignore the position of the leaves on the stem, the degree of correlation is remarkably high. Thus, in the case of *Mercurialis perennis*, the correlation coefficient for leaf area and stomatal frequency based on an examination of 121 leaves was — 0·400 and its probable error 0·0514, indicating that, despite other factors, there is a distinct connection between the two features.

It is in striking contrast to the marked negative correlation presented by the leaves of *Statice binervosa* growing on shingle, that the leaves of *Ficaria verna* show little if any relation between leaf area and stomatal frequency, as is indicated by the following data, and the negative correlation is not very pronounced in the broad-leaved form of *S. binervosa*.

TABLE III.—Leaf area and stomatal frequency in *Ficaria verna*.

| Area of leaf. | Average number of stomata (both surfaces). | Area of leaf. | Average number of stomata (both surfaces). |
|---|---|---|---|
| sq. cms. | | sq. cms. | |
| 1·06 | 97·0 | 3·35 | 54·5 |
| 1·27 | 97·0 | 3·80 | 75·0 |
| 1·32 | 119·0 | 3·82 | 61·6 |
| 1·69 | 104·0 | 4·09 | 45·6 |
| 2·63 | 57·0 | 4·13 | 70·0 |
| 2·70 | 64·0 | 4·24 | 129·3 |
| 2·90 | 147·0 | 4·25 | 65·2 |
| 3·02 | 58·6 | 4·45 | 80·5 |
| 3·05 | 47·6 | 4·59 | 59·7 |
| 3·05 | 49·0 | 4·97 | 83·4 |
| 3·07 | 63·9 | 5·25 | 66·3 |
| 3·08 | 102·0 | 5·48 | 73·6 |
| 3·32 | 76·0 | 8·97 | 105·0 |

The correlation coefficient is here positive, instead of negative as in the previous cases considered. That is, so far as these data are concerned, decrease in the size of the leaf tends to be accompanied by a decrease, instead of an increase, in the number of stomata, and *vice-versa*. As, however, the correlation coefficient is only $+0{\cdot}074$, which is less than the probable error, no importance can be attached to this feature. The fact that there is no "significant" correlation is, however, to be emphasised, as despite the comparatively small number of leaves examined, viz., 26, the contrast with *Statice binervosa* (30 leaves) is so marked. The *Ficaria* leaves were obtained from eight different plants, grown under uniform conditions as to soil and in a moist atmosphere, four being well illuminated and the other four in shade. It would, therefore, seem that the relation between leaf size and stomatal frequency, which is so pronounced when we compare leaves of the same node, may be entirely masked under humid conditions. This is borne out by examination of *Mercurialis* leaves from plants growing in very damp air under bell-jars. Since the different parts of the plant will naturally be growing under differing conditions of humidity it will be convenient to consider next the relation which stomatal frequency exhibits to the varying positions in which the leaf is borne upon the plant.

### 2.—*Stomatal frequency in relation to the position of the leaf upon the plant.*

YAPP, in his study of *Ulmaria palustris* ('Ann. Bot.,' vol. XXVI, p. 826, 1912), drew attention to the fact that there appeared to be a regular increase in the number of stomata per unit area as one passed from the basal to the apical leaves, and raised the question as to whether the phenomenon might not be a general one. Inasmuch as the leaves of most plants tend to become smaller as one passes from the base to the apex, it is apparent that the phenomenon may be but an expression of the negative correlation dealt with in the preceding section, but, before considering this aspect, we may enquire as to whether this gradient of stomatal frequency is a general one or not.

Woodland grasses offer a suitable field of enquiry owing to the ease with which leaves of comparable width and length can be obtained from varying heights on the same plant. Data from several species are given in the accompanying table.

TABLE IV.—Stomatal frequency in relation to height above the ground.

| Height above ground. | Stomatal frequency per sq. mm. | | | | |
|---|---|---|---|---|---|
| | Upper surface. | | Lower surface. | | Total average. |
| | Range. | Average. | Range. | Average. | |
| I. *Holcus mollis.* | | | | | |
| cms. | | | | | |
| 16·5 | 48–65 | 54 | 18–29 | 24 | 79 |
| 22·5 | 55–61 | 57 | 24–34 | 27 | 84 |
| 29·0 | 57–84 | 71 | 34–43 | 41 | 113 |
| 39·0 | 60–71 | 65 | 49–60 | 53 | 118 |

| | Height above ground | Stomatal frequency per sq. mm. | |
|---|---|---|---|
| | | Range. | Average. |
| II. *Brachypodium sylvaticum* (stomata confined to upper surface). | | | |
| | cms. | | |
| Plant A . . . . . . . | 18·5 | 186–229 | 206 |
| | 29·0 | 226–234 | 229 |
| | 43·0 | 279–324 | 299 |
| | 64·0 | 293–313 | 301 |
| Plant B . . . . . . . | 17·0 | 130–136 | 133 |
| | 26·0 | 185–200 | 193 |
| | 35·0 | 255–272 | 266 |
| | 49·0 | 310–334 | 319 |
| Plant C . . . . . . . | 24·0 | 180–209 | 196 |
| | 36·0 | 214–219 | 216 |
| | 56·0 | 270–291 | 283 |
| | 78·0 | 273–297 | 289 |

Table IV (continued).

| Height above ground. | Stomatal frequency per sq. mm. | | |
|---|---|---|---|
| | Upper surface. | Lower surface. | Total average. |
| III. *Bromus asper*. | | | |
| cms. | | | |
| 33·5 | 36–37 | 4–5 | 41 |
| 46·0 | 42 | 0–4 | 44 |
| 62·0 | 36–42 | 0–2 | 40 |
| 90·0 | 47–53 | 0–2 | 51 |

| Height above ground. | Range. | Average. |
|---|---|---|
| IV. *Hordeum sylvaticum* (upper surface only, lower with 0–16 stomata per sq. mm.). | | |
| cms. | | |
| 3·0 | 64–65 | 64 |
| 5·0 | 60–66 | 63 |
| 7·0 | 65–71 | 68 |
| 16·0 | 80–86 | 83 |
| 36·0 | 83–104 | 91 |

V. *Festuca gigantea* (upper surface only, lower with 0–7 stomata per sq. mm.).

| | Height above ground. | Average. | | Height above ground. | Average. |
|---|---|---|---|---|---|
| | cms. | | | cms. | |
| Plant A . . . | 24·0 | 37 | Plant B . . . | 17·0 | 28 |
| | 38·0 | 34 | | 30·0 | 29 |
| | 57·0 | 44 | | 46·0 | 29 |
| | 99·0 | 62 | | 65·0 | 39 |
| | | | | 93·0 | 41 |

In all the above grasses it will be noted there is an almost uniform increase of stomatal frequency from below upwards, and the same feature is encountered in other groups, where, however, the decrease in size of leaves from below upwards is usually too rapid to admit of comparison of equal sized leaves.





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TABLE V.—Stomatal frequency in relation to height of leaf above ground.

*Polygonatum multiflorum* (stomata on lower surface only).

| Height of leaf. | Stomatal frequency per sq. mm. | |
|---|---|---|
| | Range. | Average. |
| *Polygonatum multiflorum* (stomata on lower surface only). | | |
| cms. | | |
| 21·5 | 50–57 | 53·6 |
| 27·0 | 52–60 | 59·0 |
| 30·2 | 75–78 | 76·5 |
| 50·2 | 72–75 | 73·0 |
| 51·8 | 68–82 | 77·0 |
| 53·8 | 78–88 | 83·6 |
| 55·2 | 84–93 | 88·3 |
| 56·5 | 87–95 | 91·0 |

| Height of leaf. | Stomatal frequency per sq. mm. | | |
|---|---|---|---|
| | Average upper. | Average lower. | Total average. |
| *Epilobium montanum.* | | | |
| cms. | | | |
| 3·0 | 29 | 120 | 149·0 |
| 7·5 | 12 | 145 | 157·0 |
| 14·0 | 8 | 256 | 264·0 |
| 24·0 | 23 | 293 | 321·0 |
| 36·0 | 6 | 312 | 318·0 |
| 40·0 | 6 | 284 | 290·0 |

| Height of leaf. | Stomatal frequency per sq. mm. | |
|---|---|---|
| | Range. | Average. |
| *Campanula trachelium* (stomata only on lower surface). | | |
| cms. | | |
| 14·0 | 102–140 | 119·0 |
| 28·0 | 108–135 | 123·0 |
| 34·0 | 142–175 | 156·0 |
| 39·0 | 148–184 | 168·0 |
| 44·5 | 236–258 | 245·0 |
| Bracts 51·5 | 220–230 | 225·0 |
| 59·0 | | 254·0 |

Table V.—(continued).

| Height of leaf. | Stomatal frequency per sq. mm. | | |
|---|---|---|---|
| | Upper. | Lower. | Total average. |
| *Lychnis dioica.* | | | |
| cms. | | | |
| 5·0 (radical) | 2–5 | 43–45 | 46·5 |
| 15·0 | 3–6 | 62–74 | 71·1 |
| 14·0 (cauline) | 1–2 | 52–70 | 63·0 |
| 14·0 | 8–9 | 58–72 | 69·6 |
| 24·0 | 0–1 | 45–72 | 55·9 |
| 37·0 | 0–2 | 55–86 | 70·0 |
| 49·0 | 0–1 | 134–144 | 141·0 |

The fact that radical leaves in general have fewer stomata than cauline leaves is in conformity with the above data. A few instances from species of various habitats will sufficiently illustrate this point.

Table VI.—Frequency in radical and cauline leaves.

| | Upper surface. | Lower surface. | Total average. |
|---|---|---|---|
| *Ranunculus auricomus* (woodland)— | | | |
| Radical leaf . . . . . . . . . . . . . . . . | 12–15 | 27–35 | 45·3 |
| ,, ,, . . . . . . . . . . . . . . . . | 11–13 | 33–40 | 47·3 |
| ,, ,, . . . . . . . . . . . . . . . . | 4–15 | 27–50 | 45·0 |
| Cauline ,, . . . . . . . . . . . . . . . . | 17–25 | 49–71 | 73·0 |
| ,, ,, . . . . . . . . . . . . . . . . | 18–23 | 38–53 | 75·9 |
| ,, ,, . . . . . . . . . . . . . . . . | 17–25 | 38–71 | 69·0 |
| *Ranunculus parviflorus* (heath)— | | | |
| Radical leaf . . . . . . . . . . . . . . . . | 14–18 | 40–56 | 62·0 |
| Cauline ,, . . . . . . . . . . . . . . . . | 12–20 | 88 | 105·0 |
| *Serratula tinctoria*— | | | |
| Radical leaf (average of a number) . . . . | 8–10 | 80–132 | 114·0 |
| Cauline ,, ,, ,, ,, . . . . | 22–35 | 215–316 | 280·0 |
| *Gentiana campestris* (chalk down)— | | | |
| Radical leaf . . . . . . . . . . . . . . . . | 0 | 47–129 | 88·0 |
| Cauline ,, . . . . . . . . . . . . . . . . | 0 | 88–134 | 112·0 |
| *Ranunculus sceleratus* (marsh)— | | | |
| Radical leaf . . . . . . . . . . . . . . . . | 22–32 | 33–45 | 67·5 |
| Cauline ,, . . . . . . . . . . . . . . . . | 33–37 | 54–56 | 90·0 |

Reference to the data given for *Mercurialis* presented graphically in fig. 2 show the same general increase from below upwards. Yapp, as already mentioned, found this to hold for *Ulmaria* and Miss Rea (' New Phyt.,' vol. XX, pp. 56–72, 1921) for *Campanula rotundifolia*. It is probably, therefore, a general feature of herbaceous species. The appended data, based on the examination of fully exposed leaves from the lower and upper regions of the same individual, leave but little doubt that the generalisation holds true also for trees and shrubs, when these are growing isolated as in the specimens examined.

| Species. | Height above ground in metres. | Range of Stomatal frequency per sq. mm. | Mean for six leaves. |
|---|---|---|---|
| *Sorbus aucuparia* . . . . . | 4·56 (15 ft.) | 146–164 | 118·0 |
| " " . . . . . | 1·8 (6 ft.) | 46–80 | 67·5 |
| *Prunus avium* . . . . . . | 3·65 (12 ft.) | 216–366 | 288·0 |
| " " . . . . . . | 2·1 (7 ft.) | 120–252 | 188·0 |
| *Cratægus monogyna* . . . . | 4·2 (14 ft.) | 128–225 | 159·0 |
| " " . . . . | 1·8 (6 ft.) | 114–130 | 122·0 |

Examination of the graph in fig. 2, showing the mean stomatal frequency of a number of leaves from each of the successive nodes of *Mercurialis* plotted against the inverted " curve " for the corresponding mean leaf areas, shows that, although the general trend

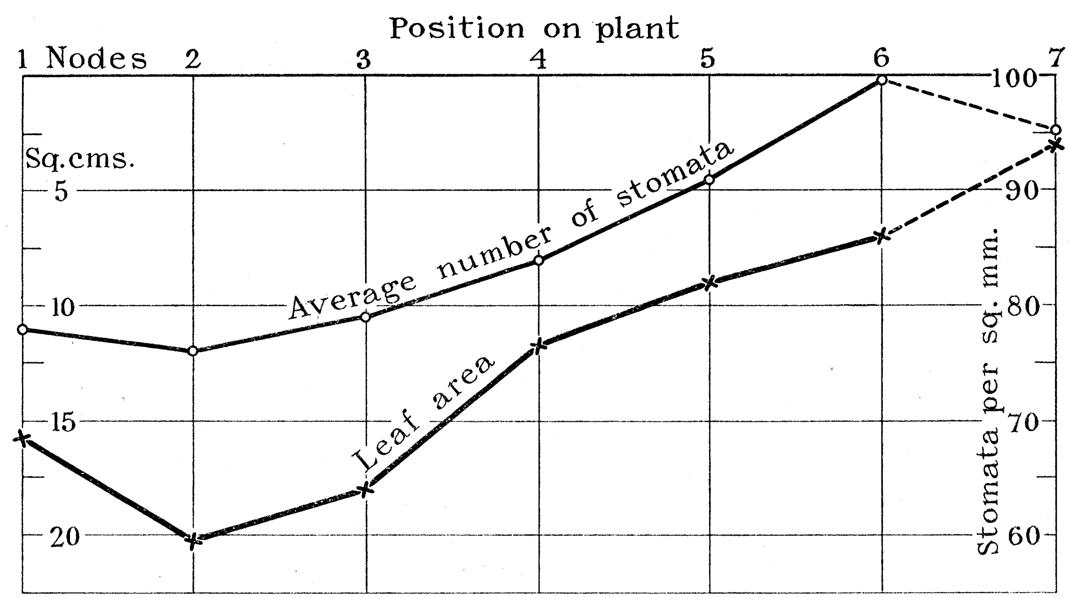


Fig. 2.—*Mercurialis perennis*, L.—Averages for leaves from equivalent nodes of 13 plants.

is an increased frequency from below upwards, the leaves of the second node have a lower mean frequency than those of the lowest node (Node 1), and as the second node has the largest mean leaf area, it would seem that the factor or factors connected with the latter feature are of more importance than actual position on the plant. Nevertheless, the importance of the factors connected with position are at once apparent in the case of specimens grown in dry conditions. For, as shown by the data given below for two plants of *Mercurialis* grown on dry soil in full sun, the middle leaves have a higher frequency than the lowest, despite their considerably larger size.

TABLE VII.—Frequency of Stomata in *Mercurialis perennis* grown in dry conditions.

| | | | | |
|---|---|---|---|---|
| Plant A . . | Size of leaf . . . . . | 3·22 sq. cms. | 9·55 sq. cms. | 6·64 sq. cms. |
| | Stomatal range . . . | 224–246 per sq. mm. | 137–150 | 134–140 |
| | Average frequency per sq. mm. . . . . . . | 234 | 144 | 137 |
| | Position of leaf. | Apex of stem. | Middle. | Base. |
| Plant B . . | Size of leaf . . . . . | 3·93 sq. cms. | 9·20 sq. cms. | 5·44 sq. cms. |
| | Stomatal range . . . | 208–234 per sq. mm. | 143–163 | 110–176 |
| | Average frequency per sq. mm. . . . . . . | 222 | 154 | 153 |

The converse is also true, namely, that the frequency gradient tends to disappear when plants are grown in a moist atmosphere. This is shown by the data given below, and has been found to obtain in nearly all the plants examined (*cf.* also figs. 8 and 12).

*Mercurialis* grown in a humid atmosphere.

| Position of leaf. | Uppermost node. | Second node. | Third node. | Fourth node. | Fifth node. |
|---|---|---|---|---|---|
| Area of leaf . . . . . . . . . | 1·60 | 2·78 | 2·86 | 2·24 | 0·74 |
| Stomatal range . . . . . . . . | 184–252 | 182–248 | 209–238 | 200–220 | 85–114 |
| Average per sq. mm. . . . . . | 208 | 215 | 226 | 210 | 100 |

It would appear then from the observed facts that the frequency gradient associated with increasing height above the ground is correlated with the greater degree of exposure to which the leaf is subject the higher the level at which it is borne. It is in conformity with this view that the leaves of *Ficaria* growing in moist air, but all at about the same level, showed no correlation with leaf size, whilst those of *Statice binervosa* growing in dry conditions showed marked correlation. More significant is the absence of any marked gradient of frequency in the successive leaves of prostrate shoots growing in moist air (in dry air the shelter effect of the parent plant on the base of the prostrate shoot has an effect). In the two shoots of *Galeobdolon* for which data are given, one had the highest

Prostrate shoot of *Galeobdolon luteum* in moist air.

| — | Average frequency on leaves. | | | |
|---|---|---|---|---|
| | First node. | Second node. | Third node. | Base. |
| Shoot A . . . . . | 142 | 121 | 133 | 129 |
| Shoot B . . . . . | 106 | 125 | 131 | — |

mean frequency in the lowest leaf and the slight gradient is the reverse of that usually present although the leaf areas for both members of each pair diminished from below upwards. In this shoot, however, the axis was appreciably arched, as a consequence of which the tip was nearer the ground, whilst the basal leaves were raised a short distance above it. In the second instance it is true the uppermost leaf has the highest mean, but the sequence is irregular and the increase is small compared with the variation on individual leaves (uppermost leaf 127–150 ; lowest but one 123–142). The following figures for a prostrate shoot of *Vinca minor* growing in dry air show a very marked gradient, the only irregularity being associated with the very small leaf at the base.

Stomatal frequency in prostrate shoot of *Vinca minor* in dry air.

| Position. | Base. | Node 2. | Node 2. | Node 3. | Node 3. | Node 4. | Node 5. | Node 5, tip. |
|---|---|---|---|---|---|---|---|---|
| Leaf area . . . . | 0·5 | 1·4 | 1·5 | 4·06 | 3·0 | 4·68 | 0·83 | 0·67 |
| Mean frequency . . | 259 | 198 | 204 | 230 | 249 | 310 | 388 | 380 |

Six successive leaves of *Lysimachia nemorum* of nearly equal size except for the two ultimate ones, exhibited frequencies of 117 ; 107 ; 108 ; 110 ; 147 and 163. The last two leaves were, however, under suspicion of being immature, so that this shoot, which grew in natural surroundings, was probably no exception to the generalisation made above.

It is suggested that the humidity of the internal and external environments of a leaf are the controlling factors in determining the numerical frequency of its stomata, and, therefore, it is important to consider next to what extent the frequency varies on individual leaves and whether this variation is in any way related to the part of the leaf considered.

3.—*Stomatal frequency in relation to the position on the individual leaf.*

Examination of different parts of the same leaf shows that there is often considerable variation in the stomatal frequency, and that the frequency bears a definite relation to the position on the leaf. It is clear, therefore, that for purposes of comparison between one leaf and another, whether of different individuals or of different species, it is important that not only the position of the leaf on the plant but also the region of the leaf examined should be taken into consideration. In the section dealing with frequency in relation to leaf area, the data given were based on determinations from comparable regions of the different leaves examined.

It is clearly important to know how and to what degree the frequency varies on the individual leaf before we attempt to analyse the causes of stomatal frequency. In the accompanying data for *Statice binervosa* it will be seen that in every one of the fifteen leaves, the highest frequency is found near the leaf-tip, the lowest frequency towards the base, whilst the frequency for the middle region of the leaf is intermediate. There is thus a frequency gradient from base to apex of the individual leaf comparable to that from the base to the apex of the plant.

TABLE VIII.—Stomatal frequency in broad-leaved *Statice binervosa* in relation to position on the leaf.

| Leaf area. | —— | Base of leaf. | Middle of leaf. | Apical region of leaf. |
|---|---|---|---|---|
| sq. cms. | | | | |
| Plant A— | | | | |
| 3·36 | Upper surface . . . . . | 17 | 39 | 43 |
| | Lower surface . . . . . | 30 | 37 | 37 |
| | Total . . . | 47 | 76 | 80 |
| 3·10 | Upper surface . . . . . | 23 | 38 | 42 |
| | Lower surface . . . . . | 18 | 39 | 41 |
| | Total . . . | 41 | 77 | 83 |
| 3·08 | Upper surface . . . . . | 24 | 39 | 49 |
| | Lower surface . . . . . | 26 | 37 | 42 |
| | Total . . . | 50 | 76 | 91 |
| 1·64 | Upper surface . . . . . | 19 | 38 | 37 |
| | Lower surface . . . . . | 17 | 34 | 37 |
| | Total . . . | 36 | 72 | 74 |
| 0·84 | Upper surface . . . . . | 35 | 45 | 54 |
| | Lower surface . . . . . | 27 | 46 | 45 |
| | Total . . . | 62 | 91 | 99 |

Table VIII (continued).

| Leaf area. | — | Base of leaf. | Middle of leaf. | Apical region of leaf. |
|---|---|---|---|---|
| Plant B— 1·16 | Upper surface . . . . . | 41 | 58 | 58 |
| | Lower surface . . . . . | 23 | 50 | 55 |
| | Total . . . | 64 | 108 | 113 |
| 0·60 | Upper surface . . . . . | 43 | 44 | 48 |
| | Lower surface . . . . . | 34 | 51 | 57 |
| | Total . . . | 77 | 95 | 105 |
| 0·34 | Upper surface . . . . . | 29 | 53 | 59 |
| | Lower surface . . . . . | 27 | 53 | 60 |
| | Total . . . | 56 | 106 | 119 |
| 0·30 | Upper surface . . . . . | 50 | 58 | 63 |
| | Lower surface . . . . . | 37 | 60 | 57 |
| | Total . . . | 87 | 118 | 120 |
| 0·12 | Upper surface . . . . . | 51 | 68 | 62 |
| | Lower surface . . . . . | 36 | 57 | 62 |
| | Total . . . | 87 | 126 | 124 |
| Plant C— 3·75 | Upper surface . . . . . | 39 | 49 | 63 |
| | Lower surface . . . . . | 31 | 44 | 48 |
| | Total . . . | 70 | 93 | 111 |
| 2·55 | Upper surface . . . . . | 34 | 45 | 53 |
| | Lower surface . . . . . | 30 | 44 | 51 |
| | Total . . . | 64 | 89 | 104 |
| 2·42 | Upper surface . . . . . | 40 | 54 | 65 |
| | Lower surface . . . . . | 43 | 48 | 59 |
| | Total . . . | 83 | 102 | 124 |
| 1·51 | Upper surface . . . . . | 60 | 65 | 89 |
| | Lower surface . . . . . | 57 | 72 | 98 |
| | Total . . . | 117 | 137 | 187 |
| 0·88 | Upper surface . . . . . | 70 | 79 | 78 |
| | Lower surface . . . . . | 64 | 65 | 78 |
| | Total . . . | 134 | 144 | 156 |
| Mean for all leaves . . . | Upper surface . . . . . | 38·3 | 51·4 | 57·5 |
| | Lower surface . . . . . | 33·3 | 49·0 | 55·1 |
| | Total . . . | 71·6 | 100·4 | 112·6 |

If we consider the two surfaces separately it will be seen that, of the fifteen upper surfaces, three do not show the complete gradient, and of the fifteen lower, two are irregular. But mean values show that whilst the lower surface has a lower frequency than the corresponding upper surface, the proportional increase in stomatal numbers from the base to the apex of the leaf is approximately the same for both the upper and lower surfaces. Similar data for *Scabiosa succisa*, *Stellaria holostea* and *Adoxa moschatellina*, &c., point to the general nature of the phenomenon (*cf.* Table IX).

