Botany. — *Concerning the influence of temperature treatment on the carbohydrate metabolism, the respiration and the morphological development of the tulip.* II. By L. ALGERA. (Communicated by Prof. J. C. SCHOUTE.)

(Communicated at the meeting of September 26, 1936).

LE NÔTRE. SEASON 1934—1935.

The analyses of Le Nôtre do not embrace the whole season but begin a week after the lifting on 26th July and end about the middle of December. Besides the carbohydrates already discussed in the preceding publication (1), the quantity of sucrose was determined by means of invertase according to COOKE's method (2). On the ground of the investigations of PINKHOF (3) the remainder of the non-reducing sugars is provisionally referred to as inulin.

Table I gives a survey of the applied temperatures. From 21st July till

TABLE I.
Le Nôtre. Season 1934—1935. Survey of the temperature treatment applied.

Lot	Lifting-date	Treatment				Planting-date
		20/7—17/8	17/8—24/9	24/9—9/10	9/10-end of the determinations	
LN 20—20—op	20—7—'34	20	20	20	in the open	9—10—'34
LN 20—20—13	„	20	20	20	13	„
LN 20—17—op	„	20	17	17	in the open	„
LN 20—13—op	„	20	13	13	„	„
LN 20—13	„	20	13	13	13	unplanted
LN 20—13—13	„	20	13	13	13	9—10—'34
LN 20—9—op	„	20	9	9	in the open	„
LN 20—5/4—op	„	20	5	4	„	„
LN 20—5/7	„	20	5	7	—	—

24th July the bulbs were in a bulbhouse at about 20° C., thereafter their temperature was kept constantly at 20° C. in the laboratory.

All the lots underwent the same pre-treatment viz. 20° C. They only differed in the after-treatment. Most of the lots were planted in the open, two of them at 13° C. On 24[th] September a part of the bulbs lying in a temperature of 5° C. had to be transferred to 4° C. (LN 20-5/4-op) and another part to 7° C. (LN 20-5/7) owing to want of space.

As a rule bulb and plant were examined together. With LN 20-20-13 and LN 20-13-13 however, only the bulb. As the plant in proportion to the bulb still has a small dry weight, the per cent composition is little affected by the removal of the plant.

Reducing sugars.

As with Murillo, the percentage of reducing sugars in Le Nôtre is very small. (Table II, fig. 1).

With the exception of LN 20-5/4-op and LN 20-5/7 this is equal in all lots up to planting on 9[th] October and gradually drops in that period. In all probability the percentage of LN 20-20-op on 14 August is due to an error. The difference is difficult to explain from the fact that the other lots were lying at 17, 13, 9 or 5° C. for one day, for then these groups would also differ amongst each other. After planting the decrease in most lots comes to a standstill, or passes into a slight rise. The unplanted LN 20-13 bulbs continued falling.

During summer the percentage of reducing sugars in Le Nôtre in the temperature-range of 20—9° C. is thus also independent of the temperature applied. At 5° C. the bulbs, however, continually contain a little more of these sugars. This difference still exists when the bulbs have already been planted in the open a month.

In comparing LN 20-20-op, LN 20-13-op, LN 20-20-13 and LN 20-13-13 with each other, it firstly appears that LN 20-20-13, in the first month after being planted like LN 20-20-op had decreased somewhat and only began to increase after 15th November.

Secondly LN 20-13-13 increased more rapidly than LN 20-20-13 and LN 20-13-op.

Whereas with an after-treatment of 20° C. the temperature during the first period after being planted had no influence on the concentration of reducing sugars, the bulbs cooled at 13° C. are able to increase their concentration at 13° C. immediately after being planted. So it is also evident here, that cooling shifts to an earlier date the period, when the bulbs can increase their percentage of reducing sugars at a higher temperature.

Non-reducing sugars.

From 26th July till 14th August the quantity of non-reducing sugars remained constant at 20° C. (Table II, fig. 1). After the division of the parcel the concentration increased more rapidly according to the tempe-

TABLE II.

Le Nôtre 1934—1935. Survey of the carbohydrate metabolism in per cents of the dry weight.

