Researches on the Changes in the opening of the Stomata which occur in different species of Citrus.

by **J. D. OPPENHEIM.**

In the Summer of 1925, experiments were made in Rechoboth with the Porometer, on species of Citrus, viz. Citrus Bigaradia and Citrus Limmetta. These two species are used as stocks in the cultivation of Sweet Orange, the well-known „Jaffa-Orange", of which the local name is „Shamuti".

The Porometer used was that of F. Darwin, revised according to the model described by M. Pinkhof[1]).

The apparatus (see Fig. 1) consist of a glass chamber, which is cemented to the leaf. This chamber has a side tube, as described by Van Slogteren[2]). It is connected with a U-shaped manometer filled to a certain height with distilled water. The closed arm of the manometer is provided with a side tube of glass, to which is attached a rubber tube with a clamp. By means of suction through the rubber tube, it is possible to rarefy the enclosed air, and so to raise the water to a corresp-

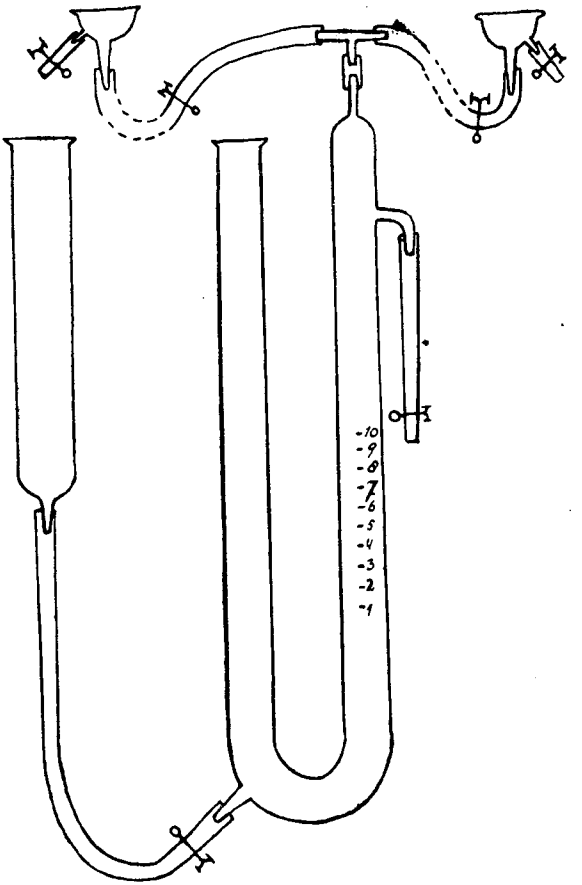
1) M. Pinkhof: A new Method of recording the modifications in aperture of Stomata. Kon. Ak. v. Wet. Amsterdam. Vol. 23 No. 8, page 1305, 1920.

2) De gasbeweging door het blad in verband met stomata en intercellulaire ruimten, E. v. Slogteren, Groningen 1917.

onding level. The open arm also has a side tube, connected to a water reservoir by a rubber tube, which can be closed by a clamp. The closed arm is further provided with a scale. The reservoir enables the water level to be fixed at Zero. After attaching the glass chamber to the underside of the leaf, it is important to make certain that air has access to the manometer through the leaf only. It is necessary to wait a quarter-of-an-hour until the gum arabic is dry, and the chamber well-scaled. Observations were made while the water level fell from 4 to 3 on the manometer scale, corresponding to from 8 to 6 mms. at pressure lower than atmospheric. The time taken was reckoned to one half-second by means of a stop-watch; readings could have been taken by the watch to one-fifth of a second, but experimental conditions precluded such accuracy. The water meniscus being concave, therefore it was impossible to note exactly, when the mark was passed, and to have given measurements to one-fifth of a second, would have resulted only in a fictitious accuracy. It is clear, that the more slowly the water falls, the more difficult it is to note the critical moment; the more rapidly the water falls, the smaller is the experimental error. Actually, however, the error very rarely amounted to more than half-a-second, so that its use as the smallest unit of time, was permissible.

At first, the observations were made on different days, as far as possible under similar conditions. Later, the observations which had to be compared, were made at the same time. This was rendered possible by placing a T-shaped tube between the glass chamber and the manometer, so that the apparatus could be provided with an additional rubber tube and a second glass chamber. The use of a clamp alternately to close the rubber tubes, enabled one observation to be made immediately after the other.

Fig. 1.



THE STOMATA.

It was, of course, important to know:

1) if the numbers of stomata found in Bigarade and Sweet Lime differ;
2) the positions of these stomata on the leaves;
3) if they are distributed on the leaves in a regular manner.

To determine these questions, leaves from several trees were examined[1]). Counts were made of the number of stomata per mm^2 in three different places on the leaf. Sections had to be taken, as it was not found possible to remove the epidermis without portions of mesophyll. No stomata having been found on the upper sides of the leaves, counts were made on the under sides only. The numbers were taken midway between

Table 1.