TABLE IX.—Average Stomatal frequency in relation to position on the leaf (Various spp.).

| — | Base of leaf. | Middle of leaf. | Apical region of leaf. | |
|---|---|---|---|---|
| *Scabiosa succisa*— | | | | |
| Upper surface | 60 | 78 | 108 | Cauline, at 4 cms. |
| Lower surface | 140 | 168 | 148 | |
| Upper surface | 90 | 106 | 137 | Cauline, at 9 cms. |
| Lower surface | 170 | 218 | 224 | |
| Upper surface | 121 | 174 | 160 | Small leaf at 19 cms. |
| Lower surface | 122 | 250 | 234 | |
| Mean U + L | 234 | 331 | 337 | |

| — | Base of leaf. | Middle of leaf. | Apical region of leaf. |
|---|---|---|---|
| *Stellaria holostea* (lower surface only, upper with 0–6 stomata). | | | |
| Large leaves near base of stem | 72 | 119 | 130 |
| | 53 | 71 | 91 |
| Small leaves near apex of stem | 106 | 164 | 198 |
| | 126 | 146 | 174 |
| Mean | 89 | 125 | 148 |

| — | Base of leaf. | Middle of leaf. | Apical region of leaf. |
|---|---|---|---|
| *Adoxa moschatellina* (stomata on lower surface only). | | | |
| Leaf A | 24 | 27 | 45 |
| Leaf B | 19 | 19 | 31 |
| Leaf C | 41 | 41 | 65 |

Table IX (continued).

| — | Base of leaf. | Middle of leaf. | Apical region of leaf. |
|---|---|---|---|
| *Paris quadrifolia* (stomata on lower surface only). | | | |
| Leaf A . . . . . . . . . . . . | 14–16 | | 15–25 |
| Leaf B . . . . . . . . . . . . | 14–19 | 12–25 | 20–39 |

| — | Base of leaf. | Middle of leaf. | | | Apical region of leaf. |
|---|---|---|---|---|---|
| *Alisma ranunculoides.* | | | | | |
| Upper surface . . . . . . . . . . | 32 | 28 | 40 | 36 | 46 |
| Lower surface . . . . . . . . | 10 | 17 | 16 | 18 | 30 |
| Upper surface . . . . . . . . | 48 | 38 | 38 | 46 | 44 |
| Lower surface . . . . . . . . | 14 | 12 | 16 | 18 | 21 |
| Mean U + L . . . | 52 | 48 | 55 | 56 | 70 |

Similarly, the stomatal frequency appears to increase, even in the middle region of the leaf, from the neighbourhood of the midrib towards the margin. Thus, in *Ranunculus Lingua* the average stomatal frequency near the midrib was 10–12 on the upper surface and 25–29 on the lower, whilst near the margin the numbers were 15–16 stomata per sq. mm. on the upper surface and 26–29 on the lower. Again in a leaf of *Paris quadrifolia*, whereas the average frequency near the midrib was 16, the average frequency near the margin was 21. Similar differences were found in other species, and it would, therefore, appear that the frequency in general increases with the distance from the main vascular supply, and that at equal distances from the midrib the frequency increases as the vascular supply of the midrib diminishes. This interpretation of the data is supported by examination of those leaves in which the base of the lamina projects backwards from the point of attachment to the petiole.

In the case of *Ficaria verna*, for example, it is apparent that we have in effect three apices to the lamina of which the basal lobes are situated nearer to the attachment of the leaf and the apex proper further away. If, therefore, the above hypothesis be correct, the stomatal frequency should be greatest at the tip of the leaf, somewhat lower on the basal lobes and least in the middle region near the midrib. The data given in the following table for twelve leaves of *Ficaria verna* show that this relation obtains in half of the leaves, although their rosette-like arrangement, which results in greater protection for the basal lobes in comparison with the upper part of the leaf, might be expected to mask any such tendency.

TABLE X.—Stomatal frequency in *Ficaria Verna*.

| Area of leaf. | Average number of stomata per sq. mm. | | | Whole leaf both surfaces. |
|---|---|---|---|---|
| | Top of leaf. | Middle of leaf. | Basal lobes. | |
| 10·9 | $\frac{24·0}{88·0}$ 112 | $\frac{16·0}{61·0}$ 77 | $\frac{16·0}{62·0}$ 78 | 88·6 |
| 9·1 | $\frac{22·0}{97·0}$ 119 | $\frac{15·0}{80·0}$ 95 | $\frac{15·3}{71·0}$ 86 | 99·4 |
| 9·0 | $\frac{18·3}{70·0}$ 88·3 | $\frac{18·0}{53·0}$ 71 | $\frac{13·0}{50·0}$ 63 | 74·0 |
| 6·2 | $\frac{23·0}{81·0}$ 104 | $\frac{18·0}{62·0}$ 80 | $\frac{13·0}{67·0}$ 80 | 88·0 |
| 6·1 | $\frac{10·3}{79·3}$ 89·6 | $\frac{11·3}{77·6}$ 88·9 | $\frac{7·0}{59·0}$ 66·0 | 81·5 |
| 4·7 | $\frac{13·3}{55·0}$ 68·3 | $\frac{15·0}{49·6}$ 64·6 | $\frac{10·0}{55·0}$ 65·0 | 66·0 |
| 4·6 | $\frac{20·0}{90·0}$ 110·0 | $\frac{13·5}{70·3}$ 83·8 | $\frac{15·0}{67·0}$ 82·0 | 91·9 |
| 4·5 | $\frac{13·0}{78·0}$ 91·0 | $\frac{16·6}{56·6}$ 73·2 | $\frac{10·2}{66·0}$ 76·2 | 80·2 |
| 4·0 | $\frac{14·0}{67·6}$ 81·6 | $\frac{10·0}{60·3}$ 70·3 | $\frac{13·0}{60·0}$ 73·0 | 74·5 |
| 3·0 | $\frac{21·6}{60·0}$ 81·6 | $\frac{15·6}{65·3}$ 80·9 | $\frac{14·3}{56·6}$ 70·9 | 77·7 |
| 1·9 | $\frac{18·0}{87·0}$ 105·0 | $\frac{12·0}{65·0}$ 77·0 | $\frac{11·0}{65·5}$ 76·5 | 86·5 |
| 1·0 | $\frac{15·0}{87·0}$ 102·0 | $\frac{12·0}{72·0}$ 84·0 | $\frac{13·0}{74·0}$ 87·0 | 91·0 |
| Averages . | 96·0 | 77·8 | 81·1 | |

If, however, the basal lobes be ignored and the frequencies determined for *Ficaria verna* near the attachment of the leaf, at the apex, and midway between, the normal gradient usually obtains.

| Base of leaf near attachment. | | Middle region of leaf. | | Apex of leaf. | | Area of leaf. |
|---|---|---|---|---|---|---|
| Range. | Mean. | Range. | Mean. | Range. | Mean. | |
| Plant A— | | | | | | sq. cms. |
| 59–73 | 68·2 | 68–79 | 74·6 | 73–83 | 78·0 | 5·48 |
| 64–76 | 70·0 | 76–92 | 83·6 | 94–100 | 96·6 | 4·97 |
| 57–86 | 72·0 | 60–71 | 65·6 | 97–110 | 104·0 | 4·45 |
| 60–68 | 62·9 | 59–72 | 64·3 | 66–71 | 67·0 | 4·25 |
| 54–67 | 60·0 | 58–64 | 60·3 | 65–78 | 72·0 | 3·07 |
| Plant B— | | | | | | |
| 59–65 | 62·0 | 54–68 | 61·0 | 72–80 | 76·0 | 5·25 |
| 44–68 | 55·0 | 57–63 | 60·0 | 60–68 | 64·3 | 4·59 |
| 44–50 | 47·0 | 37–46 | 42·0 | 42–55 | 48·0 | 4·09 |
| 54–62 | 58·0 | 55–70 | 62·6 | 58–70 | 65·0 | 3·82 |
| 48–50 | 48·5 | 51–58 | 54·0 | | 61·0 | 3·35 |

If the frequency be determined at successive intervals from the base of the more elongate Monocotyledonous leaves, interesting results are obtained. Thus the relatively short leaves of *Luzula pilosa* growing in a shady habitat exhibit an increased stomatal frequency from below upwards.

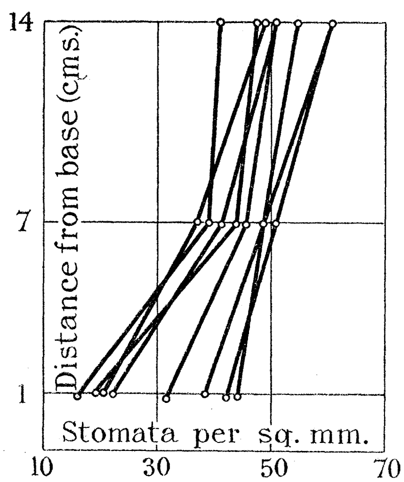


FIG. 3.—*Luzula pilosa.*

In six of the eight leaves examined (*cf.* fig. 3) the frequency (as determined at 1 cm. from the base, at 7 cms. and near the tips of the leaves at 14 cms.) shows a fairly steady increase, but in two instances the difference of frequency at 7 cms. and 14 cms. was inappreciable. The mean values for the eight leaves were :—1 cm. from base, 29 stomata per sq. mm. ; 7 cms. from base, 44·7 ; 14 cms. from base, 51·7.

Determinations at intervals of 5 cms. throughout the length of six leaves of *Carex sylvatica* (fig. 4) reveal a rapid increase in frequency a short distance above the base, followed by a gradually diminishing frequency as the tip of the leaf is approached. The frequency in the latter region, however, does not fall to so low a value as at the base. Similar examination of six leaves of *Luzula maxima* (fig. 5), of which three were from a "shade"-plant and three from a "sun"-plant, showed the same relation to hold as regards the latter (*cf.* fig. Su′. Su″. Su‴).

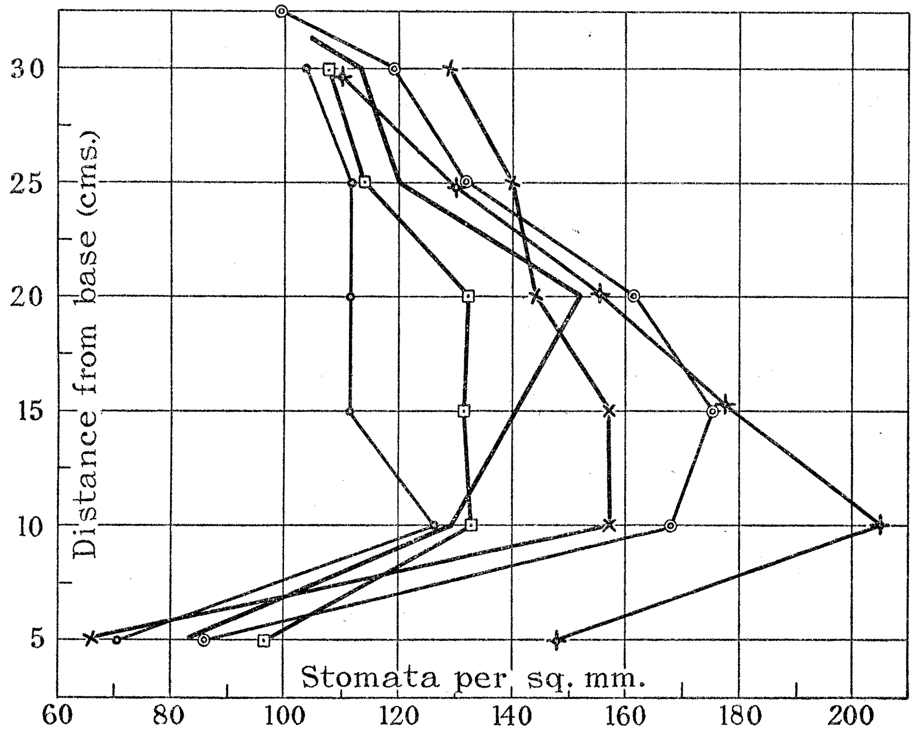


FIG. 4.—*Carex sylvatica*.

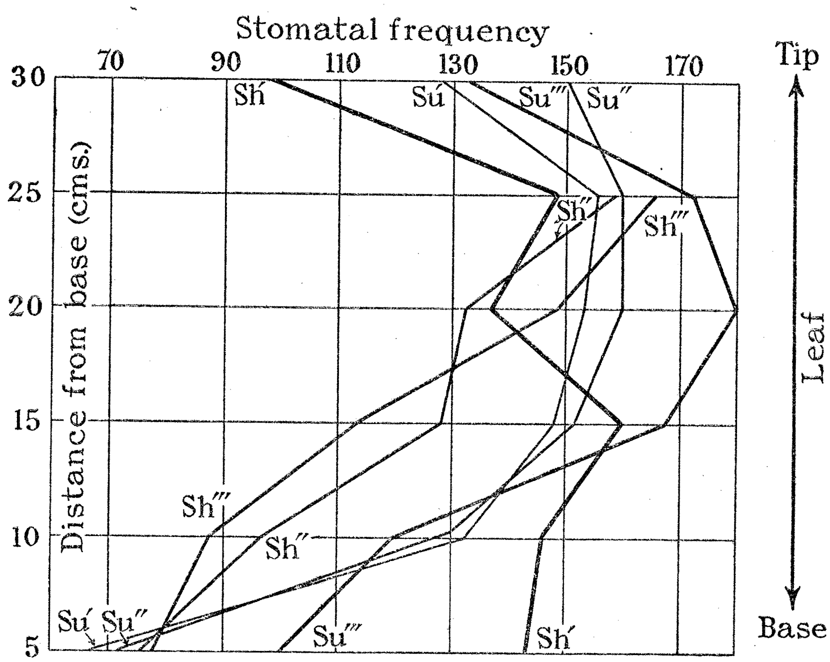


FIG. 5.—Stomatal frequency gradient in sun- and shade-leaves of *Luzula maxima*.



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Of the three shade-leaves the longest had a remarkably high stomatal frequency towards the base, which, apart from fluctuations, was more or less maintained up to 25 cms. from the base, followed by a very marked decrease at 30 cms. In the two shorter shade-leaves there is a fairly steady increase from below upwards (*cf.* fig. Sh″. Sh‴).

The same rise and fall is shown by the leaves of *Iris fœtidissima* (*cf.* fig. 7) and the axes of *Juncus effusus* (*cf.* fig. 6). The latter is noteworthy as showing the rise and

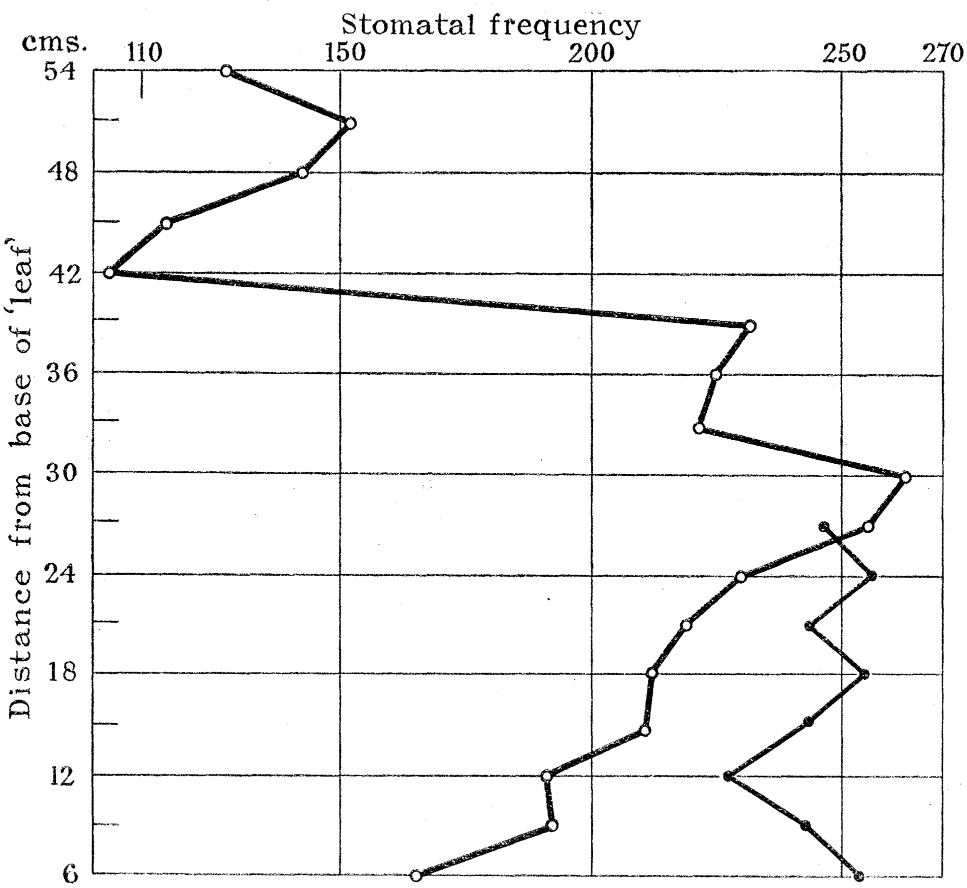


FIG. 6.—Stomatal frequency gradient in *Juncus effusus*.

fall of stomatal frequency, in the longer specimen examined, in a very marked degree despite fluctuations, whereas the shorter specimen exhibits fluctuation about a fairly constant mean.

The average frequencies for the specimens examined are given in the Table (see pages 23 and 24).



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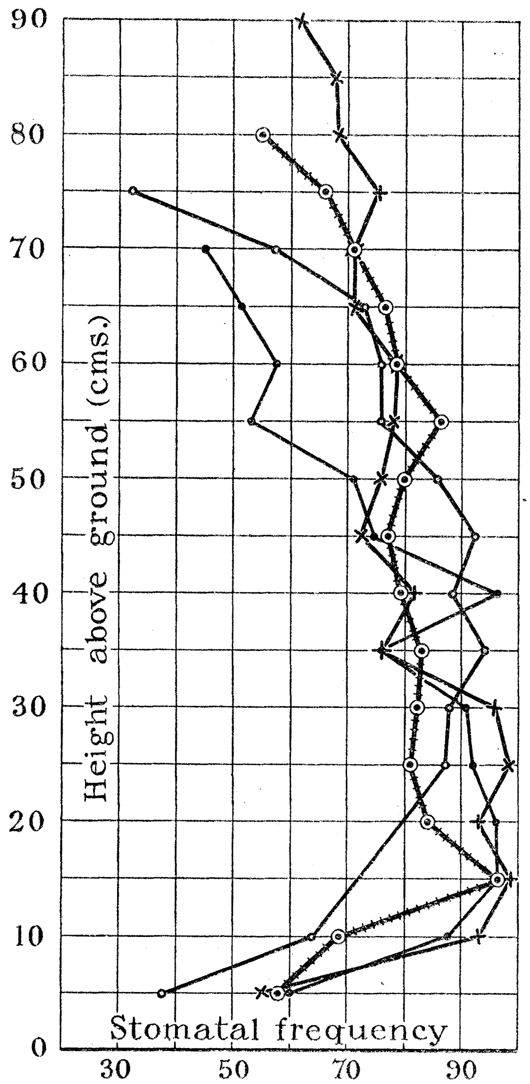


FIG. 7.—*Iris fœtidissima.*

TABLE XI.—Stomatal frequency at successive distances from the leaf base (mean values for all determinations).

| Species. | Distances in centimetres from the base of the leaf. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Iris fœtidissima*— | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 |
| Stomata per sq. mm. . . . | 53 | 77 | 87 | 89 | 93 | 89 | 81 | 84 | 75 | 76 | 72 | 66 | 57 | 62 | 70 | 62 | 68 | 61 |



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Table XI (continued).

| Species. | Distances in centimetres from the base of the leaf. | | | | | | |
|---|---|---|---|---|---|---|---|
| *Carex sylvatica*— | 5 | 10 | 15 | 20 | 25 | 30 | 35 |
| Stomata per sq. mm. . . . | 92 | 156 | 149 | 147 | 124 | 114 | 106 |
| *Luzula maxima*— | 5 | 10 | 15 | 20 | 25 | 30 | |
| Stomata per sq. mm. . . . | 81 | 116 | 143 | 151 | 160 | 124 | |
| *Luzula pilosa*— | 1 | 7 | 14 | | | | |
| Stomata per sq. mm. . . . *cf.* also *Scilla nutans* (p. 46). | 29 | 45 | 52 | | | | |

The frequency gradient is, therefore, clearly of widespread occurrence, and in the case of elongated leaves there is a fall in stomatal numbers in the apical region. Even in small Dicotyledonous leaves there is sometimes an appreciable fall in stomatal numbers at the extreme tip as compared with the penultimate region.

It naturally follows from the fact that the growth of a leaf is mainly intercalary, and that the leaf base and the leaf tip are the first formed or oldest parts, that these are also the least exposed, since they attain maturity whilst still protected by the surrounding leaves. Thus the leaf base and the leaf tip develop in a more humid atmosphere than the later formed and intercalated middle region. Moreover, in the elongated monocotyledonous leaves such as those of *Luzula maxima* it is easy to see that, as the leaf matures, its protection by the older surrounding leaves becomes less and less, so that the successive basipetally intercalated portions of the lamina develop under increasingly drier conditions. That the high frequency towards the base was not the outcome of immaturity was shown by marking the young leaves at equal intervals horizontally, when it was found that this region had ceased elongating.