	Joint	LN 20—20—op				LN 20—20—13				LN 20—17—op				LN 20—13—op				LN 20—13	LN 20—13—13				LN 20—9—op				LN 20—5/4—op				LN 20—5/7
	26/7	14/8	14/9	16/10	5/11	1/11	15/11	29/11	12/12	18/8	22/9	13/10	6/11	17/8	21/9	12/10	7/11	20/11	1/11	13/11	28/11	11/12	16/8	20/9	11/10	9/11	15/8	18/9	9/10	9/11	10/10
$\frac{\text{Dry}}{\text{Fresh}}$ Weight	0.388	0.394	0.401	0.436	0.422	0.406	0.376	0.381	0.343	0.407	0.421	0.415	0.385	0.409	0.441	0.407	0.378	0.441	0.400	0.376	0.358	0.359	0.406	0.408	0.424	0.366	0.414	0.391	0.393	0.377	0.395
Red. sugars	1.01	0.30	0.51	0.59	0.48	0.60	0.35	0.48	0.88	0.84	0.63	0.45	0.54	0.91	0.68	0.42	0.52	0.27	0.71	0.90	0.68	1.06	0.87	0.58	0.42	0.42	0.87	1.15	0.80	0.73	0.86
Non—red. sugars .	6.4	6.4	7.9	7.7	17.7	10.7	13.5	11.7	17.2	7.5	7.8	8.9	18.0	6.2	13.0	14.0	20.2	11.8	14.2	14.9	11.3	16.2	6.4	13.7	12.8	18.7	6.5	19.3	21.3	20.1	15.7
Sucrose.	3.0×	3.2×	4.5×	5.3	14.1	7.0	10.2	9.8	11.7	1.7	5.4	7.1×	12.6	3.7	11.2	13.3	18.6	10.1	9.9	10.2	10.5	9.9	3.4	12.8	12.9	17.6	5.7	17.8	21.4	12.3	15.8
Inulin	3.4+	3.2×	3.4×	2.4	3.6	3.7	3.2	2.0	5.5	5.8	2.4	1.8×	5.3	2.6	1.8	0.7	1.6	1.7	4.4	4.7	0.7	6.3	3.0	0.9	0.0	1.1	0.8	1.5	0.0	7.8	0.0
Starch	82	81	80	85	71	67	63	63	61	57	83	82	67	86	77	81	69	45	65	64	65	59	80	78	73	66	82	71	65	72	66

TABLE III.

Le Nôtre 1934—1935.

