Botany. — *"On the Regulation of the transpiration of Viscum album and Rhipsalis Cassytha". A contribution to the knowledge of the antagonism between the guard cells of the stomata and the adjacent cells of the epidermis.* By Z. KAMERLING. (Communicated by Prof. BEIJERINCK).

(Communicated in the meeting of Jan. 31, 1914).

In recent years I have conducted numerous experiments to obtain an insight into the need for water and the consumption of water in various tropical plants. In continuation of these investigations, carried out in Java and in Brazil, similar experiments have been undertaken during the last few months in Holland with native plants. The method of inquiry was almost always the same: leafy boughs were cut off and hung up in the laboratory in the shade or exposed to the sun in the habitat of the plant investigated; they were weighed periodically at shorter or longer intervals.

By this method of experiment the extent of transpiration can be determined when the tissues of the plant still possess their normal water-content and also the nature of its modification, when the tissues of the plant gradually lose water.

These experiments show that in many plants there is a continuous and very considerable transpiration from the beginning of the experiment until the leaves of the bough are dried up. In other species the transpiration is more or less great at the beginning, but decreases gradually so that finally it sinks to a minimum-value. This decrease or regulation of transpiration is evidently due to the narrowing or closing of the stomata, which in the different plants investigated may occur more or less quickly and more or less extensively.

In some plants I found that in contrast to the normal course of the regulation, the intensity of transpiration increases distinctly at the beginning and only afterwards diminishes in the usual way. Such an irregularity, at least when there is no external influence at work, such as temperature, illumination or conditions of humidity or movement of the atmosphere, can hardly be explained otherwise than by assuming that the aperture of the stomata in these cases first dilates when the plant begins to wither and only afterwards constricts.

I observed this phenomenon very clearly in *Viscum album* in a comparative experiment which I performed on the transpiration of this plant and of some deciduous and evergreen woody plants.

It is unnecessary to publish here the detailed results. I append only the figures which have reference to the above-mentioned irregularity in the regulation of transpiration.

The boughs for experiment were arranged at an open window, where they were not reached by the sun before mid-day, and only for a short time afterwards.

6 September	First bough experimented on Viscum album. Weight in grammes	6 September	Second bough experimented on Viscum album. Weight in grammes	6 September	Third bough experimented on Viscum album. Weight in grammes
9.25	21.5 grm.	9.35	15.75 grm.	9.40	15.55 grm.
9.45	21.2 „	10.	15.55 „	10.	15.50 „
10.10	21.0 „	10.15	15.4 „	10.20	15.42 „
10.45	20.7 „	10.55	15 0 „	10.55	15.24 „
11.15	20.27 „	11.25	14.57 „	11.25	14.94 „
11.45	19.82 „	11.55	14.12 „	12.	14.53 „
2.	18.3 „	2.15	13.22 „	2.15	13.15 „
3.35	17.2 „	3.35	12.92 „	3.35	12.75 „

The results of these experiments are more intelligible when represented in the following way:

First bough experimented on.			
	Length of period	Total transpiration	Average transpiration in 5 minutes
9.25 till 9.45	20 minutes	300 milligrm.	75 milligrm.
9.45 „ 10.10	25 „	200 „	40 „
10.10 „ 10.45	35 „	300 „	43 „
10 45 „ 11.15	30 „	430 „	72 „
11.15 „ 11.45	30 „	450 „	75 „
11.45 „ 2.	135 „	1520 „	56 „
2. „ 3.35	95 „	1100 „	58 „

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Second bough experimented on.

	Length of period	Total transpiration	Average transpiration in 5 minutes
9.35 till 10.	25 minutes	200 milligrams	40 milligrams
10. „ 10.15	15 „	150 „	50 „
10.15 „ 10.55	40 „	400 „	50 „
10.55 „ 11.25	30 „	430 „	72 „
11.25 „ 11.55	30 „	450 „	75 „
11.55 „ 2.15	140 „	900 „	32 „
2.15 „ 3.35	80 „	300 „	19 „

Third bough experimented on.