Thus the data as to frequency distribution upon the individual leaf are consistent with the view that this is determined by the water relations ; conditions favouring high rates of transpiration or a low rate of water intake (*e.g.*, distance from vascular supply) favour high stomatal frequencies. If this be true then, under constantly humid conditions the frequency gradient should tend to disappear. This is confirmed by the study of *Scilla nutans* leaves grown respectively in moist air in the dark and in moist air in the light. The air was maintained close to saturation point and, as is seen from fig. 8, the gradient both in light and darkness is almost obliterated, whereas in dry air the gradient is very marked (fig. 8 right-hand " curve ").

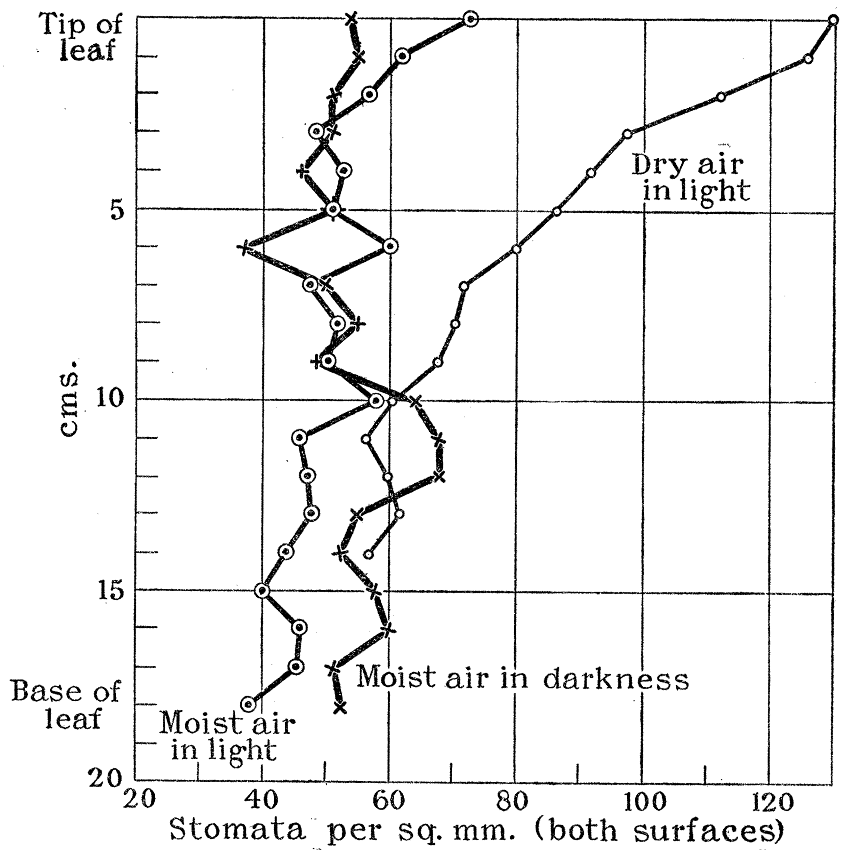


FIG. 8.—*Scilla nutans.*

## 4.—*Stomatal frequency in relation to habitat.*

Most of the data that have been collected with respect to stomatal frequency have been obtained in relation to plant communities, the assumption in most instances being that there is some correlation between habitat and the stomatal numbers observed. Most writers have regarded the number of stomata as an adaptative character which adjusts the transpiration of the plant to its environment, thus following the views of ZIEGELER and SPITZER already referred to. On this view the more arid the conditions the fewer should be the number of stomata, but actually, as we have seen, the reverse appears to be the case, so that before considering the effect of the individual habitat factors in the next section, it will be helpful to see how far detailed analysis of a single habitat in this respect bears out the above contention. To this end there are set forth in the following tables data accumulated from time to time over a number of years respecting most of the members of the British woodland flora. Although these data represent several thousands of determinations, since more than two hundred species are involved, it is obvious that no great stress is to be laid on the extreme values giving the observed range, though specimens from comparatively extreme conditions and leaves




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from diverse parts of different individuals have been examined. Moreover, the number of specimens examined of the different species is very diverse, ranging from only a few in those cases where a query is placed after the average (*e.g.*, *Phyteuma spicatum*) to others such as *Mercurialis perennis* and *Sambucus nigra*, where the number of leaves examined was several hundred. Nevertheless, having regard to the numbers of species in each section of the habitat, viz., Tree-layer, shrub-layer, marginal flora and ground flora, and that, for purposes of statistical analysis, only the averages for each species have been considered, it is felt that the conclusions arrived at can be accepted with a reasonable amount of confidence.

TABLE XII.—Number of stomata per sq. mm. of leaf-surface in British woodland plants. (Author's records unless otherwise stated.)

| — | Upper surface. | Under surface. | Average. |
|---|---|---|---|
| (A) TREE LAYER— | | | |
| *Acer campestre* . . . . . . . | 0–1 | 216–474 | 270 |
| ,, *pseudo-platanus* . . . . . | 0 | | 400 (WEISS) |
| *Alnus glutinosa* . . . . . . . | 0 | | 108 (CZECH) |
| *Betula alba* . . . . . . . . . | 0 | 76–254 | 184 |
| ,, *pubescens* . . . . . . | 0 | 95–189 | 147 |
| *Carpinus betulus* . . . . . . . | 0–1 | 135–200 | 160 |
| *Fagus sylvatica* . . . . . . . | 0 | 94–450 | 236 |
| *Fraxinus excelsior* . . . . . . | 0 | 150–186 (Dufour) | 176 |
| *Ilex aquifolium* . . . . . . . | 0 | 148–280 | 180 |
| *Populus alba* . . . . . . . . | 0 | | 315 (CZECH) |
| ,, *nigra* . . . . . . . . . | 20 | | 115 |
| ,, *tremula* . . . . . . . . | 0 | 306–369 | 330 ? |
| *Prunus avium* . . . . . . . . | 0 | 120–414 | 282 |
| *Pyrus communis* . . . . . . . | 0 | | 91 (CROCKER) |
| ,, *malus* . . . . . . . . | 0 | | 275 |
| ,, *torminalis* . . . . . . . | 0 | | 110 |
| *Quercus robur* . . . . . . . . | 0 | 220–810 | 450 |
| ,, *sessiliflora* . . . . . . | 0 | 350–656 | 400 |
| *Sorbus aria* . . . . . . . . . | 0 | | 225 ? |
| ,, *aucuparia* . . . . . . . | 0 | | 224 |
| *Taxus baccata* . . . . . . . . | 0 | 118–184 | 115 |
| *Tilia parvifolia* . . . . . . . | 0 | 185–263 | 214 |
| ,, *platyphyllos* . . . . . . | 0 | 107–145 | 119 |
| ,, *vulgaris* . . . . . . . . | 0 | | 130 |
| *Ulmus montana* . . . . . . . | 0 | 183–250 | 218 |
| *Castanea vulgaris* . . . . . . | 0 | 266–408 | 330 |

Total, 26 species.

Average number of stomata, upper surface, 0.
Average number of stomata, lower surface, 223. } *Both*, 224.

"Standard deviation," 98·5. "Standard error of mean," 19·3. (This value is really too large, but owing to the varying number of specimens of each species examined each specific mean has been treated as a single observation.)

Table XII (continued).

| —— | Upper surface. | Under surface. | Average. |
|---|---|---|---|
| (B) SHRUB LAYER— | | | |
| *Berberis vulgaris* | 0 | 229–595 | 400 |
| *Buxus sempervirens* | 0 | 126–164 | 160 (*cf.* MORREN, CZECH and DUFOUR) |
| *Clematis vitalba* | 0 | 52–139 | 115 |
| *Cornus sanguinea* | 0 | 103–288 | 246 |
| *Corylus avellana* | 0 | 98–398 | 225 |
| *Cratægus monogyna* | 0 | 93–225 | 154 |
| ,, *oxycanthoides* | 0 | 93–192 | 147 |
| *Cytisus scoparius* | 90 | | 90 |
| *Daphne laureola* | 0 | 43–138 | 94 |
| ,, *mezereum* | 0 | 65–153 | 99 |
| *Euonymus europæus* | 0–1 | 145–340 | 254 |
| *Hedera helix* | 0 | 114–139 | 125 |
| *Hypericum androsæmum* | 0 | 144–513 | 200 |
| *Ligustrum vulgare* | 0 | 100–680 | 300 |
| *Lonicera periclymenum* | 0 | 73–176 | 130 |
| *Prunus cerasus* | 0 | | 216 (CROCKER) |
| ,, *spinosa* | 0 | 202–515 | 375 |
| *Rhamnus catharticus* | 0 | 96–250 | 167 |
| ,, *frangula* | 0 | 336–572 | 477 |
| *Ribes grossularia* | 0 | 90–270 | 195 |
| ,, *nigrum* | 0 | 114–176 | 144 |
| *Rosa canina* | 0 | 50–116 | 89 |
| *Rubus corylifolius* | 0 | 256–360 | 292 |
| *Salix caprea* | 0 | | 400 |
| *Sambucus nigra* | 0 | 12–260 | 90 |
| *Solanum dulcamara* | 0 | 74–298 | 156 |
| *Vaccinium myrtillus* | 10–26 | 61–218 | 120 |
| *Viburnum lantana* | 0 | 100–180 | 136 |
| ,, *opulus* | 0 | 70–88 | 79 |

Total, 29 species.
Stomatal mode for upper surface, 0.
Average number of stomata, lower surface, 196. } *Both*, 199.
" Standard deviation," 106·8. " Standard error of mean," 20·1.

The mean value for the tree layer shows an increase of 25 stomata per sq. mm. as compared with the shrub layer. As, however, the standard error of difference between the two means is 27·80, it is quite possible that with a much larger representation of each class, as for example if we examined the whole European woodland flora, the difference would disappear. Examination of the lists will show, however, that the high values for the standard deviations and standard errors of the means are to some extent the outcome of the bridging species which partake of the characters of both layers. Actually the tree layer consists of tall trees and others intermediate in character and position between trees and shrubs. In the shrub layer also we find the same overlap in habit. *Salix caprea* and *Euonymus*, which both have exceptionally high means as compared with the other shrubs, are often more in the nature of trees than shrubs. Again, too, we may note the low means of those shrubs which can tolerate great shade as compared with those shrubs which belong to the marginal flora. The former are exemplified by *Clematis vitalba*, *Daphne* spp., *Sambucus nigra*, *Lonicera*, and *Viburnum opulus*.

If we consider the tall trees alone the mean value is 316, whilst if we ignore the less typical shrubs (*Clematis*, *Hedera*, *Lonicera*, *Salix caprea*), the mean value for the shrub layer is 199, an average difference of 117. We are probably, therefore, justified in believing that the mean frequency of stomata per unit area is appreciably higher for the leaves of trees than for the leaves of shrubs. To appreciate the significance of this conclusion it is important to recognise that the means represent averages based on the examination of both sun-leaves and shade-leaves, and, therefore, the mean difference between the two layers is not to be attributed to comparison of exposed sun-leaves from the tree layer with partially shaded leaves of the shrub layer.

There is, in fact, an inherent tendency for the members of the higher tree layer to exhibit a higher stomatal frequency than that shown by the members of the shrub layer, even when both are growing in the open. This is best shown by comparison of extreme values given below :—

| —— | Tree layer. | Shrub layer. | Difference of means. |
|---|---|---|---|
| Minima (range) . . . . . . . . . | 76–350 | 37–336 | (T.-S.) |
| Mean of minima . . . . . . . . . | 175·8 | 115·1 | 60·7 |
| Maxima (range) . . . . . . . . . | 145–810 | 88–680 | |
| Mean of maxima . . . . . . . . | 356·4 | 286·0 | 70·4 |

The maximum observed frequencies for the tree layer were, therefore, over 24 per cent. in excess of those observed for the shrub layer, whilst the minimum frequencies for the shrub layer were, on the average, 34·5 per cent. lower than those for the tree layer. This tendency for the higher story to exhibit a higher frequency of stomata is paralleled by the frequency exhibited on different parts of the same plant or even by different parts of the same organ (*cf.* p. 15).

A striking feature shared in common by trees and shrubs is, that with few exceptions (*Populus nigra*, *Cytisus scoparius*, and rarely in three others of the species examined) stomata are entirely absent from the upper epidermis. This is in marked contrast to the frequency of stomata in the upper epidermis of the leaves of the members of the ground flora, where they are normally present in more than half the marginal flora (*cf.* Table XIII).

TABLE XIII.

| Species. | Upper surface. | | Under surface. | |
|---|---|---|---|---|
| (C) HERBACEOUS PLANTS— | | | | |
| (*a*) Shade flora— | | | | |
| *Actæa spicata* | 0 | | 28–62 | Av. 45 |
| *Adoxa moschatellina* | 0 | | 10–68 | Av. 33 |
| *Ajuga reptans* | 4–44 | Av. 17 | 60–210 | Av. 147 |
| *Allium ursinum* | 0 | | 55–65 | Av. 60 |
| *Anemone nemorosa v. genuina* | 0 | | 34–67 | Av. 45 |
| ,, ,, *v. robusta* | 0 | | 30–58 | Av. 42 |
| *Arum maculatum* | 21–63 | Av. 45 | 65–105 | Av. 76 |
| *Asperula odorata* | 0 | | 48–224 | Av. 119 |
| *Cephalanthera pallens* | 0 | | 40–60 | Av. 48 |
| *Circæa alpina* | 0 | | 42–60 | Av. 56 |
| ,, *lutetiana* | 0 | (a few rarely present) | 84–260 | Av. 154 |
| *Conopodium denudatum* | 0 | | 99–147 | Av. 130 |
| *Convallaria majalis* | 13–62 | Av. 60 | 30–60 | Av. 47 |
| *Dentaria bulbifera* | 0 | (0–1 on radical leaves) | 20–130 | Av. 104 |
| *Epipactis latifolia* | 0 | | 30–52 | Av. 41 |
| *Ficaria verna* | 7–86 | Av. 21 | 17–152 | Av. 75 |
| *Helleborus fœtidus* | 0 | | | Av. 65 |
| ,, *viridis* | 0 | | 35–50 | Av. 41 |
| *Iris fœtidissima* | Both surfaces | | 38–99 | Av. 78 |
| *Lamium galeobdolon* | 0 | | 132–233 | Av. 175 |
| *Linnæa borealis* | 0 | | 144–282 | Av. 200 |
| *Listera ovata* | 0 | | 26–34 | Av. 29 |
| *Luzula maxima* | 0 | | 57–180 | Av. 129 |
| *Mercurialis perennis* | 0–12 | (stomata on basal leaves only) | 53–240 | Av. 82 |
| *Narcissus pseudo-narcissus* | 40–91 | | 26–110 | |
| *Orchis mascula* | 0 | | 33–67 | Av. 45 |
| *Oxalis acetosella* | 0 | | 44–97 | Av. 56 |
| *Paris quadrifolia* | 0 | | 18–39 | Av. 20 |
| *Phyteuma spicata* | 0–4 | | 146–218 | Av. 176 ? |
| *Polygonatum multiflorum* | 0 | | 59–95 | Av. 73 |
| ,, *officinale* | 0 | | 44–104 | Av. 66 |
| ,, *verticillatum* | 0 | | 73–105 | Av. 88 ? |
| *Primula acaulis* | 0 | | | Av. 140 |
| *Pyrola minor* | 9–24 | Av. 17 | 142–208 | Av. 183 |
| ,, *rotundifolia* | 0 | | 203–316 | Av. 200 |
| *Sanicula europæa* | 0 | | 116–174 | Av. 143 |
| *Scilla nutans* | 10–99 | Av. 55 | 14–90 | Av. 51 |
| *Scirpus sylvaticus* | 0 | | 62–182 | Av. 108 |
| *Trientalis europæa* | 0–1 | | 32–62 | |
| *Viola sylvestris* | 0 | | | Av. 52 |

Total shade species examined, 40.

Upper surface shade species, stomata normally absent in 28 or 75·6 per cent. Normally present in 7, occasionally in basal leaves of three other species.

Lower surface, shade species, average 86, both surfaces 92. $\sigma = 43 \cdot 9$.

Table XIII (continued).

| Species. | Upper surface. | | Under surface. | |
|---|---|---|---|---|
| (D) MARGINAL FLORA— | | | | |
| *Aconitum napellus* | 0 | (Weiss records 189 for this species) | 62–80 | |
| *Aegopodium podagraria* | 0 | | | Av. 266 |
| *Agrimonia eupatoria* | 0–1 | | 160–235 | Av. 200 |
| *Agrostis alba* | 70–90 | | 37–71 | Av. 56 |
| *Alchemilla vulgaris* | 18–79 | Av. 50 | 114–248 | Av. 190 |
| *Angelica sylvestris* | 0 | | | Av. 90 |
| *Anthriscus sylvestris* | 0–1 | | | Av. 160 |
| *Aquilegia vulgaris* | 0 | | | Av. 69 |
| *Arctium nemorosum* | 0 | | | Av. 99 |
| *Arenaria trinerva* | 0–8 | (on basal leaves) | | Av. 80 |
| *Arrenatherum avenaceum* | 38–85 | Av. 61 | 4–29 | Av. 13 |
| *Asarum europæum* (data from Weiss) | 50 | | 18 | |
| *Asperula odorata* | 0 | (water pores only) | 48–224 | Av. 119 |
| *Astragalus glycyphyllos* | 0 | | | Av. 108 ? |
| *Atropa belladonna* (from Weiss) | | Av. 56 | | Av. 227 |
| *Ballota nigra* | 0 | | | Av. 274 |
| *Barbarea vulgaris* | 250 | | 378 | |
| *Brachypodium sylvaticum* | 130–334 | Av. 240 | 0 | |
| *Bromus asper* | 36–53 | Av. 42 | 0–5 | Av. 2 |
| *Byronia dioica* | 0 | | 214–260 | Av. 194 |
| *Calamintha clinopodium* | 0 | | 160–310 | |
| *Campanula latifolia* | 0 | | 168–228 | |
| ,, *trachelium* | 0 | | 93–320 | Av. 165 |
| *Carex pendula* | 0 | | 171–207 | |
| ,, *sylvatica* | 0 | | 60–204 | Av. 132 |
| *Chærophyllum temulum* | 0 | | 115–235 | Av. 150 |
| *Cirsium palustre* (rosette ls.) | 2–4 | | | Av. 128 |
| *Colchicum autumnale* | 41 | | 47 | (WEISS) |
| *Crepis paludosa* | 1–4 | | 85–134 | |
| *Dentaria bulbifera* | 0–1 | (radical leaves) | 20–130 | |
| | 0 | (cauline leaves) | Av. radical, 25 ; Av. cauline, 104 | |
| *Digitalis purpurea* | 0–12 | | 89–155 | |
| *Dipsacus sylvestris* | 9–10 | | 82–104 | |
| ,, *pilosus* | 16–36 | (basal) | 145–210 | Av. 175 |
| | 16–36 | (cauline) | | |
| *Epilobium angustifolium* | | Av. 8 | 96–405 | |
| ,, *montanum* | 6–48 | Av. 16 | 116–352 | Av. 185 |
| ,, *tetragonum* | 88–200 | Av. 145 | 150–243 | Av. 200 |
| *Euphorbia amygdaloides* | 0 | | | Av. 104 |
| *Festuca gigantea* | 16–69 | Av. 36 | 0–7 | (near midrib only) |
| ,, *sylvatica* | | Av. 200 ? | 0 | |
| *Fragaria vesca* | | Only water stomata | | Av. 400 |
| *Gagea lutea* | | Av. 27 | | Av. 27 (WEISS) |
| *Galanthus nivalis* | | Av. 30 | | Av. 55 (WEISS) |
| *Galeopsis tetrahit* | 13–17 | | 138–162 | Av. 140 |
| *Galium mollugo* | 10 | | | Av. 200 |
| *Geranium dissectum* | 0 | | 233–488 | Av. 318 |
| ,, *phæum* | 0 | | 65–174 | Av. 130 |
| ,, *robertianum* | 0 | | 68–297 | Av. 190 |
| ,, *striatum* | 0 | | 93–125 | Av. 106 |

Table XIII (continued).

| Species. | Upper surface. | | Under surface. | |
|---|---|---|---|---|
| (D) MARGINAL FLORA—continued. | | | | |
| *Geum rivale* | 20 | | | Av. 220 |
| ,, *urbanum* | 32 | | | Av. 160 |
| *Heracleum sphondylium* | 34–86 | | 250–377 | |
| *Hieracium sylvaticum* | 0 | | 94–150 | |
| *Holcus lanatus* | 20–120 | Av. 55 | 9–79 | Av. 37 |
| ,, *mollis* | 48–84 | Av. 61 | 18–60 | Av. 38 |
| *Hordeum sylvaticum* | 60–104 | Av. 79 | 0–16 | Av. 5 |
| *Hypericum hirsutum* | 0 | | 276–320 | |
| ,, *humifusum* | 0 | | 320–336 | |
| ,, *perforatum* | 0 | | | Av. 233 (KROCKER) |
| ,, *pulchrum* | 0 | | 138–188 | |
| *Lactuca muralis* | 0 | | 74–129 | Av. 89 |
| *Lathyrus macrorhizus* | 0–46 | Av. 10 | 38–80 | Av. 58 |
| ,, *pratensis* | 56–86 | Av. 73 | 50–92 | Av. 76 |
| ,, *sylvestris* | 158–176 | | 100–106 | |
| *Lilium martagon* | 0 | | 19–40 | Av. 30 |
| *Lithospermum officinale* | 0 | | 216–322 | Av. 230 |
| ,, *purpureo-cœruleum* | 0 | | | Av. 170 ? |
| *Luzula forsteri* | 0 | | 74–282 | Av. 189 |
| ,, *pilosa* | 0 | | 17–60 | Av. 41 |
| *Lychnis dioica* | 0–19 | Av. 3 | 43–144 | Av. 67 |
| *Lysimachia nemorum* | 27–54 | Av. 38 | 67–119 | Av. 86 |
| ,, *nummularia* | 20–107 | Av. 60 | 87–160 | Av. 125 |
| *Melica nutans* | 100–145 | Av. 122 ? | 0 | |
| ,, *uniflora* | 70–120 | Av. 95 | 0 | |
| *Melittis melissophyllum* | 0 | | 160–234 | Av. 191 |
| *Milium effusum* | | Av. 103 | 0 | |
| *Nepeta cataria* | 0 | | 150–175 | |
| ,, *glechoma* | 0 | | 137–186 | Av. 160 |
| *Poa nemoralis* | 3–16 | Av. 3·8 | 110–123 | Av. 118 |
| *Potentilla erecta* | 0 | | | Av. 400 |
| ,, *sterilis* | 0 | | 162–181 | |
| *Prunella vulgaris* | | Av. 42 | | Av. 210 |
| *Pteridium aquilinum* | 0 | | 29–126 | Av. 63 |
| *Ranunculus auricomus* | 4–25 | Av. 16 | 27–71 | Av. 59 |
| *Rumex condylodes* | 6–24 | Av. 12 | 84–141 | Av. 103 |
| ,, *sanguineus* | 0–5 | Av. 2 | 80–224 | Av 120 ? |
| *Saponaria officinalis* | 7–32 | Av. 17 | 96–122 | Av. 103 |
| *Scabiosa succisa* | 52–174 | Av. 90 | 97–250 | Av. 142 |
| *Scrophularia nodosa* | 0–12 | Av. 2 | 85–274 | Av. 181 |
| ,, *vernalis* | 3–5 | | 110–150 | Av. 130 |
| *Sedum telephium* | 7–24 | Av. 18 | 42–54 | Av. 46 |
| *Serratula tinctoria* | 8–35 | Av. 19 | 80–316 | Av. 170 |
| *Sibthorpia europæa* | 0 | | 96–128 | Av. 100 |
| *Sison amomum* | 0 | | 135–320 | |
| *Sisymbrium alliaria* | 0–8 | | 115–156 | Av. 138 |
| *Solidago virgaurea* | 35–96 | Av. 80 | 48–143 | Av. 78 |
| *Stachys betonica* | 10–52 | Av. 32 | 132–176 | Av. 145 |
| ,, *sylvatica* | 0 | | 128–144 | |
| *Stellaria graminea* | 24–46 | | 82–87 | |
| ,, *holostea* | 0–6 | | 50–198 | Av. 118 |

Table XIII (continued).

| Species. | Upper surface. | | Under surface. | |
|---|---|---|---|---|
| (D) MARGINAL FLORA—continued. | | | | |
| *Teucrium scorodonia* . . . . | 0 | | 156–320 | |
| *Verbascum thapsus* . . . . . | 48–96 | Av. 75 | 137–144 | |
| *Veronica chamædrys* . . . . | | Av. 4 | | Av. 190 |
| ,, *montana* . . . . . | 0 | | 100–180 | Av. 164 |
| ,, *officinalis* . . . . . | 19–27 | Av. 23 | | Av. 90 |
| *Vicia cracca* . . . . . . . . | | Av. 130 | | Av. 44 |
| ,, *sepium* . . . . . . . | 0 ? | | 153–168 | |
| ,, *sylvatica* . . . . . . . | | Av. 78 | 3–8 | |
| *Vinca minor* . . . . . . . | 0 | | 180–396 | Av. 270 (WEISS records 477 for this species) |
| *Viola odorata* . . . . . . . | 4–5 | | | Av. 96 |

Of the total marginal species examined, viz., 110, 58 or 52·7 per cent. have stomata normally present on the upper surface, the average number for all 58 species being 20·3 stomata per sq. mm.