BIGARADE			SWEET LIME		
B	M	A	B	M	A
210	178	210	294	273	268
210	268	242	284	252	268
268	178	200	347	284	305
294	305	284	347	347	357
239	200	221	294	284	305
189	236	200	273	252	357
200	200	200	242	326	268
189	178	189	294	268	242
242	236	268	336	357	284
268	200	210	273	284	268
242	252	242	284	284	242
200	236	236	236	284	326

[1]) All the leaves were picked on the same height of the trees.

the midrib and the margin of the leaf, at the base (B), in the middle of the lamina (M), and about 1½ cm. below the apex (A). The following numbers were found. (Table I, see page 11.)

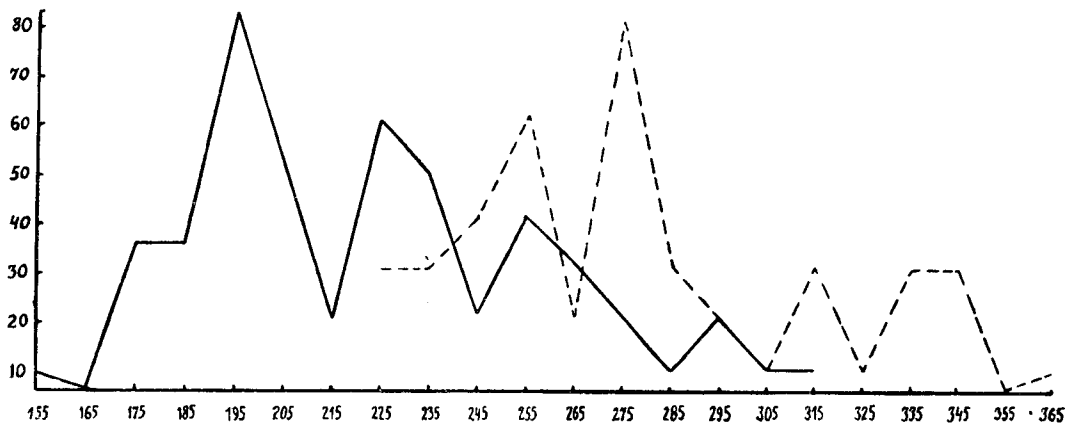
No regularity in the distribution of the stomata could be observed, and there was nothing, therefore, to prevent the attachment of the glass chamber in the middle of the leaf, avoiding the midrib. It was, moreover, easier to fasten it there, as it is the broadest part of the leaf.

The specific character of the number of the stomata in Bigarade and in Sweet Lime, is apparent from the figures in Table 1. To confirm it, further counts were necessary, and the following curve (Fig. 2) is made from the results of 894 counts. The curve shows a case of transgressive variability. The numbers were grouped in tens, i. e. every figure below the abscissa represents 10 units which belong to the group, e. g. 155 = 150 to 159, 165 = 160 to 169 etc. The frequency of each group is given on the ordinate.

Typical variation divisions can be recognised in the curves for Bigarade and Sweet Lime and the modes are clearly different from one another. The lower numbers of stomata are not found in Sweet Lime, nor the higher in Bigarade. Numbers between 225 and 315 are found in both species. In further investigations, still higher values occasionally resulted, but they are not given in the curve.

As Table 1 shows, there are great fluctuations in the number of stomata per leaf e. g. in Bigarade, 210-178-210, and in Sweet Lime, 236-284-236. It is important to know whether the fluctuations are greater per tree than per leaf. To determine this, 5 leaves from each of 20 trees were examined, and three counts were made of the stomata per leaf as previously explained. The results obtained from 3 trees are given in Table II.

Fig. II.



——— = Bigarade, - - - - - - - - = Sweet Lime.

Table II.

	BIGARADE				SWEET LIME		
	B	M	A		B	M	A
1)	168	188	200	1a)	273	268	273
	178	200	252		326	294	242
	178	221	200		252	284	268
	189	210	200		294	273	273
	221	242	221		294	305	242
2)	236	210	200	2a)	315	273	268
	221	210	273		326	284	252
	268	236	236		268	273	294
	159	189	200		326	368'	410'
	210	236	252		284	221	242
3)	273	236	236	3a)	315	389'	410'
	242	236	210		347	294	389'
	200	210	210		442'	421'	389'
	200	236	189		431'	442'	386'
	210	236	189		357	410'	378'

(Numbers marked ' are not shown in the curve. Only once, has a tree been found with such an extreme value as 3a.)

Variations in the numbers of stomata per mm².

Tree 1	between	160	and	252
,, 2	,,	157	,,	273
,, 3	,,	189	,,	273
,, 1a	,,	242	,,	326
,, 2a	,,	221	,,	410
,, 3a	,,	294	,,	442

As might be expected from the results of the examination of leaves (see table II), there are great variations in the num-

bers of stomata per tree; nevertheless it is possible to distinguish between trees of a relatively high number, and trees of a relatively low numbers of stomata.

It is difficult to give a satisfactory explanation of this phenomenon. The great fluctuations in the numbers of stomata per leaf and per tree respectively, may be proof of a strong power of modification, on the other hand, the more or less distinct differences per tree raise the suspicion that the cause is some heredity factor. This is also indicated by the curve whose many-tipped character may be explained by the division into groups.

Uphof[1]) carried out investigations of the stomatal numbers in Lucerne. Working with pure lines, he made 150 counts in each, and calculated the averages. He says: „Es hat sich nun gezeigt, dass jede reine Linie ihre eigenen Transpirationsverhältnisse hat. Das brachte mich auf den Gedanken, die Anzahl der Stomata der Ober- und Unterseite der Blätter einzelner Nummern zu studieren. Jede Nummer wies eine bestimmte mittlere Anzahl auf".