	Length of period	Total transpiration	Average transpiration in 5 minutes
9.40 till 10.	20 minutes	50 milligrams	12.5 milligrams
10. „ 10.20	20 „	80 „	20 „
10.20 „ 10.55	35 „	180 „	26 „
10.55 „ 11.25	30 „	300 „	50 „
11.25 „ 12.	35 „	410 „	59 „
12. „ 2.15	135 „	1380 „	51 „
2.15 „ 3.35	80 „	400 „	25 „

The three boughs were cut off at the same time, about 9.20; the weighing of the first bough took place therefore about five minutes, that of the other two fifteen and twenty minutes after cutting. Whether the transpiration in the interval between cutting off and the first weighing changed noticeably, cannot be ascertained, but what is important is that the behaviour of the three boughs agrees, for they all, $^3/_4$—1 hour after the cutting (when they had lost from 1% to 4% of their weight by transpiration) show a distinct rise in the intensity of transpiration, which about two hours later (when the loss by transpiration of the boughs had risen to from 6 to 10% of their original weight) reached a maximum and then declined again.

Boughs of *Pirus malus* and *Populus nigra* which were investigated in the same way at the same time as those of *Viscum* showed no similar phenomena; the intensity of transpiration was here very great and remained almost unchanged until the boughs had dried up. In an experiment conducted some days later in precisely the same way with some evergreens (*Hedera helix, Pinus spec, Abies spec.* etc.) transpiration was at first also great, but gradually declined until a minimum was reached. Therefore these evergreens showed nothing of the irregularity which was noticed in *Viscum.*

The ready conclusion that in the experiment with *Viscum* it was not a question of one or another disturbing factor, which had been overlooked, but probably of some physiological peculiarity due to the structure of the stomata, was further confirmed by the fact that on looking through the protocols of my experiments I found again very clearly the same irregularity in an experiment conducted in Brazil with *Rhipsalis Cassytha.* The structure of the stomata of this plant agrees in several very obvious characteristics with those of *Viscum album.*

For the experiment, of which I give here the results only so far as they bear upon the phenomenon in question, a large plant was carefully loosened from the tree on which it grew, and hung up in the laboratory. The weight of this plant was recorded at intervals for a period of 136 days.

The following diminution in weight was observed:

during the	first	24 hours		1,36 %	of the original weight
,, ,,	second	,,		2,10 %	
,, ,,	third	,,		2,13 %	
,, ,,	fourth	,,		1,77 %	
,, ,,	5th, 6th and 7th	,,	on the average	0,94 %	
,, ,,	8th and 9th	,,	,, ,, ,,	0,904%	
,, ,,	10th and 11th	,,	,, ,, ,,	0,86 %	
,, ,,	12th to 16th inclusive	,,	,, ,, ,,	1,01 %	
,, ,,	17th ,, 28th	,,	,, ,, ,,	0,88 %	
,, ,,	29th ,, 37th	,,	,, ,, ,,	0,68 %	
,, ,,	38th ,, 76th	,,	,, ,, ,,	0,42 %	
,, ,,	77th ,, 104th	,,	,, ,, ,,	0,23 %	
,, ,,	105th ,, 136th	,,	,, ,, ,,	0,15 %	

We see here also how the intensity of transpiration increases when the plant investigated has lost $\pm 1^1/_2$ % of its weight and then later decreases (when the loss by transpiration has risen to $\pm$ 6 %). Simultaneously with this experiment on *Rhipsalis Cassytha* some other plants (*Pothos aurea*, *Philodendron* spec., *Aechmea* spec., *Vriesea* spec. div.) were investigated by the same method, without showing this peculiar irregularity in the transpiration.

It may clearly be assumed that in the case of *Viscum album* and *Rhipsalis Cassytha* we have to deal with the antagonism between the guard-cells of the stomata and the adjacent epidermal cells, which has been often mentioned in the literature, but nevertheless remains the subject of controversy. The epidermal cells next to the stomata are in both plants differentiated from the other epidermal cells, and are developed as so-called subsidiary cells of the stoma

As my considerations and conclusions are limited to the plants investigated, it would lead us too far afield to quote the whole literature of the mechanism of the stomata; it will be enough to characterise the current view of the antagonism between the guard-cells and the adjacent epidermal cells.

MOHL and LEITGEB have assumed, partly on the evidence of experiments with isolated pieces of epidermis, that the stomata are passively closed through the turgor of the adjacent epidermal-cells.

SCHWENDENER and his school on the other hand defended the view that the subsidiary cells are of little or no importance for the stomatal mechanism.