The average number of stomata per sq. mm. in the lower epidermis for all the 110 species is 145 and for both surfaces together 166·8. $\sigma = 91 \cdot 9$.

If we regard the mean value for a given species as a single observation for statistical purposes then the mean value for the whole "population," viz., 145 stomata per sq. mm. of the lower surface, has a "standard error" of 8·6.

Owing to the small number of trees and shrubs in comparison with the herbaceous flora it will be convenient for comparison with the latter to treat the two woody layers together, the more so as the line of demarcation between the trees and shrubs, on the one hand, and the herbaceous flora on the other, is far less arbitrary than that between the two upper stories.

The average frequency of stomata per sq. mm. for shrubs and trees taken together is 211·03, with a "standard deviation" of 104·9, and a "standard error of the mean" of 14·2. For the entire ground flora (both shade and marginal species) the mean stomatal frequency is 148·5, the "standard deviation" being 85·4 and the "standard error of the mean" 7·2.

The difference in the means for the ground flora and the woody plants is, therefore, 62·53, with a "standard error of difference" of 12·2. The difference is thus rather more than five times the standard error, so that there is no doubt as to its significance. This is again well shown if we compare the observed ranges and the respective means of the maximum and minimum frequencies observed (*cf.* p. 33).

The mean value for the shade flora of 86 stomata per sq. mm. has a standard error of 9·1. The difference between the means for these two portions of the ground flora is 59, and the "standard error of the difference" is 12·5. The difference is thus 4·72 times the standard error, and hence there can be no question of the difference observed being a result of errors of sampling. We can safely say that *the stomatal frequency on the lower surface of the Shade flora is less than the stomatal frequency of the Marginal flora.*

Considering both surfaces of the leaf together, the mean number of stomata per sq. mm. for the shade flora is 92, with a "standard error" of 7·53. For the marginal species the mean for both surfaces is 166·8 and the "standard error" 9. The differences of the means is, therefore, 74·8 and the "standard error of the difference" 11·73. Here again then there can be no doubt that the frequency of stomata per unit of leaf area is appreciably greater for the leaves of marginal species in general than for those of the shade flora, in general.

This difference is in harmony with the well-known fact that the stomatal frequency is greater for "sun-leaves" than for "shade-leaves," but the fact just demonstrated must not be confused with this. The observed ranges for the individual species include both sun and shade leaves, and, therefore, the lower frequency in the shade species, as compared with the marginal species, is to be regarded as a parallel phenomenon with regard to the various species to what we know to obtain with respect to individuals. The case is analogous to the fact that xerophytes in general tend to be more hairy than mesophytes, and at the same time potentially hairy species tend to become glabrous when growing in humid environments.

A comparison of the observed ranges for the shade and marginal floras respectively further emphasises the difference.

| —— | Shade flora. | Marginal flora. | Difference of means. |
|---|---|---|---|
| Minima (range) . . . . . . . | 10–203 | 16–320 | (M.-S.) |
| Mean of minima . . . . . . . | 58·6 | 115·1 | 56·5 |
| Maxima (range) . . . . . . . | 34–316 | 53–488 | |
| Mean of maxima . . . . . . | 141·6 | 172·0 | 30·4 |

| —— | Tree and shrub layer together. | Ground flora (shade and marginal). | Difference of means. |
|---|---|---|---|
| Minima (range) . . . . . . . | 37–350 | 10–320 | (W.-S.) |
| Mean of minima . . . . . . . | 140·3 | 98·8 | 41·5 |
| Maxima (range) . . . . . . . | 88–810 | 34–488 | |
| Mean of maxima . . . . . . | 165·1 | 163·2 | 1·9 |

The general conclusion to be drawn from the study of the woodland flora is that:—The species composing the woodland flora exhibit a stomatal frequency which is correlated with their normal position in the woodland community. Broadly the potentiality for the development of a high stomatal frequency increases from the interior of the wood outwards and from the ground flora upwards. Moreover, the innate character of this feature is shown by the same relation, as to stomatal frequency, being maintained when members of the various layers are grown in conditions of equal illumination and exposure.

With regard to the relative frequency of the stomata on the two surfaces it is apparent that, in general, the upper surfaces of the leaves of trees and shrubs are devoid of stomata. Thus of the fifty-five species examined belonging to these two classes, only two normally exhibit stomata in the upper epidermis, whilst in three other species they occur but rarely. On the other hand, no less than 80 species, or over 53 per cent. of the 150 species of the ground flora examined, normally develop stomata in the upper epidermis, sometimes in greater numbers than in the lower epidermis (*e.g.*, *Vicia sylvatica*, *Scilla nutans*) and more rarely as in certain grasses (*Milium effusum*, *Festuca sylvatica*) confined thereto.

It is, however, noteworthy that the shade flora in this respect exhibits a closer approach to the tree and shrub layers than to the marginal flora. For whereas 53 per cent. of the marginal species normally possess stomata in the upper epidermis, this is the case for only 20 per cent. of the shade flora. It must be remembered in this connection that most members of the shade flora develop their foliage very early in the year (*cf.* Salisbury, "Phenology and Habitat, with special reference to the Phenology of Woodlands," 'Trans. Roy. Met. Soc.,' vol. XLVII, pp. 251–263, 1921), and many retain some or all their foliage throughout the "light phase." This, to a smaller degree, is true also for the marginal flora, but in many of the shade species the foliage borne during the early part of the year obtains no protection from the previous season's foliage, as is the case for many marginal species with precocious foliage. It is, therefore, not improbable that, taken as a whole, the shade flora endures a greater degree of exposure than the marginal flora. In this connection we may recall the observations of Erban (M. Erban: "Ueber die Verteilung der Spaltöffnungen in beziehung zur Schlafstellung der Blätter," 'Ber. d. Deut. Bot. Ges.,' vol. XXXIV, p. 880, 1916), who found that the stomata of leaves which exhibit sleep movements are more numerous on that surface which is most protected when the leaf is folded.

Examination of British woodland species thus refutes the idea that the frequency of stomata is a means of adjustment to the exposure of the habitat, but confirms the conclusions based on the frequency in different regions of the same plant, and this comparison is emphasised by the diagram in fig. 9, where the average heights of the various sections of the woodland flora are plotted with reference to their average stomatal frequencies. It will be noted that, just as the stomatal frequency was seen to increase with the height of the leaf upon the individual plant, so too the frequency augments with the height of the species.

It is noteworthy that in the preponderatingly mat-like alpine vegetation, numerous stomata on the upper surface is the rule, thus P. L. Lohr ('Unters. u. d. Blattanatomie von Alpen -und Ebenenpflanzen,' Diss., Groningen, 1919) found that out of 106 species of alpine plants examined by him all had stomata upon the upper surface. Wagner ("Zur Kenntniss des Blattbaues der Alpenpflanzen und dessen biologischer Bedeutung," 'Sitz. d. k. Akad. d. Wiss. Wien,' Bd. CI, 1892) enumerates forty-five alpines with stomata upon the upper surface, of which thirty-one have more upon the upper than the lower. He also mentions fourteen species in which the stomata are confined to the lower surface, of which eight, however, are shrubs. Taking the average of both LOHR's and WAGNER's observations, the mean number of stomata for alpine plants, adding both surfaces together, is 242 per sq. mm. of surface, as compared with 167 for the marginal flora of an English woodland, which can obviously be correlated with the much greater exposure in the mountain pasture.

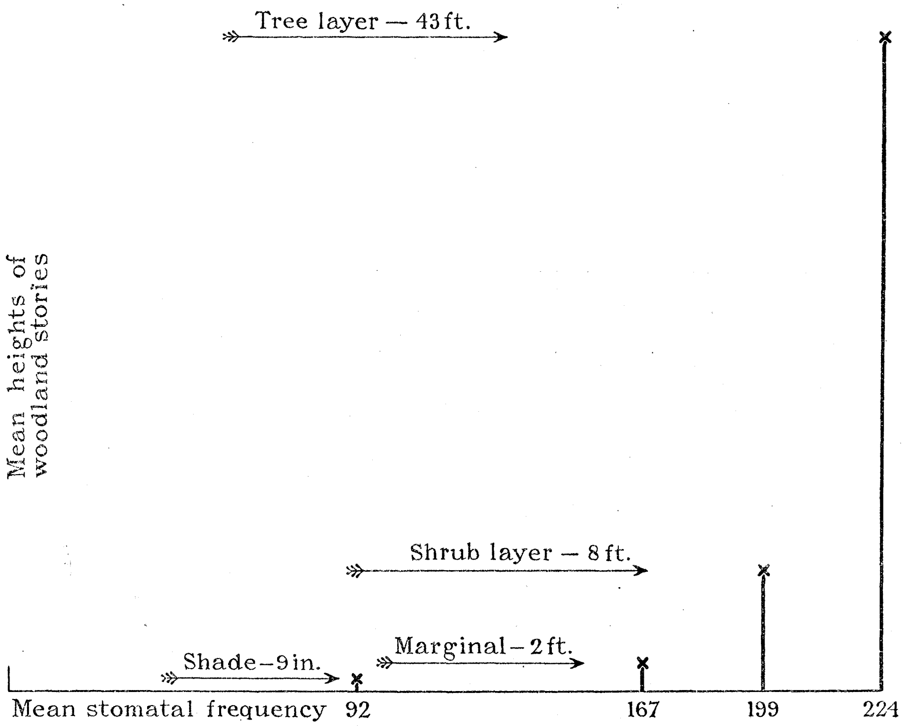


FIG. 9.

The following averages for aquatic species, though based on but few observations for some species, sufficiently indicate the order of frequency met with in plants of wet situations. It is quite clear from these that HABERLANDT's generalisation ('Physiological Anatomy,' Eng. Ed., p. 473) "that the stomata are, on the whole, least numerous where the external conditions are most xerophytic," is not borne out by comparison of the members of different habitats nor by comparison of different species within the same habitat. For we note that the more exposed types, such as *Typha* and *Juncus* are the only species with high stomatal numbers, whereas the average frequency is lower than for the woodland herbs, despite the greater insolation to which the emersed foliage of aquatic plants is exposed.





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TABLE XIV.—Stomatal frequencies for aquatic and marsh plants.

| Species. | Stomatal frequency upper surface per sq. mm. | Stomatal frequency lower surface per sq. mm. | Frequency per unit of leaf area. |
|---|---|---|---|
| *Alisma ranunculoides* . . . . . | 39 | 17 | 56 |
| ,, *plantago* . . . . . . . . | 50 | 36 | 86 |
| *Hydrocharis morsus-ranæ* . . . . | 89 | 0 | 89 |
| *Hippuris vulgaris* . . . . . . . | 106 (bracts) | 75 | 181 |
| *Ranunculus sceleratus* . . . . . | 31 | 47 | 78 |
| ,, *lingua* . . . . . . . | 13 | 27 | 40 |
| *Myosotis palustris* . . . . . . . | 7 | 91 | 98 |
| *Malachium aquaticum* . . . . . | 31 | 54 | 85 |
| *Strati otesaloides* . . . . . . . . | 38 | 49 | 87 |
| *Saxifraga stellaris* . . . . . . . | 16 | 5 | 21 |
| *Typha angustifolia* . . . . . . . | — | — | 490 |
| *Juncus effusus* . . . . . . . . | — | — | 230 |
| *Caltha palustris* . . . . . . . . | — | 40 | 40 |

Comparison then of different habitats or of different parts of the same habitat tends to confirm the conclusions based on comparison of different regions of the same plant or of different individuals in humid and dry air, that the more arid the environment the higher the stomatal frequency tends to become.

*Relative frequency on the two leaf surfaces.*

In those leaves in which stomata are present on both surfaces, the question naturally arises as to whether the stomatal frequency varies independently on the two surfaces or whether there is a negative or positive correlation.

The facts that plants of moist habitats tend to have stomata in the upper epidermis more frequently than those of dry situations, that stomata in the upper epidermis are

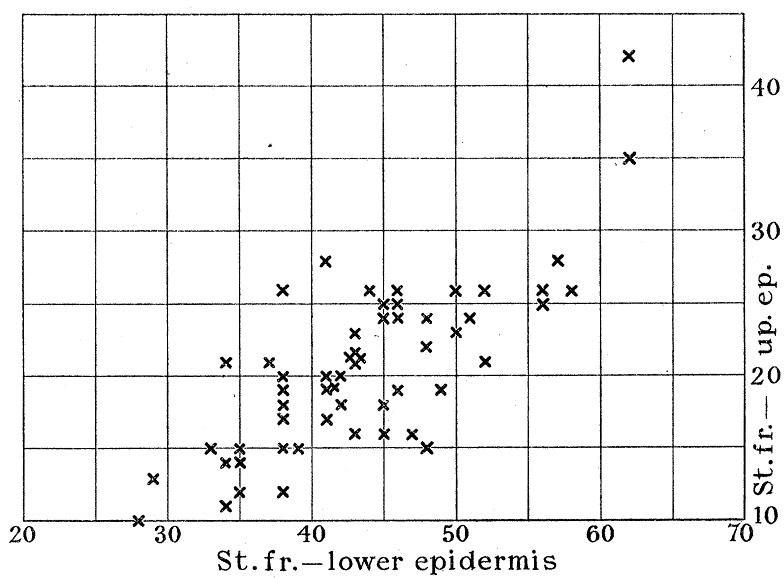


FIG. 10.—*Ficaria verna.*—Correlation of stomatal frequency in upper and lower epidermis.

also characteristic of rosette plants, whilst some plants which normally have no stomata in the upper epidermis, possess such on the lower leaves (*e.g.*, *Mercurialis perennis*), all tend to suggest the possibility of this distribution being adaptive in character, and hence, perhaps, we might expect to find that an increased frequency in the upper epidermis was accompanied by a decrease in the lower, and *vice versa*. Examination of *Scilla nutans* and *Ficaria verna*, however, reveal the fact that, as shown by the correlation diagrams (figs. 10 and 11), there is a high degree of positive correlation between the

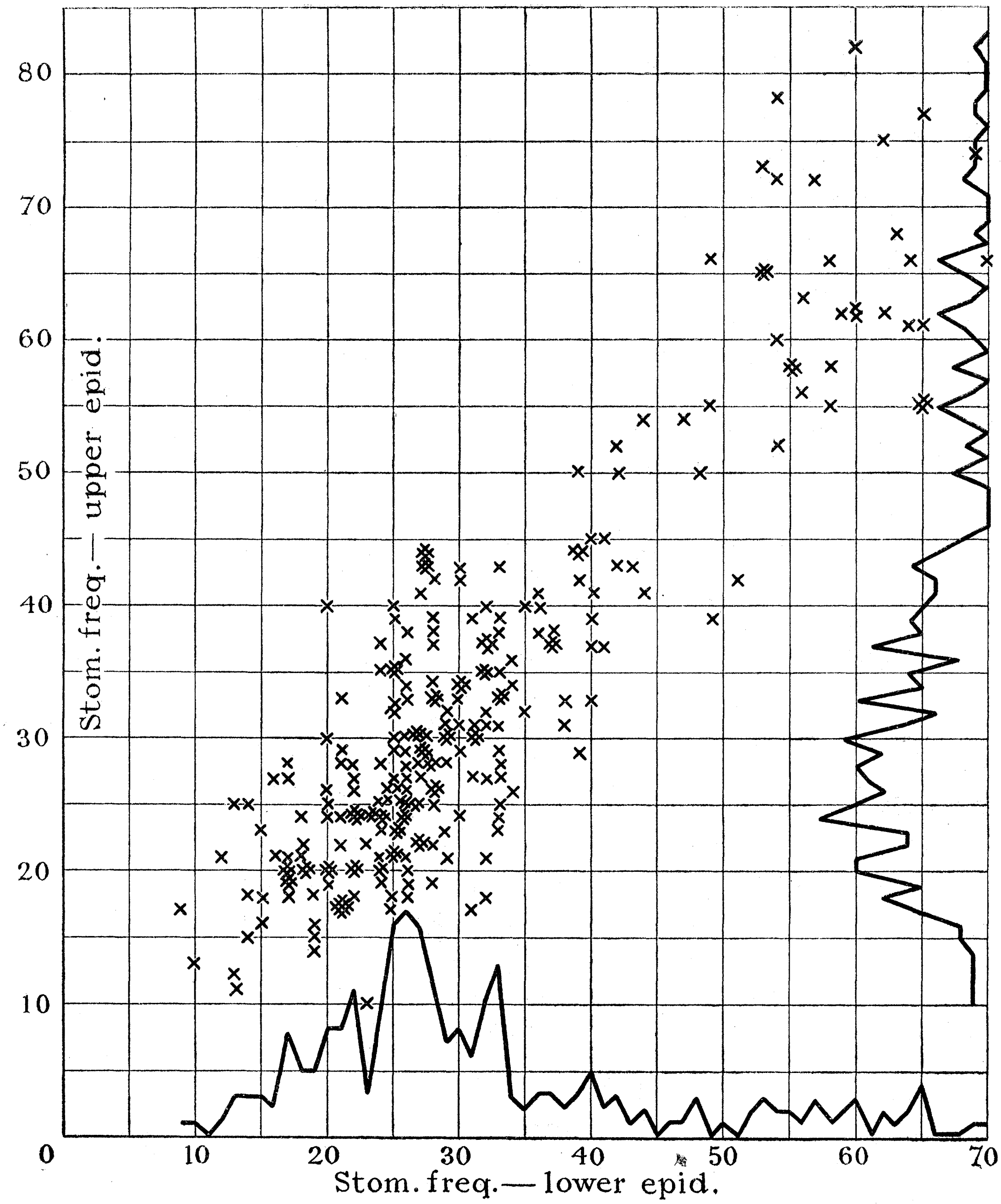


FIG. 11.—*Scilla nutans*.

stomatal frequency in the upper and lower epidermis, so that an increase in the one is accompanied by an almost equal increase in the other, and *vice versa*. Seeing that, in the case of *Ficaria verna* especially, the two surfaces of the leaf encounter very different external conditions as regards humidity, illumination and probably also as regards partial pressure of $CO_2$; it would seem probable that the determining factor or factors are internal rather than external.

Casual examination of leaves of other species from this point of view indicates that the same positive correlation between the two surfaces is of general occurrence.

### 5.—*Stomatal frequency in relation to external factors.*

(*a*) *Illumination.*—To test the influence of illumination on stomatal frequency plants of various species were grown in frames of which one-half was shaded. Any differential heating effect was obviated by darkening the upper part of the sloping glass so that the air warmed by the illumination of the lower part should rise into the darkened region, and the circulation thus effected produced a practically uniform air temperature and humidity.

*Circæa lutetiana*—In shade Stomatal frequency 116–188. Mean $140 \cdot 3 \pm 16$.
In full illumination Stomatal frequency 124–168. Mean $141 \cdot 0 \pm 11$.
*Lepidium sativum*—In shade Stomatal frequency 90–189. Mean $136 \pm 13$.
In full illumination Stomatal frequency 108–142. Mean $121 \pm 5 \cdot 4$.

The data for *Circæa lutetiana* and *Lepidium sativum* given above show that there was no significant difference in the stomatal frequencies for the plants grown in the light and in the shade. A result that at first appears surprising in view of the well-known higher stomatal frequency of sun leaves as compared with shade leaves, but whereas, in the latter case, the conditions of the environment differ in other respects beside illumination, in the experiments described the other conditions (*e.g.*, humidity of the air and soil) were as near as possible uniform.

A much more extensive series of cultures of *Scilla nutans* were made to test this point. As the leaves of this plant bear stomata on both surfaces for the purpose of comparison, the stomata on both sides were counted and added together. A total of 122 determinations were made for plants grown in moist illuminated conditions and 146 of plants grown in darkness but otherwise similar conditions. As it has been shown that some of the differences between sun and shade leaves are already recognisable in the bud stage, the plants were thoroughly established before being experimented upon, and, as the sequel shows that during the first season the normal differences are found between plants grown in the sun and others which are shaded (the conditions in other respects beside illumination not being uniform) the fact that the conditions of the experiment preclude the use of the same plants for more than one season does not invalidate the conclusions.