The last comment shows that probably the fluctuations are not so great, that it is impossible to calculate the averages.

Christiansen-Weniger[2]) says that the numbers of stomata per square millemetre in Triticum vulgare is essentially smaller than in Triticum dicoccum, and that the F_1 generation of the crossing between them, shows an intermediate value. He also examined the F_2 generation and reached a similar conclusion.

[1]) J.C.Th. Uphof. Zeitschr. f. Pflanzenzüchtung. Bd X. Heft 1 page 16. 1925.

[2]) F. Christiansen-Weniger; Anatomische Untersuchungen des Blattbaues der F_2-Generation einer Unterartkreuzung bei Triticum und der Versuch einer physiologischen Deutung der Befunde. Landw. Forsch. II Reihe 1925 pag. 81—125 I know only the report in Zeitschr. f. Pflanzenzüchtung. Bd XI Heft I pag. 43, 1925.

The averages are quoted in the review of his work, and there it is stated that: „bei den einzelnen Pflanzen fanden sich aber starke Unterschiede in Zahl und Grösse".

Alois Tavcar[1]), who investigated the stomata of Pisum sativum, also found that the numbers of stomata is a result of heredity, and that there is a fluctuation in their number between limits caused by factors of environment.

From all these investigations, it may by seen that considerable fluctuations may occur with every plant. Great cautions must be observed in the interpretation of the Citrus curve[2]), but it may be concluded that the so-called Bigarade and Sweet Lime are each a population in respect to the numbers of their stomata. The various species in these populations are distinguished by different stomatal numbers.

THE OBSERVATIONS.

The plants under observation were $1^1/_2$ years old. They are set out in beds in the nursery of Mr. Zeidin, Rechoboth. (The author is greatly indebted to Mr. Zeidin for his kindness in allowing the use of his material for the purpose of these investigations). All the plants tested were treated in the same way with regard to soil cultivation, manuring and irrigation. As far as possible, leaves of the same age were selected for examination. The Porometer was protected from the direct rays of the sun to avoid as far as possible changes of pressure in the manometer. The surface area of the glass chamber was $\pm$ 720 mm^2. In fixing it to the leaf, great care was taken that the gum-arabic did not spread over a larger area of leaf, as might easily occur through the pressure. When it was seen in removing the glass chamber, that the gum had extended beyond the rim, the observations involved were discarded. Since at

1) Zeitschrift für Pflanzenzüchtung. Band XI Heft 3 Mai 1926 page 241.

2) E. Baur, Vererbungslehre 1922 pag. 153.

every measurement, the same quantity of gum-arabic was used, all errors were probably of the same magnitude. A thermometer was fixed to the stand carrying the Porometer at the same height as the glass chamber. An anemometer[1]) was attached in the same way. The wind velocity and the time were noted at the beginning and at the end of each series of observations. In order to prevent the saturation of the air in the chamber with water vapour, the side tube was opened between each observation. Frequently a series had to be broken through extreme heat and the uncomfortable position of the observer; all the cocks were then opened to allow the free access of air.

In the following tables are given: the beginning and the end of each series of observations, the changes of temperature to $^1/_2{}^0$ C., the velocity of the wind (W.V.), and the time readed for the column of water to sink through the given space on the scale (P.M.T.)

Some observations were extended throughout one complete day in order to determine the most suitable time for further experiments. Such a series for Bigarade and for Sweet Lime is quoted in Tables III and IV.

On July 5-th, the observations were brought to a close earlier than on the previous day, as the cloudiness of the sky made it impossible to continue reading the scale in the evening.

Comparison of the Tables III and IV shows a lower P.M.T. for Sweet Lime. On July 4-th, the maximum temperature was 40° C. at 11.21 a. m. with an early morning temperature of 25.5° C. at 6.50 a.m. as the minimum. On July 5-th, the maximum temperature was 39.5° C. at 10 a. m., and the minimum 25° C. at 7 a. m.

As is well known from literature on the subject, stomata react strongly to light, a fact also demonstrated in these tables.

[1]) Schalenkreuz-Anemometer of R. Fuess.

Table III.

BIGARADE, JULY 4th 1925.

TIME a.m.	Temperature C.	W. V. m/sec.	P.M.T. Secs.	TIME a.m.	Temperature C.	W. V. m/sec.	P.M.T. Secs.
6.50	25.5	0	54.5				49.0
			56.5				55.0
			51.5				45.0
			64.0				52.0
7.00	26.0		64.0		34.5		54.0
			64.0				69.5
			61.5				64.5
			68.0				53.0
			65.0				57.5
			66.5				74.5
			77.5				59.0
		< 1.2	93,0		35.0		60.5
	26.5	Slightly cloudy	130.5				67.0
		Cloudy	158.0				74.5
		,,	171.0				66.5
		,,	147.0	11.01	35.5		58.0
		,,	182.5	11.15	39.5	1.2	55.5
	27.0		146.0				57.0
	27.5		126.5				53.0
	28.0	,,	182.5		39		56.0
		,,	181.5				49.0
	28.5		166.0		39.5		50.0
		,,	148.5				56.0
	29.0		172.5		40.0		48.5
			157.0				50.0
8.52	29.5		122.5				58.5
10.20	34.0	1.2	51.0		39.5		48.0
							42.0