PFEFFER [1]) in 1897 in a survey of these views pointed out that the results of these various investigators only differed quantitatively and that in experiments with isolated pieces of epidermis the different rate of water-absorption in the various cells may have a great influence on the phenomena which are observed in the stoma.

BENECKE [2]) in 1892 published a special study of the subsidiary cells of the stomata, and came to the conclusion that the subsidiary cells have in Succulents the function of eliminating the influence on the stomata of pressure and tension, which in consequence of the crumpling of the leaves through loss of water, are set up in the epidermis.

BENECKE scarcely touches on the question of the antagonism between guard-cells and subsidiary cells: "Wir verfolgen diese Streitfrage "über die Bedeutung der Nebenzellen hier nicht weiter sondern

1) PFEFFER, Pflanzenphysiologie. Zweite Auflage 1897, Erster Band, S. 173.

2) BENECKE, Die Nebenzellen der Spaltöffnungen, Botanische Zeitung 1892.

"präcisiren nur noch unsere Stellungsnahme zu derselben. Es sei "betont dass wir eine allgemein gültige Lösung dieser Frage hier "nicht geben wollen noch können. Unserer Ansicht nach ist eben "die Fragestellung nach der Rolle der Nebenzellen in dieser Allge- "meinheit unrichtig, weil höchst wahrscheinlich diese eine von Fall "zu Fall wechselnde ist. Im Allgemeinen richtig wird eine ver- "mittelnde Stellungsnahme sein: Die Oeffnung des Spaltes wird durch "der Turgor der Schliesszellen selbst bewirkt, die angrenzenden Epi- "dermiszellen müssen in vielen Fallen zum Verschluss mit beitragen."

In 1899 WESTERMAIER [1]) published an investigation on stomata and their accessory apparatus in which he does not deal at all with the question of the antagonism between guard-cells and adjacent epidermal cells.

In 1902 COPELAND [2]) published a detailed inquiry into the mechanism of the stomata in which he takes the view that the turgor of the adjacent epidermal cells can only play a passive part in bringing about the movement of the stomata. "In stomata, whose outline changes "with their movements, and only in these, the turgescence of the "contiguous cells must be a factor in determining the state of equilibrium, "open, closed, or intermediate. But because the pore closes with excessive "transpiration when turgescence in the leaf is least, because the "contents of the guard-cells furnish a clue to changes in turgor "which is wanting in the neighbouring cells, because some stomata "do not change their outline (surface view) in their movements, "because isolated stomata usually move like those on uninjured "leaves, and because the forms and structures of the guard-cells are "explicable and intelligible on this ground only, the conclusion cannot "be escaped that the turgescence of the neighbouring cells is a "passive factor, the active one being, as SCHWENDENER and his students "have maintained, the turgescence of the guard-cells".

HABERLANDT [3]) in the last edition of his handbook hardly says anything about this question, but also clearly ascribes very little importance to the turgor of the adjacent cells. The only reference to the antagonism between the guard-cells and the epidermal cells is as follows: "Bei einigen Gräsern (Cynosurus echinatus, Aira capillita, "Briza maxima) ist die Zentralspalte auch im turgorlosen Zustande "der Schliesszellen, nach Tötung dieser, offen. In diesen Fällen mus- "sen also, sofern die Spaltöffnungen überhaupt noch funktionsfähig

[1]) WESTERMAIER, Ueber Spaltöffnungen und ihre Nebenapparate. Festschrift für SCHWENDENER. Berlin 1899.

[2]) COPELAND, The Mechanism of Stomata. Annals of Botany XVI 1902.

[3]) HABERLANDT. Physiologische Pflanzenanatomie. Dritte Auflage 1904.

"sind, die beiden seitlich gelagerten Nebenzellen durch ihren Turgor-"druck den Spaltenverschluss herbeiführen".

JOST[1]) in a very recent handbook deals with the question in the following way: "Die Öffnungsweite der Spalte hängt übrigens "nicht allein von dem Turgordruck der Schliesszellen ab sondern "auch von dem Gegendruck der Nachbarzellen; wird dieser etwa "durch Anstecken der Zellen aufgehoben, so sieht man sofort "eine starke Spaltenöffnung in den Schliesszellen eintreten, ohne "dass in *diesen* der Druck gestiegen wäre. Umgekehrt kann aber "auch eine Druckzunahme in den Nachbarzellen einen passiven "Verschluss der Spaltöffnung herbeiführen. In wie weit indess die "Einwirkung der Nachbarzellen *in der Natur* eine Rolle spielt, dar-"über gehen die Meinungen der Autoren noch weit auseinander; "SCHWENDENER[2]) schreibt den Nebenzellen gar keine, LEITGEB[3]) eine "sehr grosse Wichtigkeit zu, DARWIN[4]) vermittelt".