	Joint	LN 20—20—op				LN 20—20—13				LN 20—17—op				LN 20—13—op				LN 20—13	LN 20—13—13				LN 20—9—op				LN 20—5/4—op				LN 20—5/7
	26/7	14/8	14/9	16/10	5/11	1/11	15/11	29/11	12/12	18/8	22/9	13/10	6 11	17/8	21/9	12/10	7/11	20/11	1/11	13/11	28/11	11/12	16/8	20/9	11/10	9/11	15/8	18/9	9/10	9/11	10/10
Carbon dioxide liberation per K.G. dry weight/hour. (cc)	46.2	27.9	32.6	37.7	33.7	45.2	57.4	47.8	58.2	23.3	34.1	52.6	37.3	13.2	28.1	44.0	34.7	35.9	51.2	80.9	52.7	63.4	7.8	17.0	21.2	37.4	5.7	13.3	14.7	29.0	14.4
Carbon dioxide liberation calculated on the basis of 2o° C. (cc)	46.2	27.9	32.6	37.7	86.4	77.9	99.0	82.5	100.3	31.0	45.5	70.2	95.6	22.8	48.5	75.9	89.1	61.9	88.3	139.5	90.8	109.4	20.1	43.7	54.2	95.9	22.9	53.2	58.8	74.4	57.6
Idem, divided by non-reducing sugars. (cc)	0.73	0.44	0.42	0.49	0.49	0.73	0.74	0.70	0.58		0.58	0.79	0.53		0.37	0.54	0.44	0.52	0.62	0.94	0.81	0.68		0.32	0.42	0.51		0.28	0.28	0.37	0.37
Oxygen consumption per K.G. dry weight/hour. (cc)	39.0	26.0	31.9	33.8	33.3	46.7	64.2	57.6	65.8	26.9	36.1	45.2	37.2	16.1	26.3	30.7	33.3	39.1	49.9	75.0	61.6	75.1	12.8	19.0	21.4	36 6	14.0	13.9	14.6	32.8	13.8
Oxygen consumption calculated on the basis of 20° C. (cc)	39.0	26.0	31.9	33.8	92.4	76.6	105.3	94 3	107.9	32.8	44.1	55.1	103.3	26.4	43.1	50.4	92.4	64.1	81.8	123.0	100.9	123.1	35.6	52.6	59.5	101.7	48.3	48.0	50.5	91.1	47.4
Idem, divided by non-reducing sugars (cc).	0.62	0.41	0.41	0.44	0.52	0.72	0.78	0.81	0.63		0.56	0.62	0.58		0.33	0.36	0.46	0.54	0.57	0.83	0.90	0.76		0.39	0.47	0.55		0 25	0.24	0.45	0.30
Since 26 July respired per K.G. dry weight. (gr).		22.6	52.8	89.0	108.6	104 8	129.6	153.2	174.7	22.6	54.9	84.1	110.0	22.6	45.9	70.2	99.7	120.3	102.4	136.4	166.4	190.7	22.6	36.6	49.5	78.6	22.6	33.7	42.4	68.2	45.0
Sugar increase since 26 July + respired food per K.G. dry weight (gr).	0.0	15.5	62.8	98.2	216.9	143 7	193.9	201.4	281.8	32.6	65.7	103.8	221.2	20.3	108.8	140.8	233.3	167.3	178.2	220.4	212.1	289.6	21.5	105.5	108.0	195.6	22.5	164.6	190.3	202.9	137.3

rature being lower. With Le Nôtre, too, the starch-decomposition was thus promoted by cooling.