TABLE XV.—Stomatal frequencies of the leaves of Plants of *Scilla nutans* grown in moist air and illuminated.

| Upper surface. | Under surface. | Total. | Upper surface. | Under surface. | Total. |
|---|---|---|---|---|---|
| 24 | 22 | 46 | 21 | 32 | 53 |
| 23 | 15 | 38 | 24 | 26 | 50 |
| 21 | 17 | 38 | 25 | 27 | 52 |
| 36 | 34 | 70 | 31 | 30 | 61 |
| 25 | 24 | 49 | 20 | 22 | 42 |
| 23 | 25 | 48 | 26 | 25 | 51 |
| 20 | 22 | 42 | 25 | 28 | 53 |
| 43 | 30 | 73 | 25 | 25 | 51 |
| 25 | 26 | 51 | 28 | 17 | 45 |
| 23 | 24 | 47 | 28 | 22 | 50 |
| 34 | 30 | 64 | 27 | 22 | 49 |
| 25 | 24 | 49 | 21 | 29 | 50 |
| 21 | 24 | 45 | 24 | 21 | 45 |
| 10 | 23 | 33 | 26 | 25 | 51 |
| 32 | 25 | 57 | 26 | 26 | 52 |
| 25 | 26 | 51 | 20 | 17 | 37 |
| 32 | 32 | 64 | 30 | 31 | 61 |
| 21 | 26 | 47 | 28 | 27 | 55 |
| 41 | 27 | 68 | 24 | 26 | 50 |
| 28 | 26 | 54 | 18 | 26 | 44 |
| 27 | 25 | 52 | 24 | 22 | 46 |
| 24 | 24 | 48 | 18 | 17 | 35 |
| 35 | 32 | 67 | 18 | 25 | 43 |
| 31 | 33 | 64 | 25 | 24 | 49 |
| 24 | 30 | 54 | 24 | 20 | 44 |
| 23 | 29 | 52 | 27 | 16 | 43 |
| 36 | 26 | 62 | 24 | 18 | 42 |
| 30 | 27 | 57 | 15 | 15 | 30 |
| 25 | 20 | 45 | 34 | 26 | 60 |
| 28 | 24 | 52 | 39 | 25 | 64 |
| 20 | 17 | 37 | 40 | 20 | 60 |
| 25 | 13 | 38 | 37 | 24 | 61 |
| 21 | 16 | 37 | 29 | 21 | 50 |
| 21 | 18 | 39 | 25 | 26 | 51 |
| 38 | 36 | 74 | 22 | 28 | 50 |
| 35 | 33 | 68 | 37 | 28 | 65 |
| 23 | 23 | 46 | 26 | 20 | 46 |
| 26 | 25 | 51 | 28 | 27 | 55 |
| 24 | 24 | 48 | 33 | 33 | 66 |
| 22 | 26 | 48 | 28 | 21 | 49 |
| 18 | 26 | 44 | 33 | 26 | 59 |
| 22 | 18 | 40 | 24 | 33 | 57 |
| 32 | 28 | 60 | 44 | 27 | 71 |
| 34 | 30 | 64 | 38 | 26 | 64 |
| 44 | 27 | 71 | 23 | 29 | 52 |
| 43 | 33 | 76 | 30 | 20 | 50 |
| 26 | 28 | 54 | 35 | 25 | 60 |
| 27 | 33 | 60 | 25 | 24 | 49 |
| 26 | 26 | 52 | 26 | 28 | 52 |
| 24 | 23 | 47 | 30 | 31 | 61 |
| 26 | 32 | 58 | 35 | 32 | 67 |
| 24 | 24 | 48 | 22 | 27 | 49 |
| 20 | 24 | 44 | 21 | 25 | 46 |

Table XV (continued).

| Upper surface. | Under surface. | Total. | Upper surface. | Under surface. | Total. |
|---|---|---|---|---|---|
| 42 | 30 | 72 | 26 | 34 | 60 |
| 40 | 25 | 65 | 31 | 29 | 60 |
| 27 | 25 | 52 | 31 | 38 | 69 |
| 29 | 27 | 56 | 18 | 32 | 50 |
| 20 | 26 | 46 | 27 | 27 | 54 |
| 35 | 25 | 60 | | | |
| 26 | 25 | 51 | | | |
| 22 | 23 | 45 | | | |
| 40 | 32 | 72 | | | |
| 31 | 32 | 63 | | | |
| 23 | 25 | 48 | | | |

Standard deviation of total, 9·6.
Means (of 122), 27·3, 24·8, 52·1.
Range, 10–44, 13–38, 33–76.
Standard error of mean, 0·872.

Table XVI.—Stomatal frequencies of the leaves of plants of *Scilla nutans* grown in moist air *in Darkness*.

| Upper surface. | Lower surface. | Total. | Upper surface. | Lower surface. | Total. |
|---|---|---|---|---|---|
| 30 | 27 | 57 | 22 | 26 | 48 |
| 27 | 25 | 52 | 30 | 31 | 61 |
| 30 | 28 | 58 | 25 | 26 | 51 |
| 22 | 30 | 52 | 32 | 24 | 56 |
| 24 | 29 | 53 | 33 | 28 | 61 |
| 26 | 24 | 50 | 30 | 31 | 61 |
| 29 | 24 | 53 | 29 | 32 | 61 |
| 22 | 26 | 48 | 28 | 27 | 55 |
| 25 | 23 | 48 | 21 | 25 | 46 |
| 24 | 21 | 45 | 26 | 24 | 50 |
| 26 | 21 | 47 | 31 | 24 | 55 |
| 32 | 26 | 58 | 28 | 24 | 52 |
| 14 | 19 | 33 | 30 | 20 | 50 |
| 24 | 18 | 42 | 23 | 29 | 52 |
| 23 | 27 | 50 | 31 | 26 | 57 |
| 27 | 23 | 50 | 30 | 33 | 63 |
| 27 | 25 | 52 | 31 | 35 | 66 |
| 31 | 26 | 57 | 27 | 29 | 56 |
| 27 | 26 | 53 | 30 | 32 | 62 |
| 27 | 22 | 49 | 32 | 34 | 66 |
| 26 | 24 | 50 | 30 | 29 | 59 |
| 33 | 28 | 61 | 32 | 32 | 64 |
| 36 | 31 | 67 | 31 | 30 | 61 |
| 40 | 30 | 70 | 31 | 33 | 64 |
| 34 | 33 | 67 | 24 | 41 | 65 |
| 30 | 39 | 69 | 28 | 26 | 54 |
| 35 | 33 | 68 | 30 | 32 | 62 |
| 29 | 40 | 69 | 25 | 24 | 49 |
| 33 | 33 | 66 | 22 | 25¾ | 47 |
| 21 | 24 | 45 | 27 | 24 | 51 |
| 28 | 26 | 54 | 23 | 27 | 50 |

Table XVI (continued).

| Upper surface. | Lower surface. | Total. | Upper surface. | Lower surface. | Total. |
|---|---|---|---|---|---|
| 24 | 25 | 49 | 23 | 23 | 46 |
| 25 | 22 | 47 | 18 | 15 | 33 |
| 22 | 24 | 46 | 27 | 18 | 45 |
| 27 | 27 | 54 | 28 | 30 | 58 |
| 19 | 28 | 47 | 15 | 14 | 29 |
| 33 | 25 | 58 | 12 | 13 | 25 |
| 32 | 24 | 56 | 17 | 21 | 38 |
| 27 | 27 | 54 | 25 | 23 | 48 |
| 31 | 23 | 54 | 27 | $24\frac{3}{4}$ | 51 |
| 37 | 26 | 63 | 25 | 17 | 42 |
| 34 | 24 | 58 | 26 | 21 | 47 |
| 24 | 24 | 48 | 22 | 22 | 44 |
| 30 | 24 | 54 | 27 | 23 | 50 |
| 17 | 31 | 48 | 17 | 21 | 38 |
| 19 | 26 | 45 | 16 | 19 | 35 |
| 31 | 22 | 53 | 19 | 17 | 36 |
| 17 | 21 | 38 | 20 | 21 | 41 |
| 22 | 21 | 43 | 22 | 19 | 41 |
| 18 | 21 | 39 | 20 | 24 | 44 |
| 35 | 28 | 63 | 26 | 25 | 51 |
| 29 | 23 | 52 | 20 | 20 | 40 |
| 21 | 32 | 53 | 29 | 31 | 60 |
| 25 | 33 | 58 | 20 | 18 | 38 |
| 37 | 24 | 61 | 20 | 14 | 34 |
| 29 | 23 | 52 | 25 | 17 | 42 |
| 32 | 21 | 53 | 24 | 22 | 46 |
| 29 | 22 | 51 | 28 | 27 | 55 |
| 40 | 22 | 62 | 22 | 31 | 53 |
| 27 | 25 | 52 | 24 | 31 | 55 |
| 35 | 20 | 55 | 16 | 19 | 35 |
| 30 | 14 | 44 | 18 | 19 | 37 |
| 31 | 27 | 58 | 28 | 29 | 57 |
| 27 | 22 | 49 | 34 | 34 | 68 |
| 24 | 31 | 55 | 21 | 28 | 49 |
| 34 | 22 | 56 | 43 | 27 | 70 |
| 31 | 22 | 53 | 26 | 22 | 48 |
| 39 | 31 | 70 | 30 | 25 | 55 |
| 25 | 14 | 39 | 28 | 27 | 55 |
| 19 | 20 | 39 | 18 | 14 | 32 |
| 22 | 19 | 41 | 21 | 12 | 33 |
| 21 | 25 | 46 | 15 | 19 | 34 |
| 24 | 24 | 48 | 16 | 15 | 31 |

Mean (of 146).—Upper surface, 26·3 ; lower surface, 24·8 ; Total, 51·1, S.E.M. 0·78.
Range.—Upper surface, 14–43 ; lower surface, 12–41 ; Total, 31–70.
Difference of mean totals for leaves in moist air in light and moist air in darkness = 1·00. Standard error of difference = 1·16.

It should be emphasised that these data for *Scilla nutans* were obtained from a number of different leaves and plants, and that from each leaf the same number of counts were made from the tip, the middle and the base, so that the considerable variation in frequency from one part of the leaf to another does not introduce any error into our comparison; moreover, in *Scilla* the question of variation according to the level at which the leaves are borne does not arise. As, however, there is the possibility that sequence of production, in other words the time at which the leaf is developed, might affect the frequency, leaves comparable in this respect were as far as possible chosen from each plant.

It will be seen that, despite the fact that the one set of plants was grown in darkness and the other in light, the conditions of humidity being the same, *there is no significant difference between the stomatal frequencies of the two sets.* For the illuminated plants the mean frequency, adding together the stomata on both faces, is 52·1, whereas for the non-illuminated plants the mean frequency is 51·1; a difference of only a single unit and the "standard error" of this difference is 1·16. If we consider the two surfaces separately we again find the correspondence extremely close. For the lower surfaces the average frequency is identical for the plants in darkness and those in the light, viz., 24·8, whilst for the upper surface the mean values are 27·3 in light and 26·3 in darkness.

Similar data were obtained from plants of *Ficaria verna* grown in bright light and in shade respectively, but both subject to relatively humid air and the same conditions as to soil, etc. Fifty-five determinations were made from the leaves in the light and sixty from those in the dark.

TABLE XVII.—Stomatal frequency in plants of *Ficaria verna* grown in uniform conditions as to soil and humidity but differing as to the illumination.

| — | Observed range. | Mean frequency. | |
|---|---|---|---|
| Shade-plants— | | | |
| Upper surface | 10–44 | 22·8 | |
| Lower surface | 28–70 | 46·3 | |
| Both surfaces | 38–110 | 69·1 | Standard error of mean, 1·78<br>Standard deviation, 13·8 |
| Sun-plants— | | | |
| Upper surface | 9–28 | 18·1 | |
| Lower surface | 28–57 | 40·5 | |
| Both surfaces | 37–85 | 58·6 | Standard error of mean, 1·35<br>Standard deviation, 10·1 |

Difference of mean totals = 10·5.
Standard error of difference = 2·23.

It will be seen from Table XVII that the frequency of stomata on the well-illuminated plants instead of being greater is actually less than on the plants grown in the shade, and that this difference is considerably more than three times the probable error. The same number of leaves were selected from sun- and shade-plants and each pair examined was matched as far as possible as regards size. In the case of the sun-leaves the areas ranged from 2·63 sq. cms. to 5·25 sq. cms., and for the shade-leaves from 2·7 to 5·48 sq. cms., the means being respectively 3·68 sq. cms. (sun) and 3·98 sq. cms. (shade). The sun-leaves were thus slightly the smaller, which, if it affected the result, would tend to give a higher frequency for the sun-leaves. It should further be emphasised that equivalent numbers of determinations were made from the apical region, the middle, and the base of each leaf in the two sets, so that the result cannot have been affected by the marked variation in frequency in different parts of the laminæ.

TABLE XVIII.—Stomatal frequency in green and yellow halves of leaves of *Ligustrum latifolium*.

| —— | Green half. | Yellow half. | Mean difference. |
|---|---|---|---|
| Leaf A . . . . . . . . | 174<br>196<br>190<br>188<br>Mean 187 | 284<br>220<br>230<br>280<br>Mean 253 | 66 stomata per sq. mm. |
| Leaf B . . . . . . . . | 118<br>118<br>124<br>124<br>Mean 121 | 186<br>148<br>196<br>204<br>Mean 183 | 62 stomata per sq. mm. |
| Leaf C . . . . . . . . . | 426<br>367<br>353<br>Mean 382 | 397<br>339<br>433<br>Mean 389 | 7 stomata per sq. mm. |
| Leaf D . . . . . . . . | 441 | 470<br>456<br>Mean 463 | 22 stomata per sq. mm. |
| Leaf E . . . . . . . . | 507<br>573<br>Mean 540 | 705<br>691<br>Mean 698 | 158 stomata per sq. mm. |
| Leaf F . . . . . . . . | 560<br>677<br>666<br>Mean 634 | 721<br>699<br>—<br>Mean 710 | 76 stomata per sq. mm. |
| Leaf G . . . . . . . . | 622 | 844 | 222 stomata per sq. mm. |
| Range . . . . . . .<br>Mean totals . . . . | 118–666<br>357 | 204–844<br>417 | Difference of means =<br>60 stomata per sq. mm. |

These results then for *Ficaria verna* and *Scilla nutans* especially, together with those for *Circæa lutetiana* and *Lepidium sativum*, render it extremely improbable that light plays any appreciable rôle in influencing the stomatal frequency. It has been suggested that the high frequency of stomata in sun-leaves is an accompaniment of their more active assimilation, but, as we have seen, there is no significant difference in stomatal frequency of plants of *Scilla* when grown in the dark and those illuminated, provided the external conditions are in other respects similar. In the above experiments, however, the conditions maintained were relatively humid, and it might be urged that, under comparatively dry conditions, the effect of different rates of assimilation might be more marked. Therefore, to check these conclusions, the leaves of variegated plants were examined and the frequency in the chlorophyllous and non-chlorophyllous regions determined. In view of what has already been demonstrated with regard to the variation in frequency in different parts of the same lamina, it was essential that the two regions should be strictly comparable. To ensure this only "harlequin" leaves were used, in which the lamina on one side of the midrib was green and on the other side devoid of chlorophyll. The two halves were thus exactly similar, except for their capacity for assimilation, which more than outweighed the disadvantage that such leaves are only available in small numbers.

Owing to the exceptionally wide range of stomatal frequency in *Ligustrum*, the number of determinations is too few to treat statistically. But since, in all seven leaves examined, the mean frequency for the yellow part of the leaf is higher, usually considerably so, than that for the green part, the difference ranging from 7 per sq. mm. to 222 per sq. mm., the evidence is fairly conclusive that a high assimilation rate does not tend to increase the stomatal frequency and, indeed, since the higher frequency in the yellow portions can be satisfactorily explained by the smaller size of the epidermal cells in the yellow as compared with the green region (*cf*. p. 61), it may be said that in the Privet there is no evidence that the rate of assimilation has any effect whatever on the number of stomata per unit area.

A number of pairs of leaves of *Ilex aquifolium*, the one totally albino and the other completely green, were taken from different branches and examined. The green leaves showed a stomatal frequency of from 184 to 168 stomata per sq. mm., with a mean of $157 \pm 2\cdot9$. The yellow leaves, however, showed a frequency of from 203 to 233 per sq. mm., with a mean value of $218 \pm 4\cdot2$. The difference of the means was, therefore, 61 per sq. mm. and the standard error of the difference $5\cdot1$.

It seems then quite clear from these observations on *Ligustrum* and *Ilex*, together with the experimental data with respect to *Scilla nutans* and other plants, that neither illumination nor the rate of assimilation have any direct effect upon the number of stomata in a unit area of the epidermis, and the suggestion that the high stomatal frequency of sun-leaves as compared with shade-leaves, has anything to do with their respective rates of assimilation, must be regarded as another example of unsupported teleology.

TABLE XIX.—Stomatal frequency in Harlequin leaves of *Ilex aquifolium*.

| — | Green half. | Yellow half. | Mean difference. |
|---|---|---|---|
| Leaf A . . . . . . . | 200 | 204 | |
| | 164 | 239 | |
| | 174 | 210 | |
| | 172 | 240 | |
| | 190 | 231 | |
| | Mean 180 ± 4·9 | Mean 225 ± 6·7 | 46±8·3 |
| Leaf B . . . . . . . | 184 | 246 | |
| | 200 | 230 | |
| | 216 | 236 | |
| | Mean 200 ± 7·5 | Mean 237 ± 6·5 | 37±9·9 |
| Leaf C . . . . . . . | 160 | 218 | |
| | 178 | 213 | |
| | 188 | 204 | |
| | 203 | 240 | |
| | 167 | 240 | |
| | 174 | 223 | |
| | 164 | 237 | |
| | 189 | 251 | |
| | 167 | 241 | |
| | Mean 177 ± 4·44 | Mean 230 ± 4·9 | 53 ± 6·6 |

(*b*) *Humidity.*—For comparison with the *Scilla* plants grown in moist air, but illuminated, other plants were grown in the same conditions as to soil and light, but in comparatively dry air. Naturally the difference in air humidity also involved a difference in the water content of the soil. From the data set forth in Table XX it will be at once seen that, with equal illumination but differing humidity, a very marked difference in the stomatal frequency is apparent; the mean difference between the two sets amounting to 36 stomata per sq. mm. in favour of the plants in the drier air, whilst the standard error of this difference is only 2·98. The graphic representation of the means of these data for the three sets of *Scilla* plants makes it clear that whilst both the plants grown in darkness and in light, but alike in moist air, have frequencies of a similar magnitude throughout their length, the leaves of the plants grown in dry air exhibit a much higher frequency at the base, and this difference augments as we pass towards the leaf apex (*cf.* fig. 12).

Comparable data for other species grown in dry and moist air respectively, but otherwise similar conditions, further emphasise the predominant influence of humidity upon stomatal frequency. Thus for *Circæa lutetiana* grown in dry air, the frequency ranged from 94 per sq. mm. to 246 per sq. mm. In moist air the frequency ranged from 84 to 232 per sq. mm. The difference here is only slight, perhaps correlated with the low growth of the plants which would tend to minimise the difference of the environment.

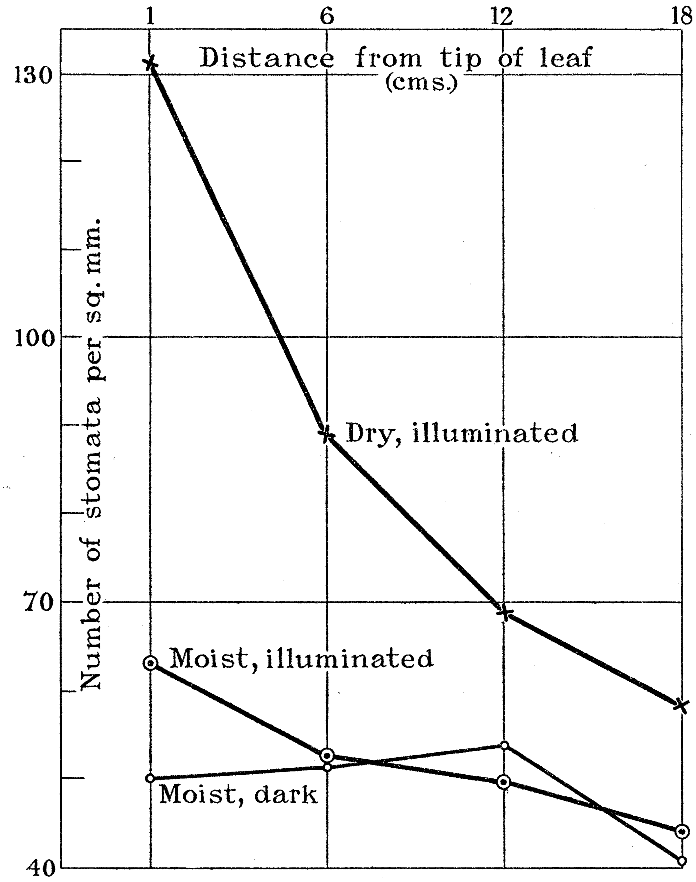


Fig. 12.—*Scilla nutans.*—Average stomatal frequencies, under various conditions. Showing almost complete suppression of normal gradient when grown in moist air.