Table III. (continued)

TIME a.m.	Temperature C.	W. V. m/sec.	P.M.T. Secs.	TIME p.m.	Temperature C.	W. V. m/sec.	P.M.T. Secs.
			50.0	5.05	29.0	> 1.9	68.0
	39.0		44.5				66.5
			45.0				60.5
			56.5		28.5		59.0
	38.5		52.5				67.5
			43.0				61.0
			47.5				63.5
11.48	38.5	> 1.2	52.0				62.5
p.m.							58.5
4.20	31.5	2.8	57.5				58.5
	31.0		59.5				55.5
	30.5		62.5				54.0
			58.0				65.5
			58.0				67.0
	30.0		54.5				74.5
			55.5				72.5
			50.5		28.0		74.5
			52.0				75.0
			51.5	5.45	28.0	1.2	82.5
			56.0		27.5	twilight	110.5
			61.0				112 5
			57.0				105.0
			57.0				120.5
			55.0		27.0	1.2	129.0
			55.0				116.5
	29.5		59.0				140.0
			60.0				157.5
			58.5				172.0
4.53	29.5	< 2.8	59.0	6.45	26.5	< 1.2	205.5

Table IV.

SWEET LIME, JULY 5th 1925.

TIME a. m.	Temperature C.	W. V. m/sec.	P.M.T. Secs.	TIME a. m.	Temperature C.	W. V. m/sec.	P.M.T. Secs.
7.00	25.0	1.2	46.5		34.5		22.0
	26.0		40.0				21.5
	27.0		39.0		35.0		22.0
	28.0		38.5				23.0
	29.0		33.5				24.0
	31.0		36.5				22.5
			35.5		35.5		21.5
			32.0				22.0
	31.5		28.5				22.0
			30.0				21.0
			31.0		36.0		22.5
			30.0				20.0
			34.0				21.0
		Clouds	36.5				21.0
		,,	39.0		35.5		22.0
		,,	35.0				21.5
			32.0				21.5
	31.0		32.5				21.0
	32.0		26.5		36.0		20.5
			28.0		36.5		23.5
	32.5		27.5				20.5
			22.0				23.5
	33.0		25.0				19.0
	33.5		22.0				21.5
			23.5				21.5
	34.0		23.0				22.0
			24.5				22.5
					37.0		25.5

Table IV. (continued)

TIME a. m.	Temperature C.	W. V. m/sec.	P.M.T. Secs.	TIME a. m.	Temperature C.	W. V. m/sec	P.M.T. Secs.
	37.5		23.5				17.0
			24.0				16.0
			23.0				16.0
			21.0		37.0		15.5
			24.0				16.5
	37.0		25.0				18.0
			24.0		37.5		17.0
			26.0				18.0
			23.5				17.0
			24.5		38.0		19.5
	37.5		23.0	10.20	38.0	1.2	17.0
			22.0	11.00	38.0	1.5	14.5
	38.0		21.5				15.5
			22.0				15.0
	37.5		23.0				14.0
			20.0		38.5		13.5
			24.5				15.0
			23.0				12.5
9.03	37.5	1.2	21.0				18.0
10.0	39.5	1.2	17.0		37.0		17.5
	38.5		16.0				17.0
			18.0				15.0
			17.0				14.5
	38.0		16.0				14.0
			15.0				15.5
			17.0				14.5
	37.5		17.0				15.0
			18.0				15.0

Table IV. (continued)