In spite of the differences at planting, all the lots, which were planted

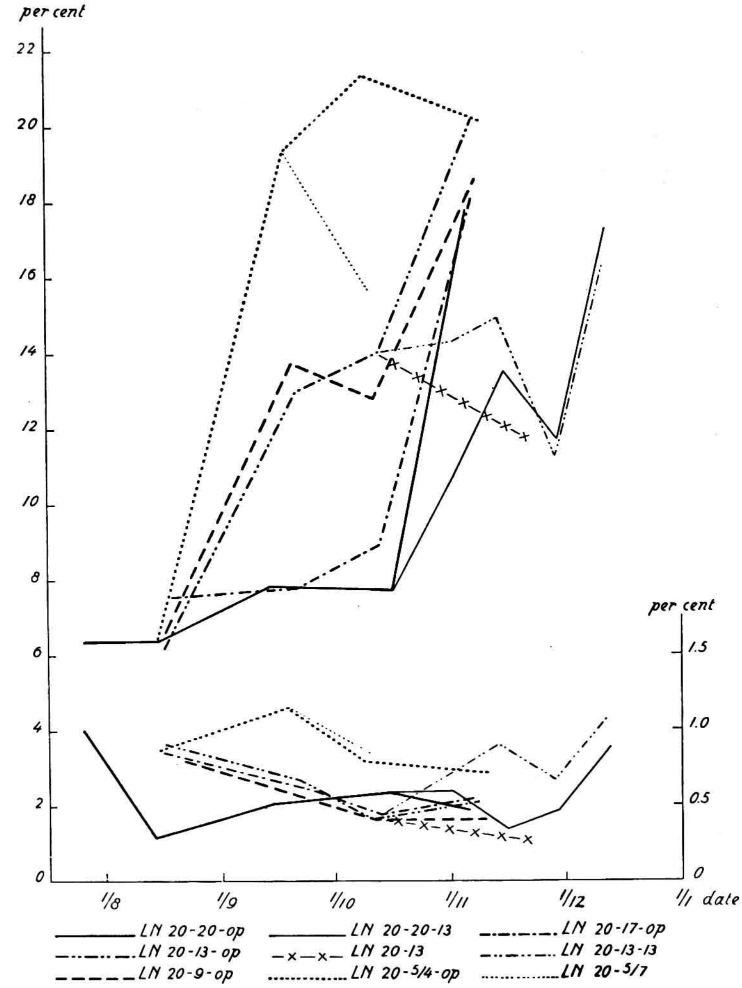

Fig. 1. Le Nôtre. Season 1934—1935. Reducing sugars (below; ordinate to the right) and non-reducing sugars (above; ordinate to the left) in per cents of the dry weight.

in the open, early in November contained about the same quantity of non-reducing sugars. The bulbs planted at 13° C. also have an equal concentration, but lower than that of the bulbs planted in the open.

These facts as well as the decrease of LN 20-5/7 in respect of LN 20-5/4-op give the impression that the temperature moves the equilibrium of starch ⇄ non-reducing sugars to such a degree that at a lower temperature this equilibrium is at a higher sugar-concentration. After a change of temperature the bulbs tend rapidly to reach a new equilibrium state.

As to the unplanted LN 20-13 bulbs the sugar-percentage of these decreases somewhat from 12th October till 20th November.

Sucrose.

Through a number of sucrose-determinations having failed, the results are not quite complete. By means of data of Le Nôtre of the season of 1935—1936, which, as far as having already been determined, corresponded to 1934—1935 the sucrose-percentages lacking were calculated. The numbers obtained in this way are indicated by a cross in Table II. As long as the bulbs were lying at 20° C., the quantity of sucrose increased slowly (fig. 2). Through cooling the percentage increases, more rapidly in proportion to the temperature being lower. The lots planted in the open again have the tendency of levelling the differences, although this is not so clear as with the non-reducing sugars. The lots which, after planting, were in a temperature of 13° C. were again equal amongst each other and contained less sucrose than in the outside temperature.

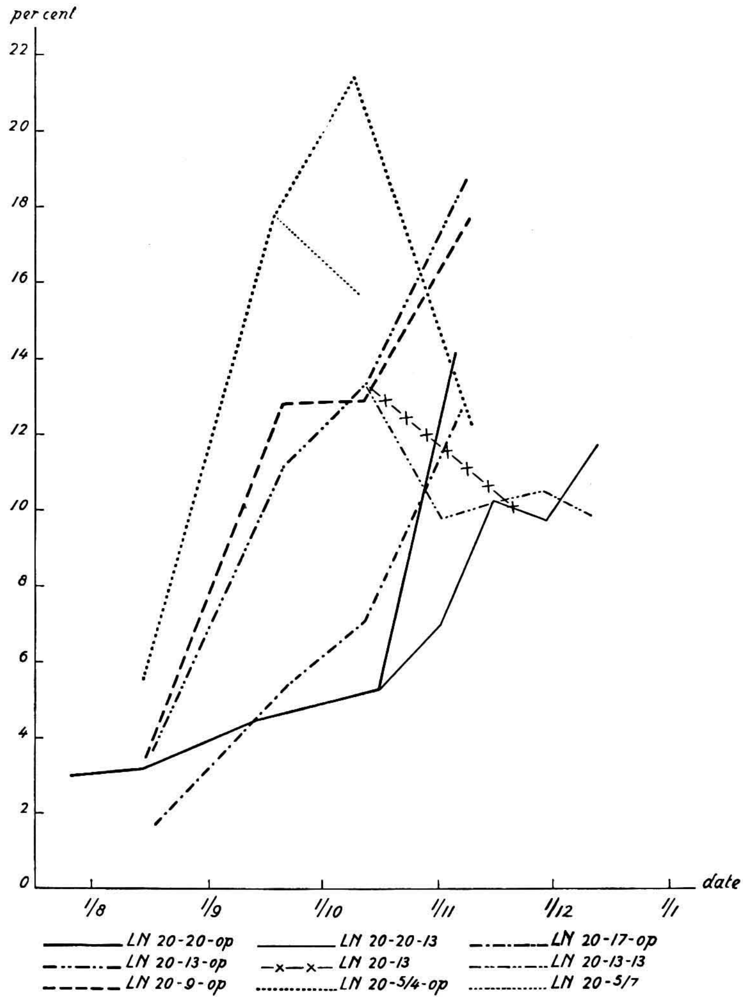

Fig. 2. Le Nôtre. Season 1934—1935. Sucrose in per cents of the dry weight.