Table XX.—Stomatal frequencies of the leaves of plants of *Scilla nutans* grown in *dry air*, but under the same conditions of illumination as those grown in moist air.

| Upper surface. | Lower surface. | Total. | Upper surface. | Lower surface. | Total. |
|---|---|---|---|---|---|
| 66 | 64 | 130 | 58 | 55 | 113 |
| 45 | 41 | 86 | 54 | 44 | 98 |
| 29 | 27 | 56 | 41 | 36 | 77 |
| 30 | 27 | 57 | 32 | 35 | 67 |
| 62 | 60 | 122 | 39 | 40 | 79 |
| 41 | 44 | 85 | 37 | 32 | 69 |
| 30 | 29 | 59 | 33 | 40 | 73 |
| 33 | 28 | 61 | 42 | 51 | 93 |
| 38 | 33 | 71 | 38 | 37 | 75 |
| 72 | 57 | 129 | 29 | 39 | 68 |
| 61 | 64 | 125 | 33 | 38 | 71 |
| 39 | 33 | 72 | 55 | 49 | 104 |
| 32 | 25 | 57 | 37 | 37 | 74 |
| 54 | 47 | 101 | 37 | 41 | 78 |

Table XX (continued).

| Upper surface. | Lower surface. | Total. | Upper surface. | Lower surface. | Total. |
|---|---|---|---|---|---|
| 75 | 62 | 137 | 33 | 30 | 63 |
| 72 | 54 | 126 | 33 | 27 | 60 |
| 77 | 65 | 142 | 42 | 41 | 83 |
| 62 | 62 | 124 | 37 | 43 | 80 |
| 50 | 48 | 98 | 38 | 37 | 75 |
| 56 | 50 | 106 | 32 | 37 | 69 |
| 50 | 39 | 89 | 34 | 36 | 70 |
| 45 | 40 | 85 | 43 | 29 | 72 |
| 43 | 42 | 85 | 36 | 35 | 71 |
| 28 | 33 | 61 | 31 | 33 | 64 |
| 29 | 33 | 62 | 26 | 29 | 55 |
| 29 | 30 | 59 | 32 | 33 | 65 |
| 31 | 26 | 57 | 34 | 22 | 56 |
| 99 | 90 | 189 | 31 | 33 | 64 |
| 91 | 84 | 175 | 28 | 25 | 53 |
| 88 | 84 | 172 | 33 | 28 | 61 |
| 58 | 58 | 116 | 61 | 65 | 126 |
| 58 | 55 | 113 | 63 | 56 | 119 |
| 65 | 53 | 118 | 44 | 39 | 83 |
| 31 | 31 | 62 | 39 | 49 | 88 |
| 37 | 40 | 77 | 27 | 31 | 58 |
| 40 | 35 | 75 | 33 | 28 | 61 |
| 17 | 9 | 26 | 55 | 65 | 120 |
| 13 | 10 | 26 | 68 | 63 | 131 |
| 11 | 13 | 24 | 61 | 63 | 124 |
| 62 | 59 | 121 | 37 | 28 | 65 |
| 66 | 49 | 115 | 44 | 39 | 83 |
| 73 | 53 | 126 | 36 | 31 | 67 |
| 52 | 42 | 94 | 33 | 21 | 54 |
| 52 | 54 | 106 | 30 | 26 | 56 |
| 42 | 39 | 81 | 34 | 27 | 61 |
| 43 | 43 | 86 | 55 | 58 | 113 |
| 44 | 27 | 71 | 82 | 60 | 142 |
| 40 | 36 | 76 | 78 | 54 | 132 |
| 66 | 58 | 124 | 37 | 32 | 69 |
| 66 | 70 | 136 | 39 | 31 | 70 |
| 66 | 66 | 132 | 37 | 37 | 74 |
| 50 | 42 | 92 | 30 | 29 | 59 |
| 41 | 40 | 81 | 35 | 24 | 59 |
| 60 | 54 | 114 | 33 | 30 | 63 |
| 56 | 56 | 112 | | | |
| 29 | 27 | 56 | Means 45·5 | 42·0 | 87 ± 1·941 |
| 53 | 45 | 98 | | | |
| 52 | 43 | 95 | Range 11–99 | 9–90 | σ = 30·73 |
| 43 | 47 | 90 | | | 24–189 |
| 51 | 42 | 93 | | | |

Difference of means (both surfaces together) for moist air and dry air = 36.
Standard error of difference = 2·98.

*Epilobium montanum* in dry air showed frequencies of from 126 to 198 as compared with a range of from 96 to 142 in moist air.

Comparison of equally illuminated plants subject to marked differences in the humidity of the environment is furnished by marginal aquatics such as *Alisma plantago*, when growing on submerged and exposed mud. The following data show that the differences between the wet-mud and dry-mud forms of the Water Plantain are quite comparable to those between sun- and shade-forms of a woodland plant. It will be seen that the plants growing on the drier mud had an average of 39 more stomata per sq. mm. than those on the wet mud, whilst the standard error of this difference is only 3·52.

TABLE XXI.—Stomatal frequency for mud- and water-forms of *Alisma plantago*.

| — | Upper surface. | | Lower surface. | | Total. |
|---|---|---|---|---|---|
| | Observed range. | Average. | Observed range. | Average. | |
| A. Leaves from twelve plants on exposed mud (102 determinations) | 49–95 | 74·7 | 32–64 | 53·3 | 128±2·0 |
| B. Leaves from eight plants growing in shallow water (66 determinations) . | 33–78 | 50·9 | 24–49 | 38·1 | 89±2·9 |
| Difference of averages . . . . | | 23·8 | | 15·2 | 39±3·52 |

Since the leaves of the water-forms were generally larger than those of the land forms the increased frequency of the stomata in the latter might be due to their smaller size, but, as the leaves examined were taken in pairs of approximately equal size from the two habitat conditions, this factor was eliminated. The evidence from *Alisma plantago* is confirmed by examination of leaves of comparable size from plants of *Veronica anagallis* and *V. beccabunga* growing on wet and dry mud.

TABLE XXII.—Stomatal frequency in *Veronica spp.* growing on dry and wet mud.

| Species. | Stomatal frequency. |
|---|---|
| *Veronica anagallis*— | |
| On dry mud . . . . . . . . . . . . . . | 152 to 162 |
| On wet mud . . . . . . . . . . . . . . | 88 to 100 |
| *Veronica beccabunga*— | |
| On dry mud . . . . . . . . . . . . . . | 134 to 152 |
| On wet mud . . . . . . . . . . . . . . | 112 to 128 |

Under the more xerophytic conditions, therefore, we find a higher and not a lower frequency.

It is, therefore, evident that the high frequency of sun- as compared with shade-forms (*cf.* fig. 13) or of land- as compared with water-forms is correlated with the

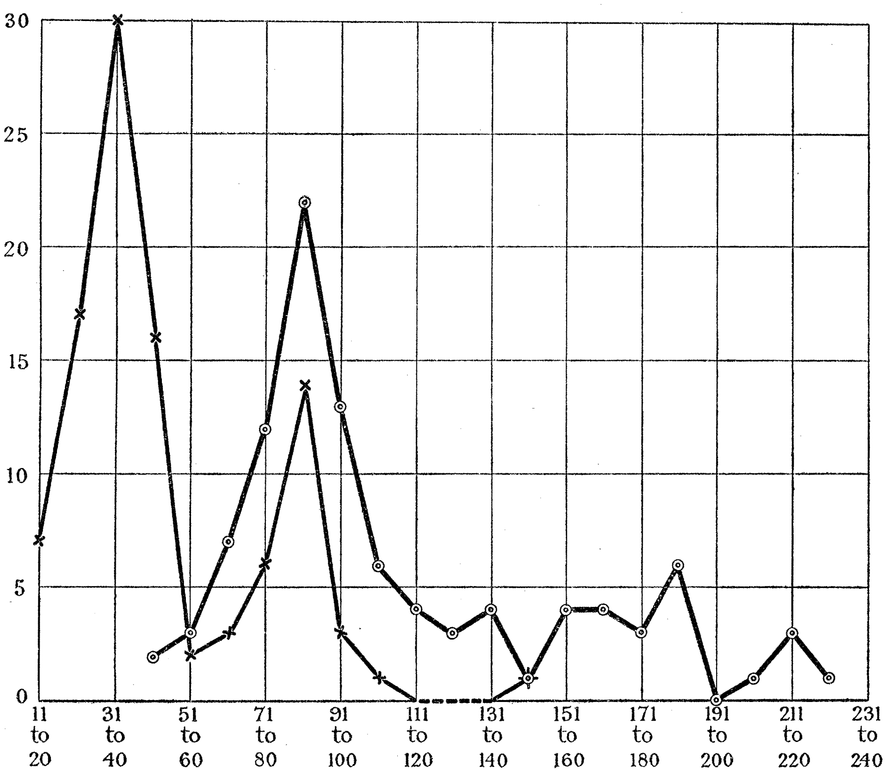


FIG. 13.—Comparison of variation in stomatal frequency in sun- and shade-leaves of *Sambucus nigra*. Ordinates, frequencies of each class interval. Abscissæ, stomatal frequencies, grouped as shown. × = shade-leaves; ⊙ = sun-leaves.

differences in the humidity of the environment and not with differences in illumination or rates of assimilation. It will, however, be evident from the data presented graphically in fig. 13 that the variation in any one type of habitat is considerable and reliance cannot be placed on results based on the examination of only a small number of leaves.

It might be urged that, though the number of stomata per unit is larger the drier the habitat, yet what the leaf lacks in number of stomata when growing in a humid situation it makes up for in their size. It is, indeed, true that the stomata of sun-leaves are smaller than those of shade-leaves, and those of plants growing in dry habitats smaller than those of plants growing in moist. Thus, in the plants of *Scilla nutans* grown in moist air, the average size of the stomatal apertures when fully open was 0·00036 sq. mm.,* whilst the average stomatal apertures of those grown in dry air was

* The average size of the apertures was determined by making camera-lucida drawings of these when fully open. The drawings of a number were so made as to form a continuous series, the total area of which was determined by means of a planimeter. In this way a high degree of accuracy was attained.

0·000301. Multiplying these values by the respective average frequencies we have for the leaves grown in dry air a total area of stomatal apertures per sq. mm. of 0·017638 sq. mm. as compared with 0·017532 sq. mm. for the leaves of plants grown in moist air. Actually, then, there is a very slightly larger area of pore space per unit area for the leaf grown in dry conditions, whilst this being distributed in smaller and more numerous units, is of much greater efficiency for transpiration. So even taking the actual apertures into consideration, we are forced to admit that the stomatal openings neither as to their number per unit area nor as to their distribution can be regarded as an adaptive feature.

### *The Concept of the Stomatal Index.*

So far it has been sufficient for the purposes of our enquiry into the ecological significance of stomatal frequency to treat of the absolute frequency as observed. This is the aspect that has been almost exclusively considered by previous writers on this subject. It is, however, apparent that a high stomatal frequency may be either the outcome of the formation of a larger proportion of stomate mother-cells in the epidermis or the high frequency may be an outcome of the fact that, though the proportion of stomata is no greater, their absolute number on a given area is raised by reason of the small size of the normal epidermal cells. Conversely a low stomatal frequency may be due to exceptional growth of the epidermal cells, thus increasing the distance between the stomata and diminishing their number per unit area; or it may be due to a failure to form the normal proportion of stomata.

It is, therefore, convenient to have some method of expressing stomatal frequency independent of their spacing through growth of the intervening unspecialised epidermal cells. As stomata originate from the epidermis, it is patent that the more stomata that are formed the fewer unspecialised cells may remain, so that a mere ratio between the number of epidermal cells and the number of stomata will not suffice. It is, therefore, proposed to employ the following expression, which we shall term the STOMATAL INDEX, viz.:—

$$I = \frac{S}{E + S} \times 100$$

where S = the number of stomata per unit area and E the number of epidermal cells in the same unit area. The Stomatal Index, therefore, aims at expressing the percentage proportion of the ultimate divisions of the dermatogen of the leaf which have been converted into stomata. Clearly the value of the Index is independent of the size attained by the epidermal cells, and is based upon the assumption that, under uniform conditions at least, there is a correlation between the number of epidermal cells per unit area and the number of stomata in the same area. In other words, that under a given set of conditions a species tends to form a definite proportion of stomatal initials.

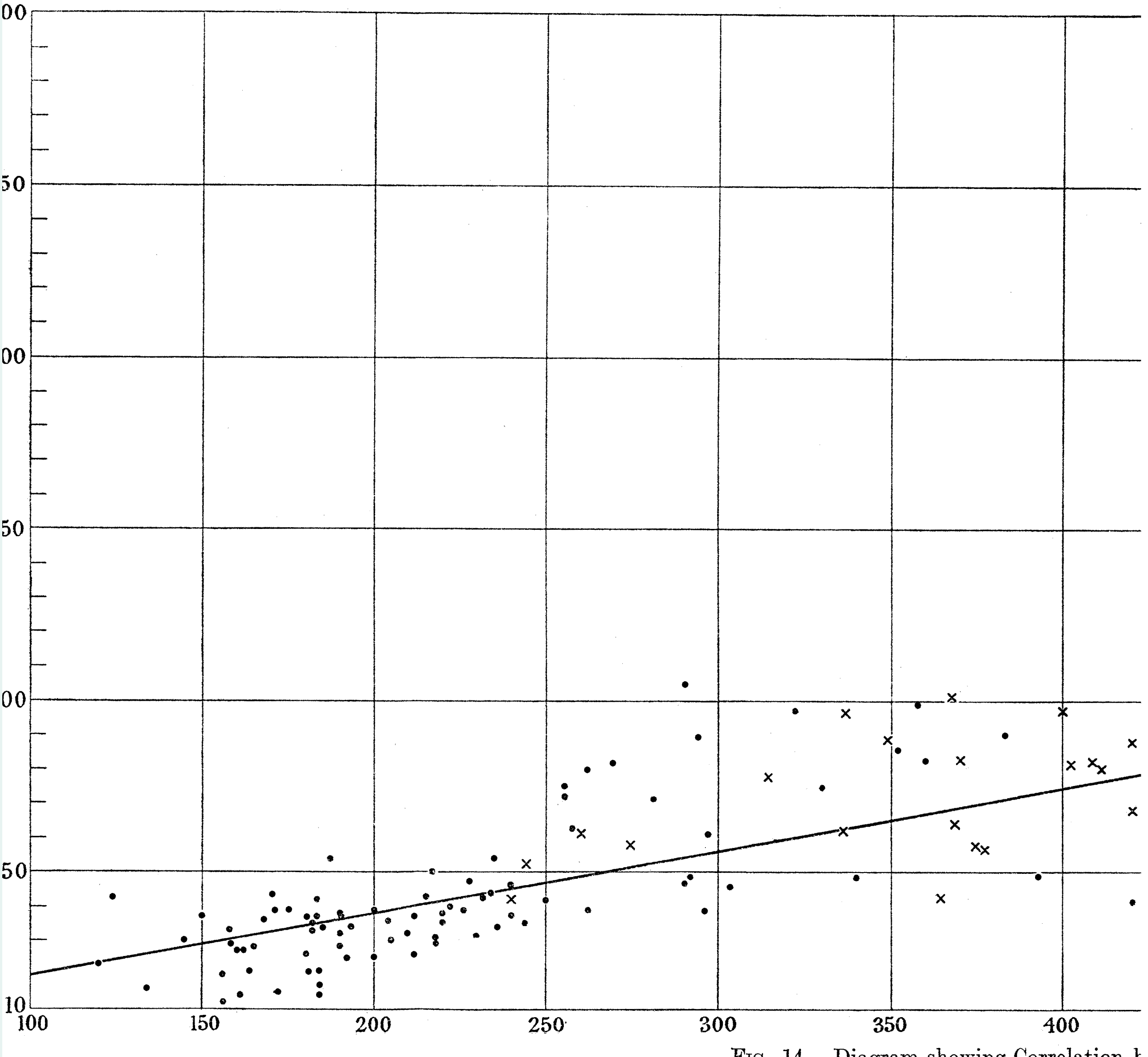


FIG. 14.—Diagram showing Correlation b

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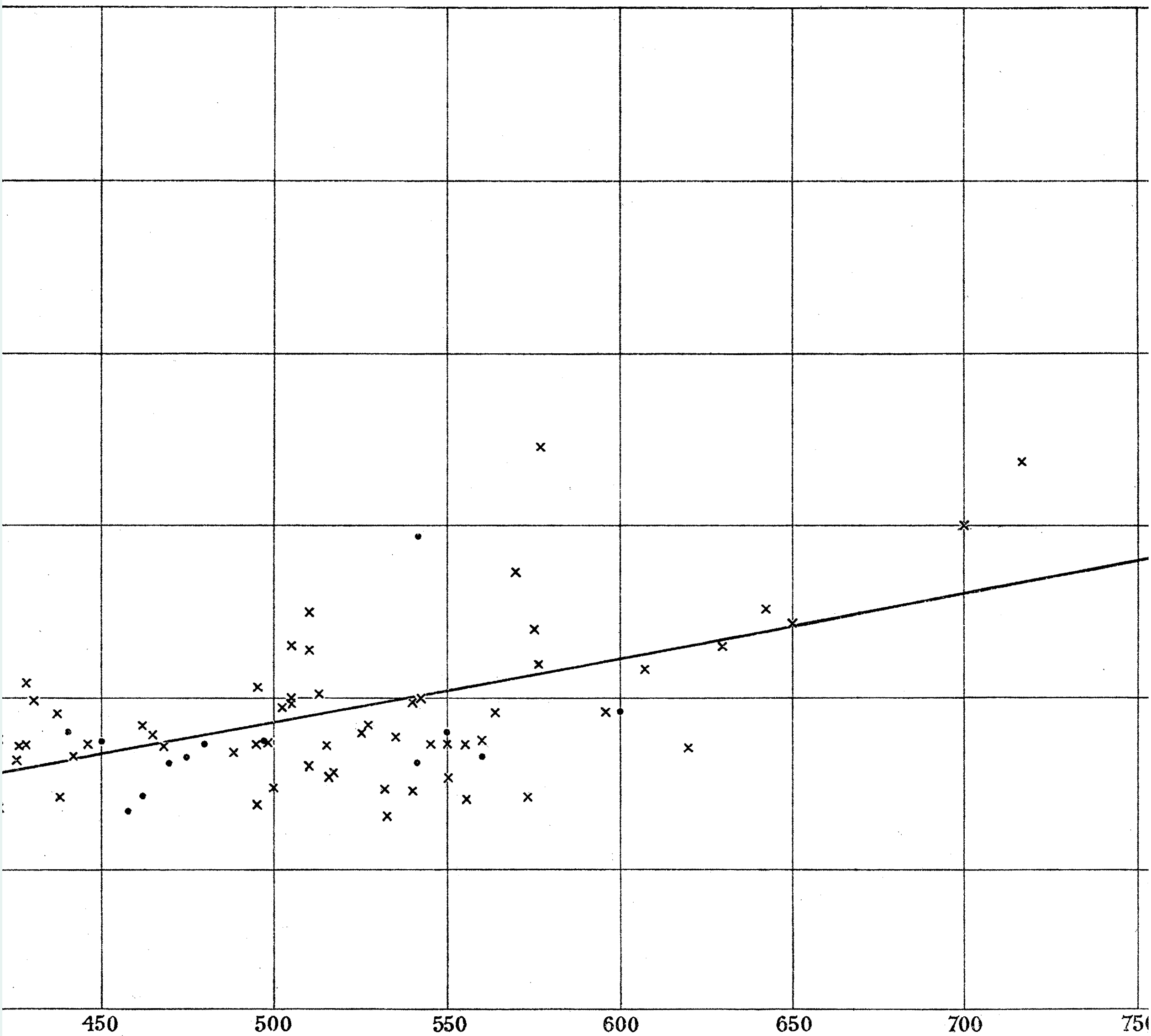


between the number of stomata (ordinates) and the number of epidermal calls (abscissæ) in 200 leaves of *Sambucus*

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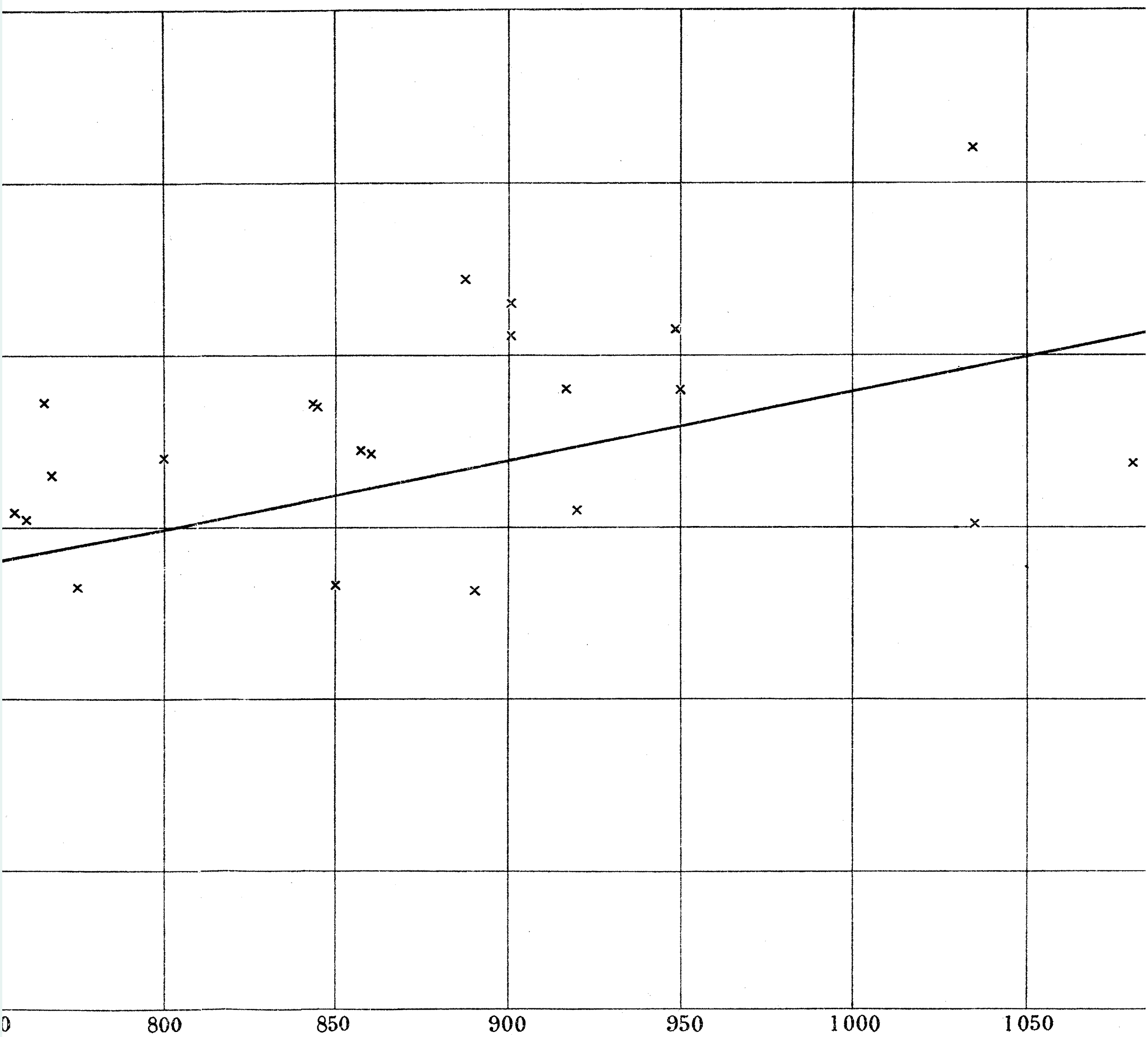


: *nigra.* Shade-leav:s are represented by a dot; Sun-leaves by a cross.