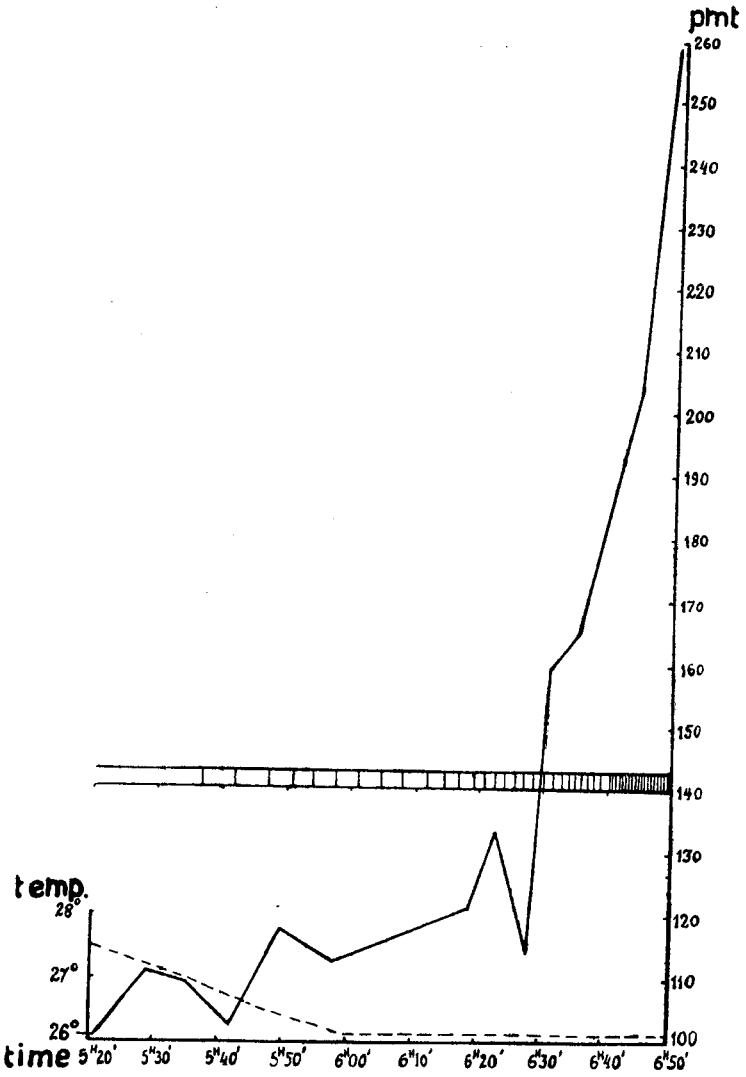
TIME a. m.	Temperature C.	W. V. m/sec.	P.M.T. Secs.	W. V. m/sec.	Temperature C.	TIME p. m.	P.M.T. Secs.
11.17	36.5	1.2	15.0				25.5
p.m.							24.5
4.20	34.0	1.9	19.0				24.0
	32.0		21.5				23.5
			20.5				25.5
	31.5		21.5				24.0
			19.0				24.0
			20.5				24.0
			21.0				25.0
			19.5		29.0		24.0
	31.0		20.5				23.5
			22.5				24.0
			18.5				25.0
			20.0				25.5
			21.0		29.0		24.5
			21.0			twilight	60.0
	30.5		20.0		28.0		55.0
			20.0		27.0		59.0
			18.0		26.5		73.5
			20.0				72.0
			20.5				87.0
4.38	30.5	1.2	20.5				89.0
5.00	31.0	> 2.4	28.0		26.0		100.0
	30.5		22.5				109.0
			24.0				103.0
	30.0		23.5				113.0
			24.0				115.0
	29.5		24.5	6.30	26.0	< 1.2	123.0

Except for the cloudiness mentioned, the intensity of the light was the same on both days. The author was not able to make measurements of the atmospheric humidity at the same time as the experiments.

The tables show that the opening of the stomata commences early in the morning and increases rapidly, reaching a maximum soon after 10 a. m. After 4.20 p. m., the maximum is passed. With sunset, the closing of the stomata, becomes progressively more apparent, until after twilight has set in, it proceeds somewhat suddenly.

For further proof of the influence of light on the apertures of the stomata, a series of observations was made on August 10-th, and is given in the table below. The experiment was carried out on Bigarade, and the relative intensity of the light was measured at the same time according to the following method. In a photographic printing frame 9×12 c. m. fastened a piece of cardboard, out of which 12 squares of 1 cm^2 are cut; they are pasted on squares slightly larger, so that they can easily be refitted into the twelve holes formed by their removal. A sheet of photographic printing paper is inserted in the frame beneath the cardboard, and, removing one cardboard square at a time, the apparatus is exposed to the light for the same length of time on separate occasions. In this way, a comparative measurement of the intensity of light can be obtained, for the same kind of printing paper is used the whole time (Leonar Gaslicht). By employing the results of this measurement of the intensity of the light, the curve (Fig. 3). could be made. Beneath the abscissa are written the times of observation, and on the ordinates the temperature and the P. M. T. Between two horizontal lines, the intensity of the light is marked by vertical strokes, in such a way that the closer they are to one another, the weaker is the intensity of the light.

Fig. III.



(for explanations see text page 22).

Table V. (fig. III)

TIME p. m.	TEMPERATURE C.	W. V. m/sec.	P. M. T. Seconds
5.20	27.5	1.2	101
5.25	27		110.5
5.35	26.5	1.2	109.5
5.41			102.5
5.49	26		118
5.57			112.5
6.19			121.5
6.23		1.2	134
6.28			114
6.31			159
6.35			165
6.45			203.5
6.49	26	1.2	259.5

The strongest closure of the stomata began after 5.57 p. m. i. e. at a time when the temperature ceased to vary more than $^1/_2{}^0$ C and, as may by seen from the curve, when the intensity of the light greatly diminishes.

From these and from similar measurements, it is clear that the best time for observation, is about 10 a. m. when great differences in the intensity of light are no longer found. The velocity of the daily wind, which invariably sets in at 9 a. m. becomes so high and so variable about 12 noon, that it was difficult to compare the series of observations. Before midday, the wind is less strong, and, what is more important, more steady.

Table VI.

BIGARADE, JUNE 21-th 1925.			SWEET LIME, JUNE 21-th 1925.		
Time a. m.	Temperature C.	P. M. T. Secs.	Time a. m.	Temperature C.	P. M. T. Secs.
10.5	36	42	11.25	39	38
		45			31.5
	36.5	40			37
		38		38.5	34.5
	37	40			35
	37.5	37.5			35
	38	38			29
		34.5		38	31
	38.5	38			28
	39	37			27
	39.5	35.5			27
		36.5			27.5
		37.5		37.5	28.5
		34			25
		32			30.5
		36			25
		34.5		37	26
		37			22
		36.5			25.5
	40				26
		38			27
		40			23.5
		33.5			27
	39.5	38		6.5 3	23
		33			27
		45			28

Table VI. (continued)

BIGARADE, JUNE 21-th 1925			SWEET LIME, JUNE 21-th 1925.		
Time a. m.	Temperature C.	P. M. T. Secs.	Time a. m.	Temperature C.	P. M. T. Secs.
		38.5			27
		34		36	28.5
		33			24
		32.5			22
		34			21
		37			28
		32			24
		34			22.5
		38.5			25.5
		34			27.5
		36.5	12.10	36	25
		31.5			
		32			
10.48	39.5	32			

COMPARATIVE OBSERVATIONS WITH BIGARADE AND SWEET LIME.