It seems to me that the peculiar irregularity in the regulation of evaporation in *Viscum album* and *Rhipsalis Cassytha* may be simply and naturally explained by assuming that when these plants begin to wither the pore of the stoma first widens under the influence of the antagonism of the guard-cells and subsidiary cells and is subsequently constricted.

The stomata of *Viscum album* are rather large and transverse to the long axis of the stem and leaves. In order to survey the complicated structure it is necessary to consider sections which have been cut from the apparatus in different directions and at different levels.

A section which divides the stoma into two halves in a direction perpendicular to the slit gives a view as represented in Fig. 4. The thickened ventral side of the epidermal-cells is seen, the thick ridges which surround the outer porch ("Vorhof") of the stoma; the subsidiary cells which to some extent surround the guard-cells above and below, and the very thick collenchymatous ridge which surrounds the inner cavity of the stoma. When a section of the stoma is made in the same direction, not however through the middle, but close to one of the extremities, the figure is different.

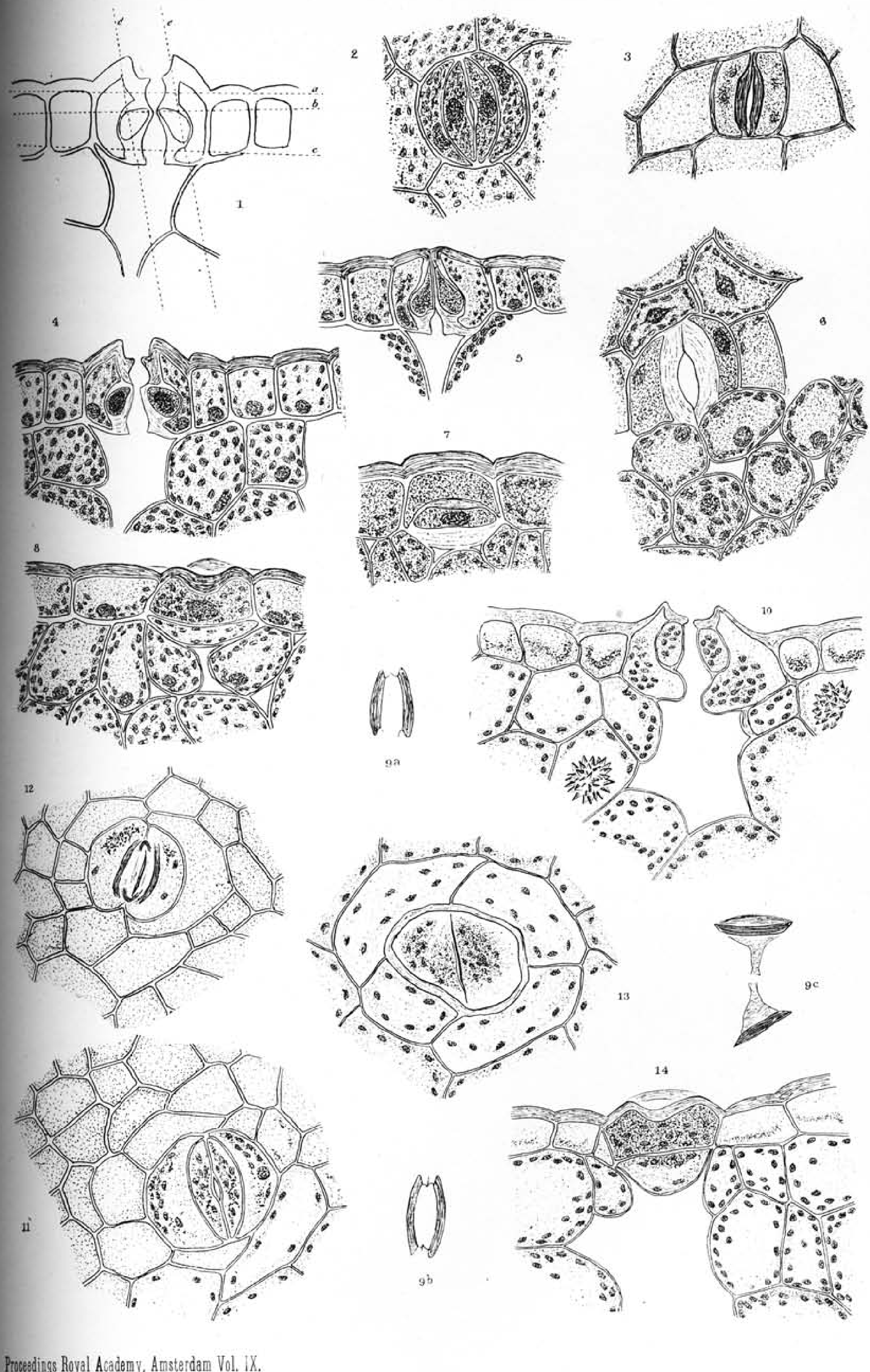
The lumen of the guard-cells is then (Fig. 5) seen to continue upwards in the shape of a wedge. The dividing-wall between the subsidiary cell and this wedge-shaped continuation of the guard-cell is thickened. In the diagrammatic Fig. 1, the lines *a*, *b*, and *c*