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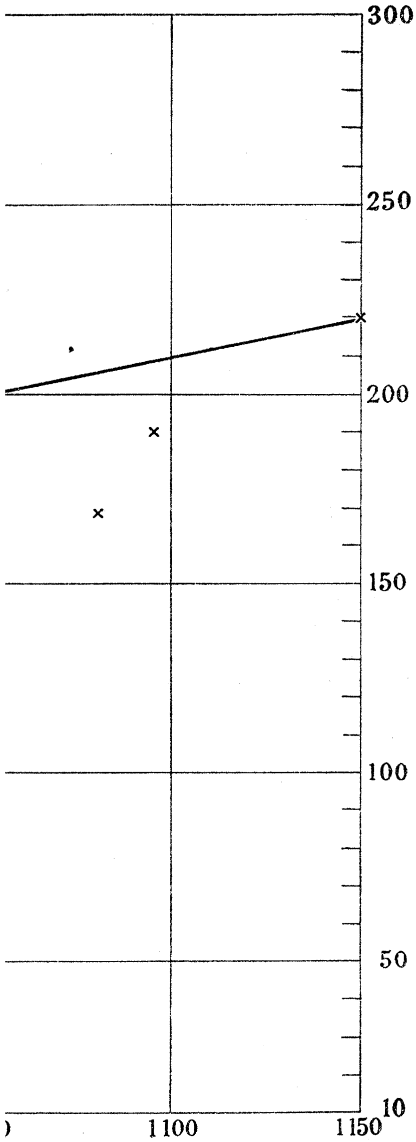


(*Facing p.* 51.)

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It will, of course, vary about this "mode" and its utility is dependent on the standard deviation not being of too great a magnitude.

The important assumption that there is a correlation between the number of epidermal cells and the number of stomata is borne out by examination of leaves of *Sambucus nigra* (*cf.* fig. 14). Utilising the formula $r = \frac{\Sigma(xy)}{n(\sigma_x \sigma_y)}$, where $x$ and $y$ are the respective deviations from the mean values for the stomatal frequency and the number of epidermal cells per sq. mm. and $n$ the number of observations, we find that two hundred determinations give a "correlation coefficient" of no less than $+0\cdot930$, whilst the "probable error" is only $0\cdot00645$. Since a correlation coefficient always lies between $\pm 1$, with perfect correlation the value cannot be greater than unity, so that we see here that the correlation is of a very high order.

Examination of *Ficaria verna* leaves confirms the results obtained with *Sambucus nigra*. Only twenty-five determinations from eleven different leaves were made of this species, but the leaves were gathered from as many different plants growing in rather varied conditions. Despite the small number, however, the correlation coefficient between stomatal frequency and number of epidermal cells was $0\cdot257$, with a probable error of $0\cdot0361$ (*cf.* fig. 15). The correlation, though not high, is here quite "significant," especially in view of the varied conditions from which the leaves were taken. As a third

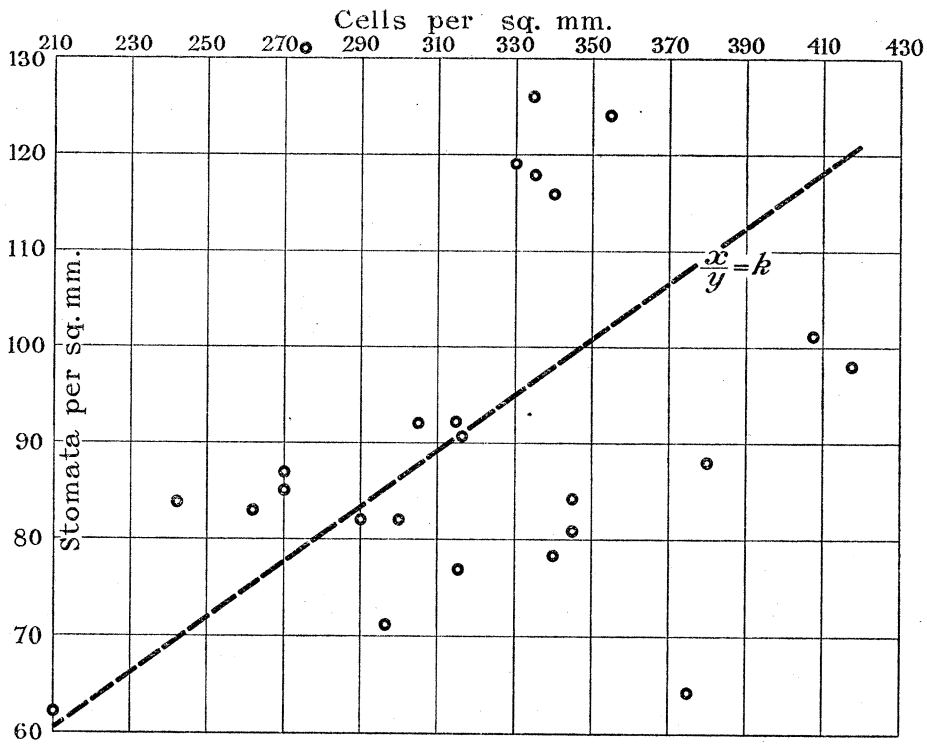


FIG. 15.—*Ficaria verna*.—Correlation of stomatal frequency and number of epidermal cells. Correlation coefficient $r = 0\cdot257$. Probable error $= 0\cdot036$.

instance we may take the leaves of *Scilla nutans* growing in moist air. These showed a correlation coefficient, between stomatal frequency and the number of epidermal cells per unit area, of 0·681 with a probable error of 0·0602 (*cf.* fig. 16). Here we have

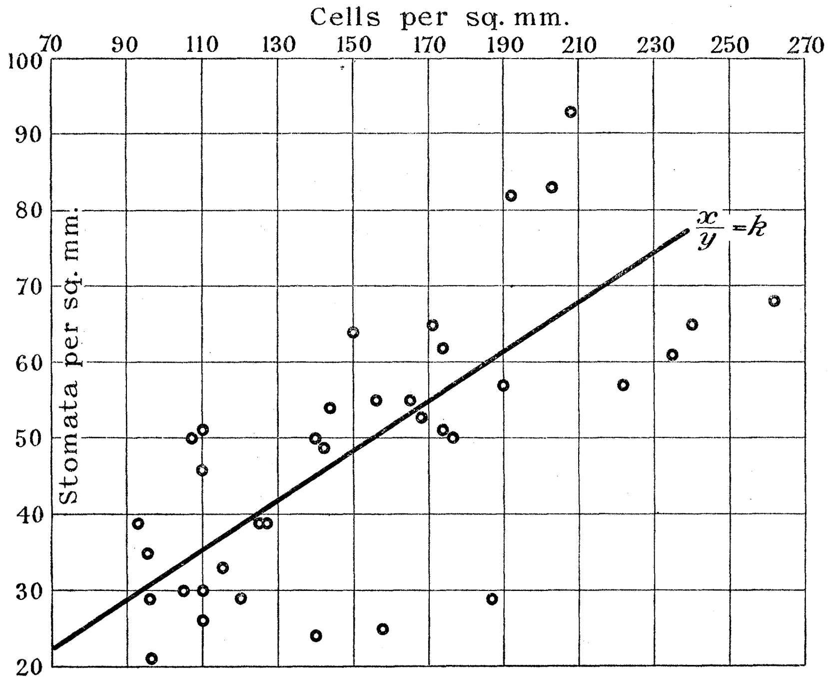


FIG. 16.—*Scilla nutans*. As in fig. 15. Correlation coefficient = 0·681. Probable error = 0·06.

a high degree of correlation with a very low probable error. This high degree of correlation between the number of stomata and the number of epidermal cells per unit area of the leaf is shown graphically in the diagrams, where the ordinates represent the stomatal frequencies and the abscissæ the number of epidermal cells. The more the dots representing individual observations tend to approximate to a diagonal line, the closer is the correlation. Representing the number of stomata by $y$, and the number of epidermal cells in the same area by $x$, then, if there were perfect correlation between $x$ and $y$ the value $x/y$ would be a constant and all observations would lie on the line representing $x/y = k$, drawn through the point given by the mean values for $x$ and $y$. This line is represented in the figures and it will be seen that the observed values do tend to be grouped in this manner. Other species show the same feature ; thus twenty-four determinations for *Scabiosa succisa* showed a correlation coefficient of 0·673 with a probable error of 0·0748, a high correlation that is the more striking from the wide range in number of the epidermal cells per square millimetre in the leaves examined, viz., from 513 to 1,161 per sq. mm.

Other species examined which showed a marked correlation between the number of stomata and number of epidermal cells were *Iris fœtidissima, Dipsacus pilosus, Hypericum androsæmum, Ligustrum vulgare, Mercurialis perennis,* and *Fagus sylvatica.*

We can, therefore, safely assume that it is of general occurrence, but consideration of the significance of this high correlation may be deferred until we have dealt with the variation exhibited by the Stomatal Index as defined above.

(*a*) *Comparison of the Stomatal Index in Sun- and Shade-leaves.*—No better known difference in stomatal frequency can be cited than that exhibited by the sun-leaves and shade-leaves of the same species. The very pronounced differences in stomatal frequency which sun-leaves and shade-leaves exhibit render them peculiarly suited to examination by means of the Stomatal Index. For this purpose two hundred determinations were made from sun-leaves and shade-leaves of *Sambucus nigra*. In the case of the shade-leaves the number of stomata per sq. mm. varied between twelve and one hundred and forty-seven, an amplitude of 135. The mean was 47 per sq. mm. (46·8). For the sun-leaves the range was 42 to 260, or an amplitude of 218, with a mean value of 112 stomata per sq. mm. (*cf.* figs. 14 and 17).

If we consider the two groups together the range is very large, viz., from 12 to 260 per sq. mm., with a mean value of 79·5 and a standard deviation of 48·4. When we turn to consider the number of epidermal cells per sq. mm., there is found to be a similar wide range, namely, from 124 to as many as 1,150 per sq. mm., with a mean value of 424·9 and a standard deviation of 231·4.

For the shade-leaves the mean number of epidermal cells was 268·5 and for the sun-leaves 581·4. Both stomatal frequency and the frequency of the epidermal cells is greater in the sun-leaves, and indeed, despite the difference in the conditions under which the two sets of leaves have developed, we find a remarkably high correlation coefficient between the number of stomata and the number of epidermal cells, viz., 0·930, with a probable error of only 0·00645.

As the full data for the two hundred determinations would occupy too much space, they are presented in the form of graphs (figs. 14 and 17). The stomatal indices based on these data are given below and presented graphically in fig. 17.

The mean value for the Index for the sun-leaves is 16·1, with a standard deviation of 2·6 and a standard error of 0·26, whilst the mean index for the shade-leaves is 15·46, with a standard deviation of 4·23 and a standard error of 0·42. The difference of these means is, therefore, only 0·64, with a standard error of 0·493, so that no significance can be attached to this difference and, indeed, it is very probable that with larger numbers of observations the difference would disappear. Having regard to the great differences in stomatal frequency (*cf.* fig. 13), this result is of great importance since it shows that in this species *the difference in stomatal frequency between the sun-leaves and shade-leaves is almost entirely to be accounted for by the extent of growth of the epidermal cells and not to differences in the proportion of stomata produced.*

The sun-leaf does not, therefore, produce more stomata, but these are developed in much the same numbers as in the shade-leaves. Whereas in the former the stomata, however, remain crowded together by reason of the small amount of growth made by the

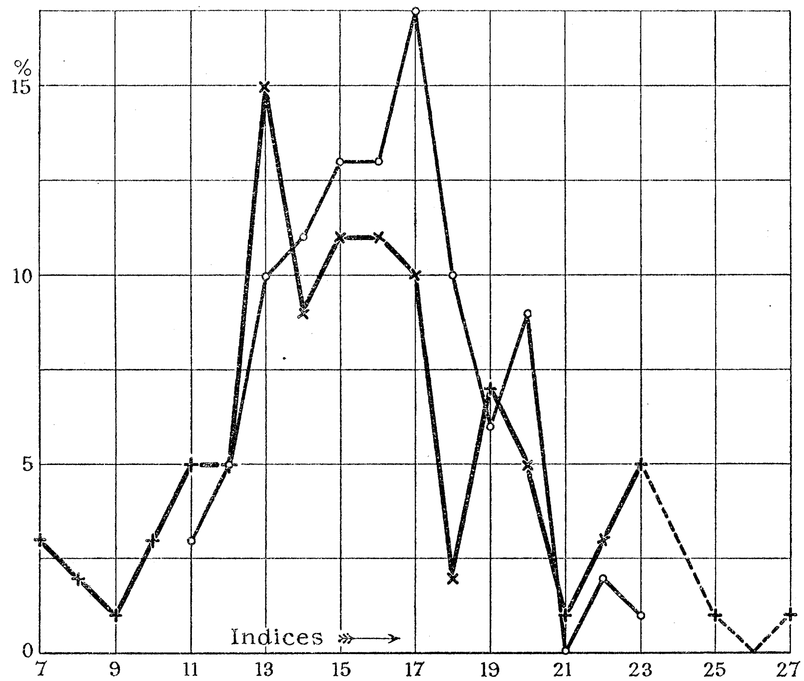


Fig. 17.—*Sambucus nigra.*—Stomatal indices for 200 sun- and shade-leaves. O = sun ; × = shade.

Table XXIII.—Stomatal Indices for sun- and shade-leaves of *Sambucus nigra*.

| Index. | Frequency shade-leaves. | Frequency sun-leaves. | Totals. |
|---|---|---|---|
| 7 | 3 | — | 3 |
| 8 | 2 | — | 2 |
| 9 | 1 | — | 1 |
| 10 | 3 | — | 3 |
| 11 | 5 | 3 | 8 |
| 12 | 5 | 5 | 10 |
| 13 | 15 | 10 | 25 |
| 14 | 9 | 11 | 20 |
| 15 | 11 | 13 | 24 |
| 16 | 11 | 13 | 24 |
| 17 | 10 | 17 | 27 |
| 18 | 2 | 10 | 12 |
| 19 | 7 | 6 | 13 |
| 20 | 5 | 9 | 14 |
| 21 | 1 | — | 1 |
| 22 | 3 | 2 | 5 |
| 23 | 5 | 1 | 6 |
| 24 | — | — | — |
| 25 | 1 | — | 1 |
| 26 | — | — | — |
| 27 | 1 | — | 1 |
| | 100 | 100 | 200 |

intervening epidermal cells, in the latter the stomata are pushed apart and the frequency correspondingly diminishes.

Examination of the sun-leaves and shade-leaves of *Fagus sylvatica* yielded results confirmatory of those obtained for *Sambucus nigra*. The number of stomata per sq. mm. for the shade-leaves examined varied between 94 and 177, whilst in the sun-leaves the range was from 130 to 295. The mean values were, respectively, 145 and 222. So that the stomatal frequency for the sun-leaves was slightly more than one-and-a-half times that for the shade-leaves. The epidermal cells numbered from 920 to 1,416 per sq. mm. for the shade-leaves and from 1,428 to 2,348 per sq. mm. for the sun-leaves, the respective means being 1,157 and 1,905. The Stomatal Index for the sun-leaves varied from 7 per cent. to 12·36 per cent., with a mean value of 10·4. For the shade-leaves the index varied between 9·8 and 11·7 per cent., with a mean value of 10·7 per cent. The difference is thus only 0·3 per cent., whilst the standard error of this difference is 0·72 per cent. We may then conclude that there is no " significant " difference between the indices of the sun-leaves and shade-leaves, and that, therefore, the proportion of stomata produced is approximately the same for the two conditions of growth. Hence here, as in *Sambucus*, the marked differences in frequency must be attributed almost entirely to the difference in development of the epidermal cells.

Examination of the sun- and shade-leaves of *Vaccinium myrtillus* showed a stomatal frequency in the former of 100 to 178 (mean 132) and in the latter of 64 to 133 (mean 90). The number of epidermal cells ranged from 433 to 699 in the shade-leaves and from 788 to 1,731 per sq. mm. in the sun-leaves, the mean values being, respectively, 545 and 1,049.

The Stomatal Index for the sun-leaves ranged from 8·2 to 18·1, with a mean value of 12·2 and a standard error of 0·24 ; whilst for the shade-leaves it ranged from 10·3 to 18·9, with a mean value of 14·5 and a standard error of 0·78. The difference of the means is in favour of the shade-leaves and amounts to 2·3, but since the standard error of this difference is 1·22, it cannot be regarded as significant.

It will be realised that the leaves examined in this connection have shown very striking differences in stomatal frequency, and, indeed, extreme examples of sun-leaves and shade-leaves were chosen for comparison, yet in three species of very diverse habit, viz., a tree, a shrub, and an under-shrub, no significant difference in the stomatal index under the different conditions was found. We are probably, therefore, justified in regarding this as a general phenomenon, and examination of isolated specimens of a variety of other species appears to confirm this view. The increased frequency in the sun-leaves is clearly correlated with more numerous epidermal cells per sq. mm., or, in other words, with their smaller size, and the data given seem to warrant the conclusion that growth of these cells is the main factor which governs the stomatal frequency. This conclusion is borne out by the form of the epidermal cells in *Sambucus nigra*, for in this species there is a marked positive correlation between the degree of irregularity in outline of the lower epidermal cells and their size. In other words, the greater the superficial growth of the cell the more wavy its outline tends to become, and in *Sambucus*, as in many other plants, the cells of the lower epidermis of the shade-leaves are usually far more wavy in outline than those of the sun-leaves. It should, perhaps, be emphasised that in all the above comparisons the greatest care was taken to ensure that equivalent numbers of determinations were made from the different parts of the sun-leaves and shade-leaves; a very necessary precaution in view of the striking differences in one and the same leaf.

(*b*) *Comparison of stomatal indices of plants grown in wet and dry soil.*—For comparison of the stomatal indices of plants grown in dry and wet soil, plants of *Caltha palustris* were grown for three years in ordinary garden soil and in the same type of soil covered with water. The effect on the stomatal ratio is shown below. Only seven determinations were however made of the dry-soil plants and thirteen of the wet-soil plants, so

Table XXIV.—Stomatal indices in *Caltha palustris*.

| — | Stomata per sq. mm. | Epidermal cells per sq. mm. | Stomatal Indices. |
|---|---|---|---|
| In water . . . . . . . . | 32–51<br>Mean 39·3 | 150–314<br>Mean 207 | 12·5–19·5<br>Mean 16·0 (0·604)* |
| In relatively dry soil . . | 53–125<br>Mean 82·4 | 332–782<br>Mean 568 | 10·3–15·7<br>Mean 12·8 (0·657)* |

Difference of mean indices . . . . 3·2
Standard error of difference . . . ±0·892

that no great stress can be laid on the difference, though the data suggest that there is a real increase in the proportion of stomata in the plants grown in water. The fact, however, that the mean frequency of stomata in the dry-soil plants is more than double that of the wet-soil plants, whilst the indices are generally higher for the wet-soil plants, shows that here again the increased frequency is an outcome of the greater crowding of the stomata in the dry-soil plants through the small size of the epidermal cells, and is not indicative of any increase in the number of stomata formed.

(*c*) *Stomatal indices in Scilla nutans in various parts of the leaf and in various conditions.*—The stomatal indices were determined for the middle, basal and apical regions of a number of leaves of *Scilla nutans* grown, respectively, in the dark in moist air, in the light in moist air, and in the light in dry air. The results are given in the subjoined table.

* Standard errors.

TABLE XXV.—Stomatal Indices for *Scilla nutans*.

| — | Tip of leaf. | Middle of leaf. | Base of leaf. | Whole leaf. |
|---|---|---|---|---|
| Grown in moist air in the dark . | 14·7–37·5<br>Mean 23·7<br>Standard error of mean ± 2·5 | 27·2–29·0<br>Mean 28·0<br>Standard error of mean ± 0·32 | 22·4–32·2<br>Mean 27·4<br>Standard error of mean ± 1·45 | 26·3 |
| | Tip of leaf. | Middle of leaf. | Base of leaf. | Whole leaf. |
| Grown in moist air in the light . | 20·6–33·1<br>Mean 26·3<br>Standard error of mean ± 1·11 | 22·6–31·6<br>Mean 27·6<br>Standard error of mean ± 1·36 | 13·0–31·8<br>Mean 21·5<br>Standard error of mean ± 1·14 | 25·1 |
| | Tip of leaf. | Middle of leaf. | Base of leaf. | Whole leaf. |
| Grown in dry air in the light | 24·8–33·8<br>Mean 29·6<br>Standard error of mean ± 1·07 | 29·0–35·7<br>Mean 33·4<br>Standard error of mean ± 1·12 | 25·7–35·6<br>Mean 29·1<br>Standard error of mean ± 1·06 | 30·7 |

| | Dry air in light—moist air in light. | | |
|---|---|---|---|
| | Tip of leaf. | Middle of leaf. | Base of leaf. |
| Difference of means . . . . . .<br>Standard error of difference . . . | 3·3<br>±1·52 | 5·8<br>±1·73 | 7·6<br>±1·55 |

| | Moist air in light—moist air in darkness. | | |
|---|---|---|---|
| | Tip of leaf. | Middle of leaf. | Base of leaf. |
| Difference of means . . . . . .<br>Standard error of difference . . . | +2·6<br>±2·73 | −0·4<br>±1·39 | −5·9<br>±1·84 |

Except as regards the base of the leaf the difference of the means for moist air and dry air is less than three times the standard error of the difference, and cannot, therefore, be regarded as significant. But it is important to note that, in so far as the difference exists, it indicates a tendency for a greater *proportion* of stomata to be formed in the dry air than in the moist air, showing that neither with regard to the absolute frequency of the stomata nor yet with respect to their relative frequency, can their incidence be considered an adaptation to the humidity relations of the environment. On the contrary it would appear that *the more xerophytic the conditions the more stomatal frequency increases, absolutely, and perhaps relatively.*

Comparing the indices for plants grown in moist air in darkness and in moist air but illuminated, it will be noted that there is no constant difference. Whilst the mean index for the tip of the leaf is greater for the leaves grown in the light, the indices for the middle and base are greater for those grown in the dark. Actually it is only the difference for the basal region that is sufficiently large in comparison with the standard error to be regarded as significant. If anything then the leaves in darkness tend to produce a higher rather than a lower proportion of stomata, so that the suggestion that the greater stomatal frequency in the light is in the nature of an adaptation to increased rate of assimilation or gaseous exchange either for assimilation or respiration is quite unwarranted.

The following data show that the range of frequency in the leaves examined was large.