The plants were raised from seed sown in January 1924, and all received the same treatment. Leaves about one season old, and consequently mature and still in a period of full activity, were used.

At the time of these observations, which were made one immediately following the other, no anemometer was used. It may be seen from the figures given, that the temperature fell after 11.25 a. m. During the summer this is a daily phenomenon caused by a sea-wind which increases in velocity

Table VII.

SWEET LIME, JUNE 29-th 1925.			BIGARADE, JUNE 29-th 1925.		
Time a. m.	Temperature C.	P. M. T. Secs.	Time a. m.	Temperature C.	P. M. T. Secs.
10.26	34	59.5	11.45	35	145
		51.5		35.5	119
		66.5		36	118
		59.5			101
		56			104
		62			146
		58.5			104
		58.5			90.5
		52.5		35.5	74.5
		53.5			92.5
		50.5			88.5
		49.5		35	89
		46.5			98.5
		49			88
		45.5			81
		47		35.5	83.5
		54.5			82
10.26		47			61.5
		47.5			85.5
		50.5			83.5
		46.5		36	71
	34.5	47			88.5
		41.5			77
11.25	34.5	43.5			77
		41		36	80

Table VIII.

BIGARADE.				SWEET LIME.
Time a. m.	P. M. T. Secs.	W. V. m/sec.	Temperature C.	P. M. T. Secs.
10.45	34	2.8	38	35
	33			31
	34			27
	32			22
	31.5		37.5	28
	31.5			28
	30			27
	35			24
	29			23
10.45	29.5	2.8	37	26
	32.5			23
	29			24
	30			21
	28		36.5	21
	34			22
	27			22
	28			29
	27			24
	30		36	20
	28		36.5	25
	27			22
	31		37	21
	29		37.5	20
	28			23
	33	2.4	38	19

during the course of the day. Although the plants were in a sheltered place, it was doubtful whether the series could be used and the question arose, what was the influence of the alterations in wind? To determine this, therefore, further series of observations on Bigarade and Sweet Lime were made, but reversed in order of time.

A comparison of the two Tables (VI and VII) shows that, the lower P. M. T. of Sweet Lime is not caused by the strength of the wind. The figures for Sweet Lime are specifically much lower than those for Bigarade. The high initial values in the series are striking. The question may be raised whether they are to be explained by the shock resulting from the attachment of the glass chamber to the leaf. Knight[1]) observed that the stomata, which close after such a shock return to their previous state after some hours. If such a shock-reaction exists here, its influence is of much shorter duration.

The results of later observations made on one and the same occasion with Bigarade and Sweet Lime, taking alternate readings, are clearer than those previously quoted. The following series will serve as an example:

Table IX.

		Number of stomata per mm²
Table VI	Bigarade	306
	Sweet Lime	350
Table VII	Bigarade	245
	Sweet Lime	300
Table VIII	Bigarade	288
	Sweet Lime	235

1) A. Burgerstein: Transpiration 1920. Bd. 2 Page 21.

In all the tables given so far, the P. M. T. of Sweet Lime is lower than that of Bigarade. This may be the result of higher stomatal numbers in Sweet Lime, or because the stomatal aperture is larger than in Bigarade. The following table shows some comparative stomatal numbers, which should be compared together with the rest of the data given in the tables mentioned.

Unlike tables VI and VII in which the numbers are roughly proportional, the figures for table VIII do not fit in with this explanation.

Measurements of the stomata could not be made at first. Later, Dr. Reichert, Tel Aviv, lent the writer his micrometer for a short time. It was only possible to determine the length of the stomata, since the width could not be measured in situ, and fixation was very difficult. As already mentioned, the epidermis could not be removed without mesophyll, and sections take too long, so that it was impossible to fix the right stage with certainty, even by the method of E. Shreve[1]).

The P. M. T. of Bigarade was not always however higher than that of Sweet Lime. Table X shows this. (See page 30).

Out of 351 series of observations, 14.3% gave a lower P. M. T. in Bigarade than in Sweet Lime.

As can be seen from the curve drawn from the stomatal numbers, no number greater than the maximum per unit of surface in Sweet Lime, has ever been found in Bigarade. Those numbers which are found in both Bigarade and Sweet Lime (225—315) are already rare in Bigarade, when, according to the curve, they are most frequent in Sweet Lime.

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1) Cited by A. Burgerstein: Transpiration 1920. Bd. 2 Page 19.

Table X.