It appears from the numbers that the non-reducing sugars chiefly consist of sucrose, so that the entire aspect of these two is about the same. It will be observed that the ratio $\frac{\text{sucrose}}{\text{non-reducing sugars}}$ becomes greater at a lower temperature.

Inulin.

The difference between the non-reducing sugars and the sucrose is considered to be inulin. The quantity of this polysaccharide is evident from Table II and fig. 3.

At 20° C. the quantity only changes a little. Up to planting there is a slight decrease. This decrease is more evident at a lower temperature.

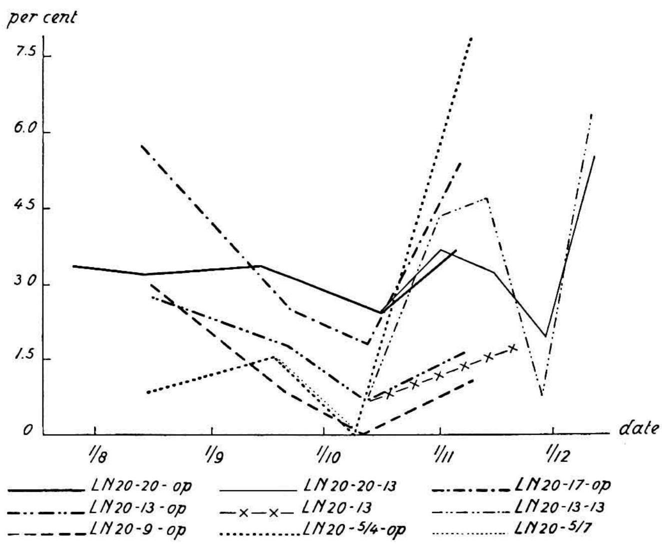

Fig. 3. Le Nôtre. Season 1934—1935. Inulin in per cents of the dry weight.

At 9 and 5° C. there is no inulin, whatsoever early in October. After this date the quantity increases again, also with the unplanted lot LN 20-13. Presumably this increase is not exclusively due to the planting.

It appears from the behaviour of LN 20-20-13 and LN 20-13-13 that this increase is followed by a decrease later on, after which an increase again ensues.

Starch.

In many respects the lines of the starch run like the reflection of those of the non-reducing sugars (Table II and fig. 4). Thus the percentage of starch at planting is for instance greater in proportion to the quantity of sugar being smaller.

The lots too, planted in the open, early in November contain each about the same amount of starch.