1) JOST. Pflanzenphysiologie. Dritte Auflage 1913. Seite 58.

2) SCHWENDENER 1881. Monatsberichte Berliner Akademie. S. 833.

3) LEITGEB 1886. Mittheilungen aus dem botanischen Institut zu Graz 1.

4) DARWIN 1898. Philos. Transactions (B) 190.


1
2
3
4
5
6
7
8
9a
9b
9c
10
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12
13
14

indicate three possible levels at which the stoma may be cut in sections parallel to the surface; the lines *d* and *e* indicate two possible ways in which in a transverse section through the leaf a razor directed more or less parallel to the slit opening of the stoma, may hit the different parts.

In sections roughly corresponding to line *a*, the slit of the "Vorhof" (Fig. 3) is seen to be bounded by two strong, very much cuticularized ridges which continue on both sides as a non-cuticularized strip united to the cell-wall of the adjacent epidermal-cells. The razor has now passed above the guard-cells without touching them; the cells seen in Fig. 3 on both sides of the opening, are the subsidiary cells. When such preparations are treated with iodine and zinc chloride, the distinction between the cuticularised ridge and the non-cuticularised strip into which the latter passes on both sides, becomes perfectly clear.

Such preparations suggest the comparison of the non-cuticularised strip to a ligament of articulation. Preparations of this nature, treated with iodine zinc chloride show that the guardcells of the stoma, wherever they border on the slit or on the respiratory cavity, are covered with a thin cuticle.

When a similar preparation, as is seen in Fig. 3, is treated with strong sulphuric acid, conditions such as those shown in Fig. 9 may result. The non-cuticularised "ligaments of articulation" are here dissolved; but the two cuticularised ridges which occur in their normal condition in *a* and *b* are still seen, but in *c* they are separated from one another and have fallen over outward. The thin cuticle which covers the inner side of the guard-cells adjoins these edges. The "ligaments of articulation" are simply the thickened strips of membrane which in Fig. 5 are shown in transverse section between the wedge-shaped continuation of the lumen of the guard cell and the subsidiary cell.

When in preparations parallel to the surface, the stoma is cut at a lower level corresponding roughly with the line *b* of Fig. 1, that is to say about in the plane of the "central slit" a view is obtained like that drawn in Fig. 2. The slit is here short and narrow, the inner wall of the guard-cell is clearly thickened where it adjoins the slit. The subsidiary-cells at this level surround the guard-cells like a crescent. A section at a lower level, roughly corresponding to line *c* of Fig 1, passes under the lumina of the guard-cells without touching them. It is then seen, as Fig. 6 represents, that the inner cavity of the stoma is surrounded by two thick ridges of cellulose. The cell-cavities which are seen on either side of these

ridges are not those of the guard-cells but of the subsidiary cel
When a transverse section through the leaf, somewhere about t line *d* of Fig. 1, cuts the stoma parallel to the long axis, a vie is obtained corresponding with Fig. 7. From above downwards t much thickened and cuticularised outer-wall, the lumen of the su sidiary cell, the wall dividing the subsidiary cell from the guai cell, the lumen of the latter and the thick ridge of cellulose he follow each other. If on the other hand the razor follows the line then as in Fig. 8, we see from above downwards the cuticularis edge which adjoins the "Vorhof", the lumen of the guard-cell, t wall separating the guard-cell from the subsidiary cell, and the lum of the subsidiary cell. In similar longitudinal sections through t stoma, the subsidiary cell can be seen either above or below t guard-cell, according to the direction in which the section is mac In Fig. 8 we may see also the peculiar shape of the guard ce somewhat like that of a dumb-bell, which can also be made o by combining the sections of Fig. 4 and Fig. 5.