BIGARADE.			SWEET LIME.
P. M. T.	Time	Temperature	P. M. T.
35	3.15	31	44.5
29.5			55.5
33		31.5	41.5
32.5			31.5
28			30.5
29			40.5
29.5		31	33.5
31.5			30
28			29.5
28.5			31
29			32
29.5			30.5
26			32.5
25			30.5
26.5			29
28.5			27.5
25			30
25	3.40	31	30

In the following table are given the results of observations made at the same time both with Sweet Lime and with Bigarade; the stomatal numbers are given. The + sign indicates a higher P. M. T. than the — sign.

A number of plants which belong to extreme types in respect to the number of their stomata per mm² are included in this table. Table XII (page 32) gives details of the measurements made on No 1 Table XI.

Table XI.

		Stomata per mm²	P. M. T.
1.	Bigarade	177	+
	Sweet Lime	301	—
2.	Bigarade	157	+
	Sweet Lime	319	—
3.	Bigarade	193	+
	Sweet Lime	339	—

Although the P. M. T. of the Bigarade is higher than that of the Sweet Lime, no connection could be found between these figures and the number of the stomata. Eleven such series for extreme types of Bigarade and of Sweet Lime were compared without revealing any connection.

Unfortunately, it has been impossible so far, to measure the width of the aperture of the stomata, which is very important. No definite conclusions can be reached on the relation between the stomatal numbers and the P. M. T. from all the foregoing figures. They do prove, however, that the P. M. T. does not depend, on the n u m b e r s only of the stomata [1]).

A study of the series reveals continual fluctuations. How do they arise? The stronger movements morning and evening can clearly be recognised as corresponding to the movements of the stomata. The smaller changes probably depend on changes in the apertures of the stomata, the latter in their turn being the result of changes in the osmotic pressure in the guard cells or in their neighbouring cells. Such osmotic changes must be very rapid; it is not likely that they are the result of

1) Christiansen — Weniger S. note 2) on page 14.

Table XII.

BIGARADE. JULY 29th 1925. SWEET LIME.							
Time	Temperature	W. V.	P.M.T.	Time	Temperature	W. V.	P.M.T.
10.15	36.5	1.5	37.5	11.15	39	1.9	31.5
			36				33.5
			44				31.5
			35		38.5		30.5
			33.5				32
			36				30.5
			36.5		38		30
			35				33.5
			36				37
			37				30
			34.5				34
	37		35				30
			36.5		38.5		29 5
			37				37.5
			33.5				33
			31				35
	37.5		32.5				34
			33.5				36.5
			35.5				37.5
			30.5				32.5
			34.5				33.5
			34.5				34
			37				34.5
10.57	38	1.9	35	12.08	39	1.9	31

alterations in the starch content, which are too slow to effect them [1]).

No satisfactory explanation can be given as yet. Further investigations are in progress.

CONCLUSIONS.

1. With regard of their stomatal numbers, Bigarade and Sweet Lime are populations of species, in which the numbers of the stomata vary greatly, and are to some extent inherited.
2. In general, the P. M. T. of Bigarade is greater than that of Sweet Lime.
3. The numbers of the stomata do not provide a satisfactory explanation of the differences in the P. M. T.

THE P. M. T. OF GRAFTED SHAMUTI.

220 series of measurements of Shamuti grafted on Bigarade, and 222 series of Shamuti on Sweet Lime were made.

Most of the grafts were $1^1/_2$—2 years old. A few experiments were made with 11—years old trees. 33% of the observations showed a higher P.M.T. in Shamuti grafted on Bigarade, than in Shamuti grafted on Sweet Lime.

Occasionally the differences in P. M. T. were very great, but small ones were also found. No clear connection with the stomatal numbers could be established.

Instances of Shamuti grafted on Bigarade are quoted in the following table. In the first part, one series of observations is given in which the P. M. T. of Shamuti on Bigarade is only slightly different from that of Shamuti on Sweet Lime; the second part of the table gives measurements showing a great difference in the P. M. T.'s.

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[1]) Benecke-Jost: Pflanzenphys. 1924 Band 1. S. 76.

Table XIII.

A.

SHAMUTI ON BIGARADE. SHAMUTI ON SWEET LIME. AUGUST 2nd 1925.				
P. M. T. Secs.	Time	Temperature C.	W. V. m/sec.	P. M. T. Secs.
47.5	11.2	35.5	1.5	43
48				47
48.5				45.5
49				49
47				45
44				49.5
45.5				47.5
42.5				46.5
41		36	1.5	44
40				42
48.5				42.5
40.5				45
36.5				41.5
41				40.5
39.5				39
40			1.9	42
41				40
40.5				—
37.5				38
43				42
40				38.5
45				40
40				38.5
40.5				37.5
40		36		39

Number of Stomata per mm² 273. Number of Stomata per mm² 261.

Table XIII. (continued).

B.

SHAMUTI ON BIGARADE. AUGUST 13th 1925.			SHAMUTI ON SWEET LIME.	
P. M. T.	Time	Temperature	W. V.	P. M. T.
202.5	11.00	32	1.9	75
142				66.5
133				66.5
124.5		31.5		66.5
123.5				61
115.5		32		55.5
121.5				52
116				64.5
118				65
110.5		32	1.9	65
119		32.5		61
104.5				56
113				51.5
90				51
102.5				48.5
99.5				51
107		33		50
90				52.5
106				49.5
107				52.5
102				47
107.5				53
115.5				47.5
114				43
109		33	1.9	50.5

Number of Stomata per mm² 287. Number of Stomata per mm² 235.