TABLE XXVI.—Frequency per sq. mm. of epidermal cells and stomata in *Scilla nutans*.

| Conditions of environment. | Number of epidermal cells. | Number of stomata. |
|---|---|---|
| Moist dark . . . . . | 75–220<br>Mean 141·6 | 20–58<br>Mean 44·0 |
| Moist light . . . . . | 93–262<br>Mean 153·1 | 21–103<br>Mean 50·0 |
| Dry light . . . . . . | 80–210<br>Mean 131·3 | 29–107<br>Mean 58·1 |

The indices and the means show a slight tendency to increase the number of stomata in the dry air as compared with the almost saturated air in which the other two sets were grown. Actually, the mean index for the entire leaf in dry air is about 20 per cent. greater than the indices for moist air, whether in light or darkness and the mean increase in stomatal frequency is of the same order. This suggests that the higher number of stomata in dry air, or probably more correctly, the fewer stomata in the moist air, is not a mere result of enlargement of the epidermal cells, but represents an actual diminution in the proportion of stomata formed; a conclusion in harmony with the more or less complete absence of stomata in submerged leaves. It should be noted that, in the case of *Caltha* cited above, the leaves of both water and land plants were surrounded by relatively dry air, whereas in these experiments with *Scilla* the plants in moist air were entirely enveloped in an almost saturated environment.

Comparing the different regions of the same leaf it will be noted that the stomatal indices show no continuous increase corresponding to the increase in stomatal frequency as one passes from the base to the apex of an elongated leaf. It is, therefore, clear that this gradient is also the outcome of differences in the development of the epidermal cells, since, as we have already seen, there is a marked correlation between the frequency of epidermal cells in *Scilla nutans* and the stomatal frequency. And what is true of *Scilla* is probably true also for other species in which a similar correlation has been noted. The stomatal indices for the different parts of the leaf do, however, show a uniformly higher value for the middle region as compared with those for the basal and the apical regions, and, inasmuch as the leaves of *Scilla* grown in moist air in the dark exhibit an increase of stomatal frequency in the middle region (*cf.* fig. 8) this increase is probably due to internal factors.

The conclusive proof that the variation in stomatal frequency in *Scilla* is mainly the outcome of variations in growth of the epidermal cells is afforded by the fact that there is no significant correlation between the stomatal indices and the stomatal frequencies. The correlation coefficient for these two variables is only 0·147, which is barely twice the standard error 0·0768. This absence of significant correlation is also shown graphically in fig. 18.

(*d*) *The stomatal index and assimilation.*—It was shown that comparing the yellow and green regions of variegated leaves, a higher frequency was associated with the yellow region; a result that indicated the absence of any adaptational significance for stomatal frequency in relation to assimilation rate. The following data respecting the stomatal index for such leaves yield a difference between the green and yellow portions of 0·4, with a standard error of difference of 0·793. There is thus no significant difference between the two regions, and we can regard the higher frequency in the yellow region as the outcome of the smaller size of the cells here as compared with the area of the leaf containing chlorophyll. This interpretation is confirmed by examination of variegated leaves in which the central region is green and the periphery yellow. Such leaves usually show a distinct puckering of the central green area, which is obviously due to failure on the part of the epidermal cells in the peripheral yellow region to keep pace with the growth of those in the central green area.

(*e*) *Stomatal index and osmotic pressure.*—Seedlings of *Salicornia annua* were grown in two strengths of sea water, corresponding to osmotic pressures of about 1 atmosphere and 30 atmospheres, respectively. The stomatal frequencies and stomatal indices are given below. There is no significant difference between the mean indices for the two cultures, though the index values tend to be higher for the culture with the higher osmotic pressure. It is very striking that there should be so little difference between the index values in view of the very striking differences between the two cultures as regards stomatal frequency and frequency of epidermal cells. It is, therefore, clear that here also the stomatal frequency is largely determined by the degree of growth of the epidermal cells.

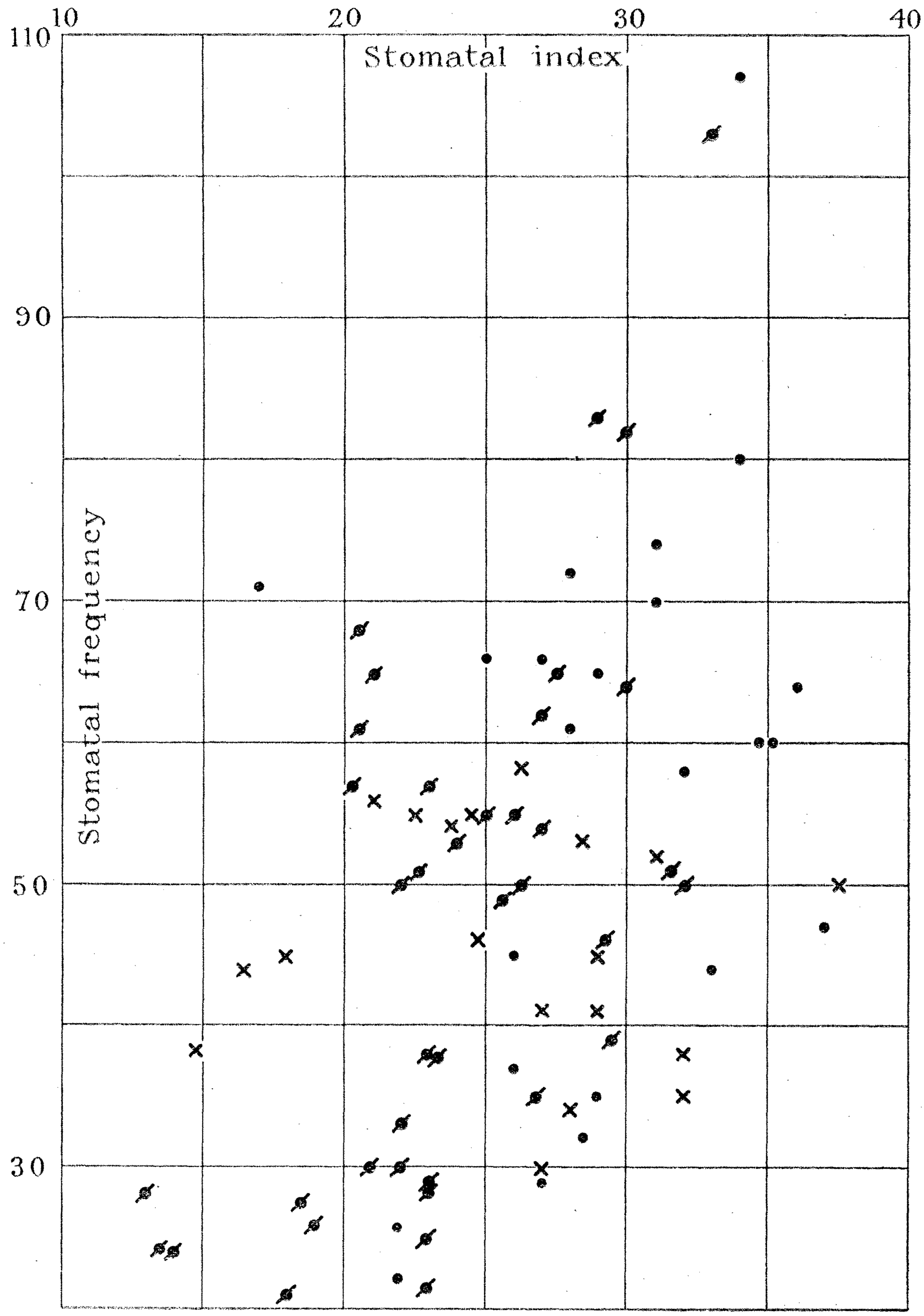


FIG. 18 and last.—*Scilla nutans*.

Cultures of *Scilla nutans* seedlings in tap-water and in sugar solutions of varying strengths gave similar results. Thus, in tap-water, the indices ranged from 18·1 to 22, whilst in sugar solution corresponding to an osmotic pressure of 7·5 atmospheres, the stomatal indices ranged from 18·7 to 25·9.



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TABLE XXVII.—Stomatal indices for yellow and green regions of variegated leaves of *Ligustrum latifolium*.

| — | Yellow portion. | Green portion. |
| --- | --- | --- |
| Leaf A . . . . . . . | 15·9<br>15·2<br>18·2 Av. 16·4 | 18·8<br>17·8<br>15·0 Av. 17·2 |
| Leaf B . . . . . . . | 16·2<br>17·0 | 15·0 |
| Leaf C . . . . . . . | 19·4<br>19·6 Av. 19·5 | 16·7<br>18·2 Av. 17·4 |
| Leaf D . . . . . . . | 19·6<br>19·2<br>Av. 19·4 | 17·4<br>20·9<br>18·3 Av. 18·8 |
| Leaf E . . . . . . . | 20·7 | 19·1 |
| Mean | 18·1 | Mean 17·7 Difference 0·4.<br>Standard error 0·793. |

| — | Epidermal cells per sq. mm. | Stomata per sq. mm. |
| --- | --- | --- |
| Yellow portion . . | 1881–3222<br>Mean 2534 | 339–844<br>Mean 576 |
| Green portion . . . | 1690–2952<br>Mean 2388 | 353–666<br>Mean 519 |

TABLE XXVIII.—Stomatal frequencies and stomatal indices of *Salicornia annua* grown in soil of different osmotic values.

| — | Stomatal frequency. | Epidermal frequency. | Index. |
| --- | --- | --- | --- |
| Osmotic pressure of soil, sol. ca. 1 atmosphere | 97 to 222<br>Mean 148 per sq. mm. | 644 to 1243<br>Mean 848 per sq. mm. | 11·7 to 20<br>Mean 14·9 |
| Osmotic pressure, ca. 30 atmospheres | 150 to 362<br>Mean 256 per sq. mm. | 854 to 1876<br>Mean 1208 per sq. mm. | 14·0 to 22·2<br>Mean 17·35 |
| | Stomatal index difference | $2 \cdot 4 \pm 0 \cdot 99$. | |

(*f*) *Stomatal index and leaf area.*—It has been shown that the stomatal frequency tends to augment with a diminution in leaf area, and, in general, one would expect that this negative correlation is an outcome of the fact that conditions favourable to the growth of the leaf as a whole will be also favourable to growth of the epidermal cells. The following data taken from several species show that there is no obvious relation between leaf size and the value of the stomatal index, which confirms the view that the frequency is determined mainly by epidermal growth. In other words, the factors which govern leaf size have apparently no influence on the proportion of stomata formed.

TABLE XXIX.—Stomatal indices in relation to leaf area.

| Species. | Leaf area. | Average stomatal index. |
|---|---|---|
| | sq. cms. | |
| *Ficaria verna* . . . . . | 8·97 | 22·16 |
| ,, ,, . . . . . | 4·64 | 19·4 |
| ,, ,, . . . . . | 1·27 | 24·1 |
| ,, ,, . . . . . | 1·06 | 20·5 |
| *Fagus sylvatica* (sun) . . | 36·30 | 11·34 |
| ,, ,, ,, . . | 26·00 | 9·4 |
| ,, ,, ,, . . | 18·8 | 10·9 |
| ,, ,, ,, . . | 13·7 | 7·0 |
| *Mercurialis perennis* . . | 35·96 | 21·3 |
| ,, ,, . . | 21·73 | 22·4 |
| ,, ,, . . | 4·64 | 20·2 |
| ,, ,, . . | 1·22 | 23·7 |
| *Sambucus* (shade) . . . | 15·6 | 17·8 |
| ,, ,, . . . | 12·5 | 16·7 |
| ,, ,, . . . | 6·4 | 15·5 |
| ,, ,, . . . | 5·6 | 16·7 |
| *Hypericum androsæmum* | 43·5 | 21·7 |
| ,, ,, | 3·7 | 22·7 |

(*g*) *The stomatal index in relation to habitat.*—Owing to the time required to make numerous determinations of indices, a large number of leaves have only been examined from this point of view in the case of a few species, but a small number of leaves have been examined of plants from various habitats and these indicate tentatively that a low index is characteristic of aquatics, whilst plants of drier habitats are characterised by higher indices. This result is in harmony with the fact that submerged aquatics tend to have few or no stomata whereas their land forms bear numerous stomata, and also with the results obtained from *Scilla* grown in moist and dry air. For a low stomatal index shows that a lower proportion of stomata are formed as distinct from diminution per unit area, consequent upon growth of the epidermal cells. The fact that some submerged aquatics form stomata upon their leaves when well nourished, whereas under conditions of malnutrition stomata may be absent (*e.g.*, *Stratiotes*, *cf.* 'Montesantos Flora,' Bd. V., pp. 1–32, 1913), shows that nutrition probably plays a large part in determining the value of the stomatal index, perhaps associated with the higher osmotic pressure.

TABLE XXX.

| Habitat. | Species. | Observed range of stomatal indices. | Mean of stomatal index. |
|---|---|---|---|
| Aquatic | *Ranunculus lingua* | 1·3 to 6·5 | 4·0 |
| ,, | *Hydrocharis morsus-ranæ* | 3·8 to 6·6 | 5·3 |
| ,, | *Sagittaria sagittifolia* | 4·6 to 9·0 | 6·8 |
| ,, | *Caltha palustris* | 12·5 to 19·5 | 14·4 |
| ,, | *Polygonum amphibium* | 7·5 to 12·2 | 9·4 |
| Marsh | *Malachium aquaticum* | 19·5 to 27·5 | 23·8 |
| Woodland | *Fagus sylvatica* | 5·2 to 15·2 | 10·7 |
| ,, | *Sambucus nigra* | 7·0 to 27·0 | 15·7 |
| ,, | *Vaccinium myrtillus* | 8·2 to 18·9 | 13·3 |
| ,, | *Scabiosa succisa* | 13·9 to 29·3 | 18·5 |
| ,, | *Phyteuma spicata* | 17·9 to 25·0 | 21·1 |
| ,, | *Hypericum androsæmum* | 16·5 to 28·1 | 21·2 |
| ,, | *Mercurialis perennis* | 17·0 to 25·9 | 21·3 |
| ,, | *Anemone nemorosa* | 17·1 to 25·9 | 21·4 |
| ,, | *Ficaria verna* | 14·5 to 30·2 | 22·5 |
| ,, | *Helleborus viridis* | 16·2 to 23·8 | 22·8 |
| ,, | *Dipsacus pilosus* | 24·5 to 29·8 | 26·9 |
| ,, | *Viola sylvestris* | 21·0 to 34·1 | 27·0 |
| ,, | *Scilla nutans* | 13·0 to 37·5 | 27·4 |
| Dry woods | *Iris fœtidissima* | 26·8 to 40·0 | 34·5 |
| Alpine | *Sempervivum montanum* | 12·3 to 20·1 | 17·2 |
| Arctic | *Melandrium triflorum* | 17·9 to 27·1 | 19·2 |
| Salt marsh | *Salicornia herbacea* | 11·7 to 22·2 | 15·7 |
| ,, | *Salicornia ramosissima* | 11·0 to 15·5 | 12·8 |
| Dune slack | *Teucrium scordium* | — | 17·4 |
| Shingle | *Raphanus maritimus* | 29·4 to 30·5 | 30·0 |
| Dune | *Euphorbia portlandica* | — | 10·8 |

The value of the stomatal index is emphasised by its much greater constancy than the stomatal frequency. Thus, in the case of *Sambucus nigra*, of which a large number of determinations were made, the stomatal frequency ranged from 12 to 260 per sq. mm., a difference of over twenty-one times. The stomatal indices for these same leaves, however, show a difference of less than four times. Or again, for *Scilla nutans* the stomatal range is from 10 to 99, or nearly ten times, whilst the stomatal indices show a range of less than three times, and this, despite the fact that the plants examined grew under the most diverse conditions.

*Summary and Conclusions.*

It has been shown in the foregoing account that the number of stomata per unit area of the leaf surface is extremely variable, even upon the same leaf.

Comparing leaves borne at the same node, the stomatal frequency tends to be lower the larger the leaf, but this negative correlation between leaf size and stomatal frequency is found only to hold in dry conditions and when the leaves are not too crowded.

It is further shown that not only have radical leaves in general lower frequencies than the cauline leaves upon the same plant, but that other conditions being similar, the stomatal frequency tends to augment with the height above the ground at which the leaf is borne. This is true alike for trees, shrubs and herbs, and it will be realised, therefore, that the stomatal frequency increases parallel to the increase in suction force and osmotic pressure (*cf.* MOLZ, 'Amer. Jour. Bot.,' pp. 433–463, 1926). The stomatal frequency gradient in respect to position tends to disappear when the entire plant is grown in a uniformly moist atmosphere.

The stomatal frequency on the individual leaf is shown to exhibit great variation and, in general, it is found that the frequency augments from the base of the leaf to the apex and from the midrib to the leaf margin. Here, too, it will be noted that the stomatal frequency is greatest in the parts of the leaf where the suction force and osmotic pressure are highest (*cf.* MOLZ, *loc. cit.*, and LUTMAN, 'Amer. Jour. Bot.,' vol. VI, p. 193). In the case of elongated monocotyledonous leaves the youngest portion of the leaf has the highest frequency, whilst the oldest parts, viz., the basal and apical regions, which were formed under more protected conditions, have lower frequencies.

Data respecting the stomatal frequencies for the majority of the British woodland species are furnished, viz., for 26 trees, 29 shrubs, and 150 species of the ground flora, and these show that the frequencies tend to be higher for the trees and shrubs than for the herbaceous vegetation, and greater for the marginal species than for the shade flora. In this connection we may recall that HARRIS and LAWRENCE found that, in five different habitats, the trees and shrubs had a higher osmotic pressure than the perennial herbs (' Physiological Res.,' vol. 2, No. 1, 1916). Aquatic plants have relatively low frequencies, whilst plants of dry exposed situations tend to have high frequencies. It is now generally recognised that plants subject to physical or physiological drought tend to possess high osmotic pressures, and that even in the same individual the osmotic pressure in time as well as in space is negatively correlated with the humidity of the environment. The marked fluctuations in time that the osmotic pressure of a single individual may exhibit render it improbable that the general relation between stomatal frequency and osmotic pressure is more than a parallel response to differences in the humidity of the environment. In any case, this correlation emphasises the association of high stomatal frequencies with xerophytic conditions. Stomatal frequency cannot, therefore, be regarded as a means of adaptational adjustment with respect to transpiration, since the number of stomata per unit area increases with the need for transpiration checks.

The frequency on the two surfaces of the leaf tends to augment or diminish in a parallel manner, but whereas trees rarely have stomata in the upper epidermis, they are frequent in the upper epidermis of the herbaceous woodland flora.

Experimental cultures of *Circæa lutetiana*, *Scilla nutans*, *Ficaria verna*, and *Lepidium sativum*, grown in light and in darkness, or in sun and shade, but under conditions of similar humidity, showed no " significant " differences in stomatal frequency corresponding to the differences in illumination. It is, therefore, concluded that the rate of assimilation plays no direct part in determining stomatal frequency. This conclusion is confirmed by the study of harlequin and variegated leaves, which show a slightly higher frequency in the areas devoid of chlorophyll, whereas shade-leaves have an appreciably lower frequency than corresponding sun-leaves.

Comparison of plants grown in dry and moist air respectively, indicates that stomatal frequency is mainly dependent on the humidity of the environment; dry exposed conditions being associated with high frequencies and humid conditions with low frequencies. Moreover, the total pore space per unit area of leaf surface is shown to be greater in dry than in moist conditions despite the larger size attained by the stomata in a humid environment.

The term *Stomatal Index* is employed to denote $\frac{S}{E + S} \times 100$, where S denotes the number of stomata per unit area and E the number of epidermal cells in the same area. By means of this index it is shown that the *proportion* of stomata formed in the epidermis is no greater for sun-leaves than for shade-leaves, but that there is a high positive correlation coefficient between the number of stomata and the number of epidermal cells per unit area.

Similarly the increased stomatal frequencies in plants grown on dry soil as compared with those grown on wet soil, of small leaves as compared with large leaves, and the slight differences between the yellow and green regions of variegated and harlequin leaves, are all shown to be due chiefly to differences in the growth of the epidermal cells, that is, to differences in the spacing of the stomata and not to differences in the proportion of stomata developed. This appears to be true also for the variations in frequency in different parts of the same leaf.

On the other hand, there is evidence that high humidity tends to reduce the proportion of stomata formed and aquatics would appear to have low stomatal indices. Differences in the osmotic pressure of the medium surrounding the plant, although these result in marked differences of stomatal frequency, appear not appreciably to affect the stomatal index. It is, therefore, clear that with the exercise of proper precautions as to the region of the leaf examined, and with due regard to its variable character, the stomatal frequency has an important ecological significance as an indicator of the humidity of the environment. But, contrary to the generally accepted view, the correlation is negative and not positive, and has no adaptational significance in relation either to transpiration or assimilation. Variations in the stomatal index appear to be due to internal factors, of which nutritional conditions are perhaps the most important.

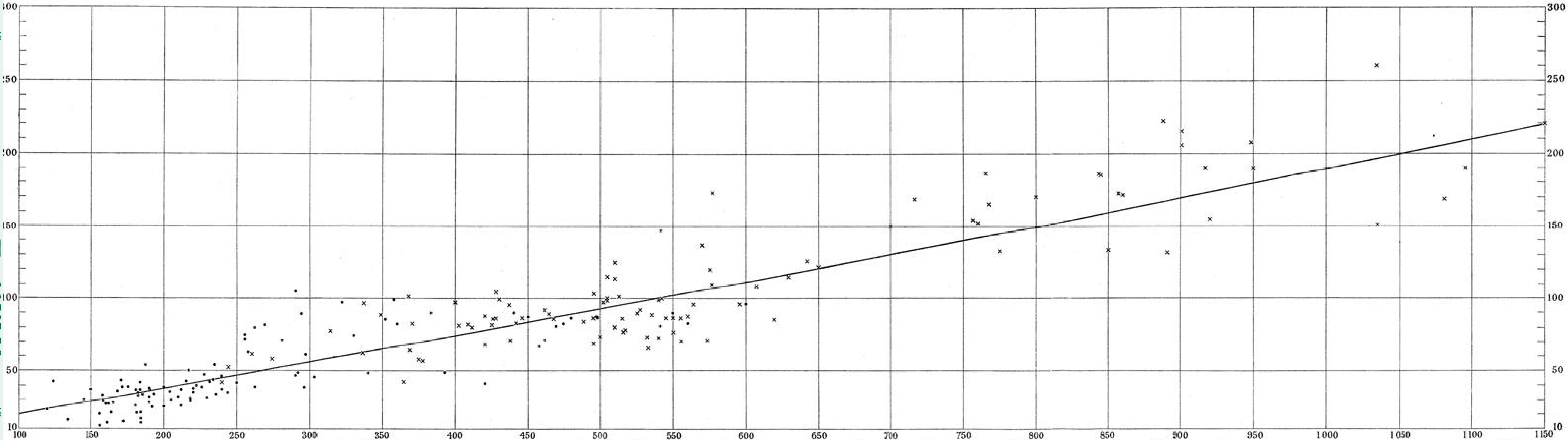


FIG. 14.—Diagram showing Correlation between the number of stomata (ordinates) and the number of epidermal calls (abscissæ) in 200 leaves of *Sambucus nigra*. Shade-leaves are represented by a dot; Sun-leaves by a cross.

(*Facing p.* 51.)