If we now wish to get an understanding of the functions of tl complicated apparatus, we must assume that the outer slit formed i the ridges of the cuticle can be considerably narrowed and widene Perhaps the inner slit, bounded by the thick edges of cellulc can also be narrowed and widened, though in all probability on to a slight extent. Variations of turgor in the subsidiary cells, mu as is at once clear from the structure, exert an influence on t width of the outer slit.

The central slit (Centralspalte) is doubtless also capable of vari tion in width; it seems however that it is never wholly closed, b that a small opening always remains as represented in Fig. 2.

As long as the subsidiary cells are turgid, they offer resistance the pressure of the guard-cells; they cannot curve as strongly a open the central slit as widely as would be the case if no count pressure were exerted by the subsidiary cells.

When in consequence of transpiration. the turgor of the subsidia cells decreases before that of the guard-cells, a stronger curvature the latter and widening of the central slit must take place.

It is likely that, when the plant first withers, the turgor inde decreases earlier in the subsidiary cells than in the guard-cells, becau the guard-cells are almost completely surrounded by the subsidiai cells and the small part of the wall of the guard-cells adjoining t opening is rather strongly cuticularised. I believe that in the fi place the chlorophyll-containing parenchyma loses water by trai piration, that these cells abstract water from the epidermal-cells a from the subsidiary cells of the stomata, and that finally the subsidiary cells draw water from the guard-cells.

The characteristic peculiarity of the stomata in question seems to me to consist in: 1. That the guard-cells have only a small free surface, the wall of which is fairly strongly cuticularised and 2. that they adjoin no epidermal cells other than the subsidiary cells so that the influx and efflux of water in the guard-cells is brought about entirely through the medium of the subsidiary cells.

When indeed turgor decreases in the subsidiary cells before it does in the guard cells there must therefore, when the plant begins to wither, be a widening of the central slit of the stomata. In this first stage when turgor in the subsidiary cells is already diminished, but is still unchanged in the guard-cells, the subsidiary cells tend to constrict the outer slit, the guard-cells tend to widen it.

When, at a later stage, turgor has also decreased in the guard-cells, the subsidiary- and guard-cells cooperate to cause the outer slit to contract and then the central slit also contracts.

In my investigation of the structure of the stomata of *Rhipsalis Cassytha* I had only at my disposal material from a hot-house, since I had omitted to bring from Brazil spirit material of this plant

The stomata are here, just as in *Loranthaceae* mostly arranged transversely to the long axis [1]) of the stem.

A median longitudinal section through the stem shows that, as in *Viscum album,* the subsidiary cells surround the guard-cells above and below. Fig. 10 represents such a section perpendicular to the direction of the slit and approximately through the middle of a stoma of *Rhipsalis Cassytha.*

In a transverse section through the stem, longitudinal sections of the stoma can be obtained as in Fig. 14 in which the bulging lower part of the subsidiary cell can be seen under the dumb-bell-shaped guard-cell and above the latter, in the background, the fold in the wall of the subsidiary cell, which fold can be recognised in Fig. 10 and projects above the level of the other epidermal cells.

A section which, parallel to the surface, passes through the plane of the outer slit above the guard-cells without touching them, is given in Fig. 12. The two folds of the cell-wall are seen and between them the narrow outer slit which is bounded at either end by a small curved fold which is not always equally distinct.

[1]) This peculiar orientation is somewhat rare. Löw found it in *Casuarina,* PFITZER, inter alia, in *Colletia,* the present writer observed it also in *Cassytha filiformis.*

The two large crescent-shaped cells which might at first sight be taken for the guard-cells, are in reality the subsidiary cells. When the stoma is similarly cut parallel to the surface but at a lower level, at about the depth of the central slit, it may be represented as in Fig. 11. The section was not completely parallel to the surface of the stem; on one side (the upper one in the figure) the ordinary epidermal cells are cut, on the other side (the lower one in the figure) the section passes through sub-epidermal cells.