The totals of the P. M. T. are as follows:

Table XIII A.

Shamuti on Bigarade	1066.5	(stomata 273)
Shamuti on Sweet Lime	1059.5	(" 261)

Table XIII B.

Shamuti on Bigarade	2893.5	(stomata 287)
Shamuti on Sweet Lime	1391.0	(" 235)

The number of stomata per mm² varies between 252 and 215 in Shamuti. On two occasions, measurements of the P.M.T. of two trees were made, with the same leaf each time. The ratios in the meanwhile had been reversed. Both trees were 11 years old.

Table XIV.

SHAMUTI ON BIGARADE. September 30th 1925		SHAMUTI ON BIGARADE. October 25th 1925
Time	9.45 — 10.23 a. m.	9.40 — 10.16 a. m.
Temperature	29°C — 30.5°C	26-28 — 27.5°C
W. V.	1.2 M/sec.	1.2 M sec.
P. M. T.	591.5 secs. (total)	1111 secs. (total)
Stomata	271 per mm²	ditto.
SHAMUTI ON SWEET LIME. September 30th 1925		**SHAMUTI ON SWEET LIME. October 25th 1925**
Time	9.45 — 10.23 a. m.	9.40 10.16 a. m.
Temperature	29° — 30.5°C	26-28° 27.5°C
W. V.	1.2 M/sec.	1.2 M/sec.
P. M. T.	900 secs. (total)	1007 secs. (total)
Stomata	315 per mm²	ditto

Fig. IV.

1 and 3 Shamuti on Bigarade
2 Shamuti on Sweet Lime.

Further, measurements of the P. M. T. of 20 leaves belonging to one stock were made twice on two separate occasions. As a result distinct differences were found, which, however, might be explained with greater probability by conditions of environment. On one occassion only was a reversal found such as this indicates.

It is certain that the extent of the opening of the stomata is an important factor in controlling transpiration. From the results previously given, it may be concluded that specific differences probably exist between the transpiration of Bigarade and of Sweet Lime. It may even be said that the degree of transpiration per leaf in general, is lower in Bigarade than in Sweet Lime, excepting 14.3% instances which showed a smaller P.M.T. Of the observations made, 33% showed that in Shamuti grafted on Bigarade, the P.M.T. was lower than in Shamuti on Sweet Lime. No correlation can, therefore, be established between the characteristics of graft and stock i. e. it is unlikely that the transpiration of the graft is influenced by the stock. The phenomenon observed in the field that trees grafted on Bigarade constantly suffer more quickly from drought, can with difficulty be explained by the influence of the transpiration of the stock. It is more readily explained by the differences in the growth of trees grafted on Bigarade and those on Sweet Lime.

The growth of the stock itself varies. Sweet Lime develops more rapidly during the first years than Bigarade.

On the other hand, it is observed in those districts of Palestine where oranges are cultivated, that with normal development, trees grafted on Bigarade are much stronger than those grafted on Sweet Lime. Illustration 4. shows this.

(The photographs were taken in Mr. Miller's plantation, Rehoboth). While the trees are planted in a sandy loam ($4^1/_2 \times 4^1/_2$ metres apart, each way) the less-advanced state of the Sha-

muti grafted on Sweet Lime is not the result of soil conditions. (It may be remarked here, that, as a rule, Bigarade is considered the more suitable stock for loamy soils).

Table XV.

SWEET LIME: Sown in January 1925, measured on October 28th 1925.	
Height of stem (cm)	106 106 124 120 91 109 98 127 98 115 120 118 131
Circumference (mm)	45 30 34 32 31 30 24 27 34 31 22 24 28
Height of stem (cm)	104 115 110 123 110 116 105
Circumference (mm)	28 29 22 23 27 27 26
BIGARADE: Sowing and date of measurement as above.	
Height of stem (c.m.)	83 82 92 97 100 90 93 85 105 85
Circumference (m.m.)	32 30 38 31 29 32 33 32 33 36
Height of stem (c.m.)	98 89 93 87 97 93 95 95 88 90
Circumference (m.m.)	21 22 20 25 26 26 26 21 21 21

The total number of leaves in Shamuti on Bigarade is greater than in Shamuti on Sweet Lime. The total amount of transpiration may, therefore, be greater in the former. Moreover, this may well explain the fact that trees propagated on Bigarade stocks suffer more readily from drought.

In conclusion, the author wishes to thank Dr. Walter Eichenberg for the friendly assistance given him in his work. Prof. Dr. O. Warburg, he wishes particularly to thank for the constant interest he has taken in this work.

SUPPLEMENTARY NOTE.

The numbers of the stomata of the lower inserted leaves are smaller than the numbers of the higher inserted leaves, as well in Bigarade as in Sweet Lime.

In the following table are classified the numbers of the stomata of young trees of Sweet Lime, 2 years old, per mm^2, on 100 cm., 50 cm., and 10 cm. above the basis of the root

SWEET LIME.		
100 c. m.	50 c. m.	10 c. m.
347	366	284
368	294	263
347	380	252
390	347	273
399	294	273
389	305	263
315	273	231
389	294	241
391	300	248
368	298	273

Rehoboth, March 1926.

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