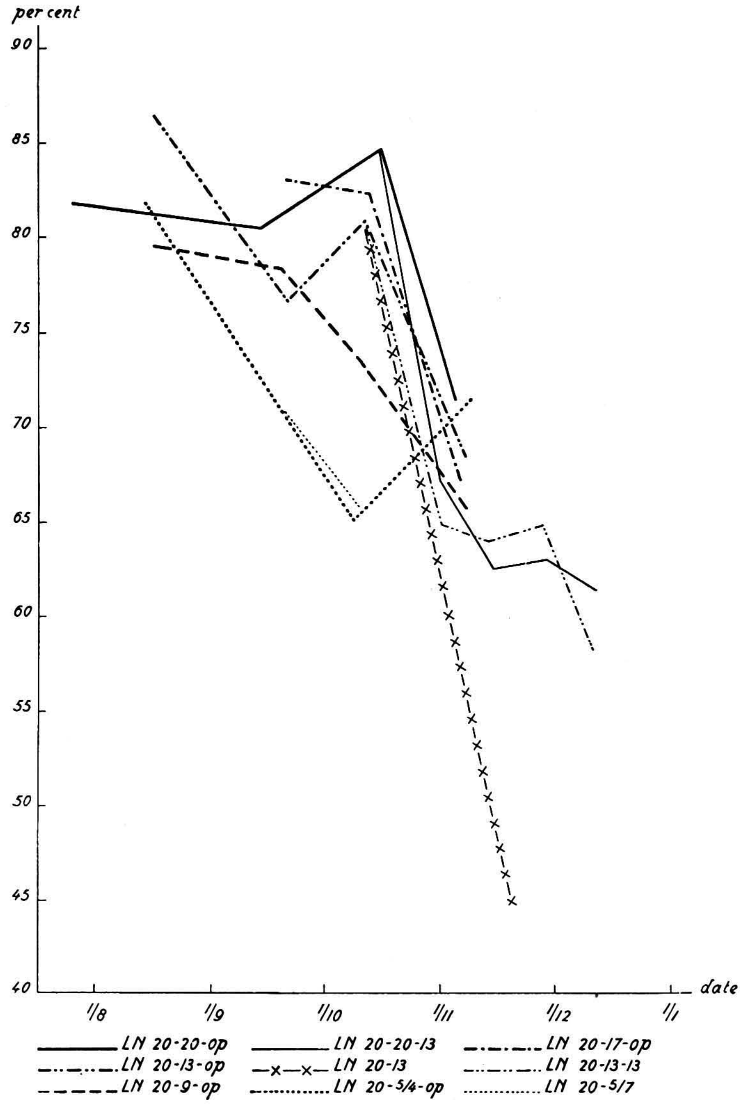

Fig. 4. Le Nôtre. Season 1934—1935. Starch in per cents of the dry weight.

Why LN 20-20-13, LN 20-13-13 and LN 20-13 contain less starch is. however, not clear.

TEMPERATURE TREATMENT AND RESPIRATION.

It goes without saying, that the temperature treatment affects the intensity of the respiration. For this process is accelerated by a rise of temperature and lowered by a decrease of temperature. The changes which the sugar percentage of the bulbs undergoes at various temperatures give rise to the surmise that the temperature also influences the respiration in another way, as the respiratory material consists of sugars. The question can be put whether the influence of the temperature treatment on the respiration can be entirely explained by the change in the percentage of sugar or whether the temperature influences the rate of the respiration in another way e.g. by a stimulation of the respiratory enzymes.

This question regarding the connection between temperature and respiration is surely of importance as the respiration is an important energy-supplying process for very many vital processes. Therefore it should be ascertained whether there is a relation between this process and the rate of the morphological development. Moreover knowledge of the intensity of the respiration is essential for a good comprehension of the carbohydrate metabolism. Of those only the percentages of the dry weight have been discussed, without taking the consumption into account. As long as the bulbs have not yet been planted the respiration is almost exclusively the only substance consumer. But very little is used for the synthesis of the young plant. With LN 20-13-13 the dry weight on 1 November, so three weeks after being planted, is only ± 2.5 % of the dry weight of the bulb.

In order to determine the respiration, both the carbon dioxide liberation as well as the oxygen absorption were determined. The method applied will be described elsewhere. The determinations were mostly performed at the temperatures in which the bulbs were kept. The results were calculated on the basis of 1 K.G. dry weight per hour and expressed in cc.

As material for these experiments was used the variety of Le Nôtre of the season of 1934—1935; the same material which was used for the determination of the carbohydrate metabolism. Table I of this publication shows the temperatures applied.

The carbon dioxide liberation.

During the time all the bulbs were at 20° C. so from 26th July till 17th August, the carbon dioxide production drops from 46.2 cc. per K.G. per hour to 27.9 cc. (Table III, fig. 5). On 17th August the parcel was divided and at about this date determinations were performed at 5, 9, 13, 17 and 20° C. The respiration of these bulbs, which with the exception of course of 20° C. had been in these temperatures for one day, was as to be expected, weaker in proportion to the temperature being lower.