If the stoma is cut at a still lower level, a view may be obtained as represented in Fig. 13. Those parts of the walls of the surrounding parenchymatosis cells which border on the internal air space are (generally though not always) somewhat considerably thickened[1]) and in the channel thus formed the two subsidiary cells are seen, separated by a long and very narrow slit. Fig. 13 was drawn from a preparation viewed from the inner side.

It appears that in the stomata of *Rhipsalis Cassytha* constriction and dilatation can take place in three places: 1. At the level of the central slit between the relatively thin parts of the wall of the two guard-cells, 2. at the level of the outer slit between the thick, strongly cuticularised edges which bound this outer slit on both sides, 3. in the plane beneath the guard-cells between the thin-walled parts of the subsidiary cells which bulge out downwards.

The assumption is obvious that in *Rhipsalis Cassytha* also, at the first withering of the plant, turgor decreases earlier in the subsidiary cells than in the guard-cells and that first, as in *Viscum album*, dilatation of the central slit and increased amount of transpiration per unit of time, results from this.

The characteristic peculiarity to which the observed irregularity in transpiration is due in both plants investigated, lies, I consider, principally in the manner in which each guard-cell is surrounded by an epidermal-cell which is developed as a subsidiary cell and separates the guard-cell from the other epidermal cells. It seems to me probable that in other plants also, whenever the stomata are constructed in a similar manner, this same irrigularity in the evaporation will be found. In *Loranthus dichrous* of Brazil this same peculiarity seems to occur, less clearly than in *Viscum album* and *Rhipsalis Cassytha*, but nevertheless in a very similar manner as the following figures show.

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[1]) This point was brought out by VÖCHTING, Beiträge zur Morphologie und Anatomie der Rhipsalideën Pringsheim's Jahrbücher IX. 1873—74.

BENECKE and WESTERMAIER (l. c.) also mention this peculiarity and state their theory of its probable significance.

Transpiration of two boughs of Loranthus dichrous Mart.
Exposed to the sun.

P. M.	First bough		Second bough	
	observed weight	loss of weight	observed weight	loss of weight
1.9	29.9 grammes		25.5 grammes	
1.14	29.1 „	0.8 gramme	24.9 „	0.6 gramme
1.19	28.4 „	0.7 „	24.5 „	0.4 „
1.24	27.8 „	0.6 „	24.05 „	0.45 „
1.29	27.0 „	0.8 „	23.6 „	0.45 „
1.34	26.5 „	0.5 „	23.1 „	0.5 „
1.39	26.05 „	0.45 „	22.75 „	0.35 „
2.9	23.5 „	0.41 gr. } average per five minutes	20.7 „	0.34 gr. } average per five minutes
2.39	20.8 „	0.45 „	18.95 „	0.29 „
3.9	18.3 „	0.42 „	17.0 „	0.325 „

These figures refer to a comparative experiment made in Campos on transpiration in *Loranthus dichrous* and *Psidium guajava*; regulation of transpiration took place regularly in the last species, but in *Loranthus dichrous* it took a noticeably irregular course. We see here also that at first the amount of transpiration per unit of time decreases, later distinctly increases (when the boughs have lost ± 5 % in weight) and finally diminishes again (when the loss in weight amounts to ± 10 %). When the experiment was finished at 3.9 both boughs already began to dry.

The results of this investigation may be summarised as follows:

When cut leafy boughs or whole plants are allowed to wither and the transpiration is followed by means of periodical weighings, it is found in most plants, either that the amount of transpiration per unit of time remains approximately constant until the bough is dried up or that these amounts decrease uniformly until the transpiration is reduced to a minimum.

In *Viscum album* and *Rhipsalis Cassytha* the peculiar phenomenon is observable when the same experiments are made, namely, that when the bough (or plant) under investigation has lost a certain proportion of its weight (varying from 1 °/₀ to 4 °/₀) the amount of transpiration per unit of time increases and then later, when the loss in weight has increased (varying from 6 to 10 °/₀), the transpiration decreases again.

We may assume that this increase in the intensity of transpiration when the plant first withers is caused by the dilatation of the openings of the stomata, a dilatation which is however only of comparatively short duration and is later again followed by constriction:

The dilatation of the stomata is probably caused by the antagonism between the guard-cells and the subsidiary cells of the stomata in such a way that turgor in the subsidiary cells begins to decrease sooner than in the guard-cells, this phenomenon causing a stronger curvature of the guard-cells and dilatation of the slit of the stomata.

The subsidiary cells of the stomata, in *Viscum album* as in *Rhipsalis Cassytha,* surround the guard-cells in a peculiar manner; probably it is in this fact that the cause must be sought for the irregularity of transpiration, with which this paper is concerned.

EXPLANATION OF THE FIGURES.

All drawings, except the diagrammatic figure 1 and figure 9, relating to a preparation treated with concentrated sulphuric acid, were made from unstained preparations of spirit material, cut by hand and treated in the usual way. The drawings were made by means of a camera lucida.

Fig. 1. Diagrammatic transverse section of a stoma of Viscum album. The lines *a*, *b*, *c*, *d*, an *e* give the various directions and levels at which the stoma was cut, line *a* corresponds roughly with Fig. 3, *b* with Fig. 2, *c* with Fig. 6, *d* with Fig. 7, *e* with Fig. 8.

Fig. 2. Viscum album. From a section parallel to the surface of the leaf. The level is roughly that of line *b* in Fig. 1.

Fig. 3. Viscum album. From a section parallel to the surface of the leaf. The level is roughly that of line *a* in Fig. 1.

Fig. 4. Viscum album. From a longitudinal section through the leaf. The stoma in transverse section, the guard-cells roughly halved.

Fig. 5. Viscum album. From a longitudinal section through the leaf Stoma cut transversely, close to the extremity.

Fig. 6. Viscum album. From a section parallel to the surface of the leaf. Level about that of line *c* in Fig. 1.

Fig 7. Viscum album. From a transverse section through the leaf. The stoma is cut parallel to the slit, roughly in the direction of the line *d* in Fig. 1.

Fig 8. Visum album. From a transverse section of the leaf. The stoma is cut parallel to the slit, in the direction roughly of line *e* in Fig. 1. The preparation was in such a position that the opening of the stoma removed by the razor, would have been found on the upper edge.

Fig. 9. Visum album. From a section parallel to the surface, treated with sulphuric acid. The cuticular ridges which are seen in Fig. 8 in relation to the cell-wall net, have been loosened by the action of the sulphuric acid. The "ligaments of articulation" are dissolved in the sulphuric acid; *a* and *b* are in normal position, at *c* the ridges have fallen outwards.

Fig. 10. Rhipsalis Cassytha. From a median longitudinal section through the stem. The stoma in transverse section, the guardcells roughly halved. The section has been somewhat distorted in cutting and preparing, so that the slit, especially in the middle and below, is wider than in the intact plant.

Fig. 11. Rhipsalis Cassytha. From a section parallel to the surface. The level is that of the central slit.

Fig. 12. Rhipsalis Cassytha. From a section parallel to the surface. The level is above the guard-cells, the outer slit is seen

Fig. 13. Rhipsalis Cassytha. From a section parallel to the surface, placed in an inverted position on the slide. The level is below the guard-cells, the canal-shaped internal air space is seen, which is closed by the two subsidiary cells.

Fig. 14. Rhipsalis Cassytha. From a transverse section through the stem. The stoma in longitudinal section parallel to the slit, in a direction roughly corresponding to line *e* in Fig. 1, the preparation is orientated in a corresponding manner to the preparation of Viscum drawn in Fig. 8.

Leiden, December 1913. *Botanical Laboratory.*

Botany. — *"The explanation of an apparent exception to* MENDEL's *law of segregation."* By Miss TINE TAMMES. (Communicated by Prof. J. W. MOLL).

(Communicated in the meeting of February 28, 1914).

In experiments on hybridisation in recent years various cases have been observed in which the numerical proportion of different forms occurring in the second generation does not agree with what might be expected according to MENDEL's law. Among these there are very many with respect to which there is no reason to assume that this law does not apply, and in the greater number of these cases it has been possible to show the causes of the discrepancy. These causes have been found to be of two kinds. Firstly, there may be deviations which are only the results of mistakes or wrong hypotheses on the part of the observer. Secondly, there are cases in which the deviations are due to the plant itself. The sources of error belonging to the first class are chiefly as follows.

1. It may happen that the deviation is the result of making too few observations.