Up to planting the carbon dioxide liberation increased with all lots. LN 20-5/4-op and LN 20-5/7 were both examined at 5° C. on 9th and 10th October. The former lot liberates somewhat more carbon dioxide than the latter. After the planting early in November a series of determinations were performed at 9° C. this being about the average ground temperature from the time of planting. The respiration of the lots was equal, except the bulbs which had been treated at 5° C. These liberate less carbon dioxide. Both the lots planted at 13° C. were examined at 13° C. Their respiration continued increasing till the middle of November; decreases

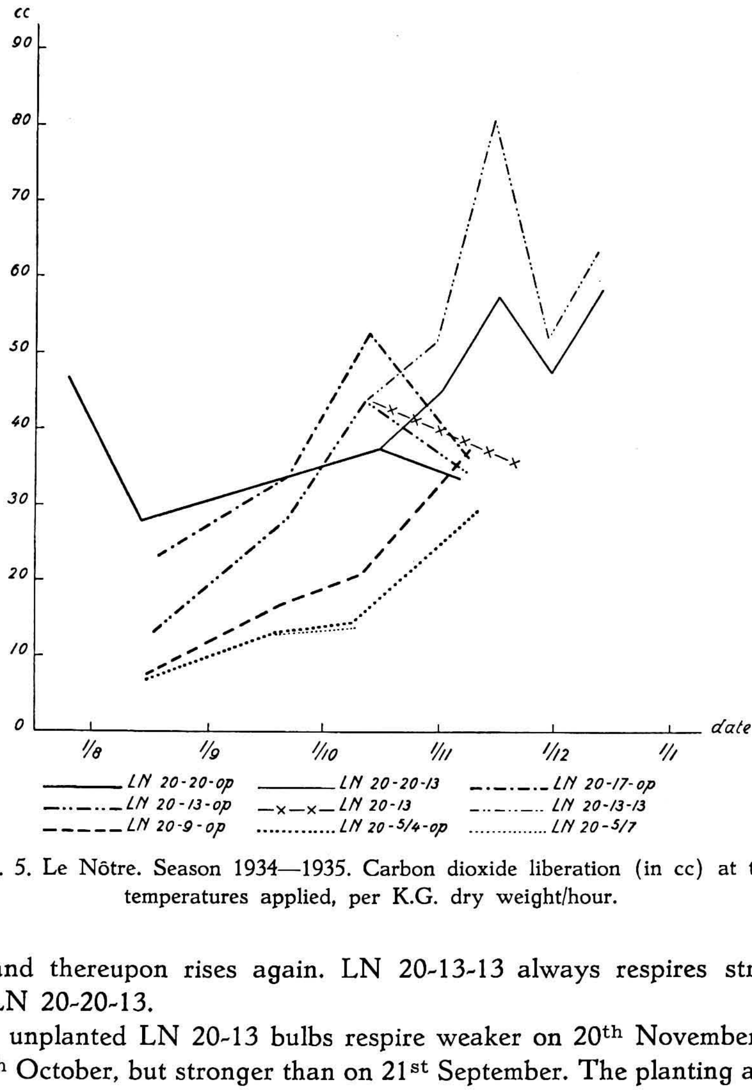

Fig. 5. Le Nôtre. Season 1934—1935. Carbon dioxide liberation (in cc) at the temperatures applied, per K.G. dry weight/hour.

then and thereupon rises again. LN 20-13-13 always respires stronger than LN 20-20-13.

The unplanted LN 20-13 bulbs respire weaker on 20th November than on 12th October, but stronger than on 21st September. The planting accelerates the carbon dioxide liberation as appears from a comparison between LN 20-13 with LN 20-13-13.

So as to compare the results better obtained at different temperatures, with each other, they have all been calculated on the basis of the same temperature viz. 20° C. by means of the temperature coefficients, which were found with Le Nôtre in the season of 1935—1936 (Table III, fig. 6). The quantities immediately after the transferrence have not been inserted in figure 6. They should be all alike. These differences discovered are, however, not only due to determination errors. Especially with the utilization of oxygen, it clearly appears that the gas exchange is abnormally large at a lower temperature. The various possible causes of this will be further gone into elsewhere.

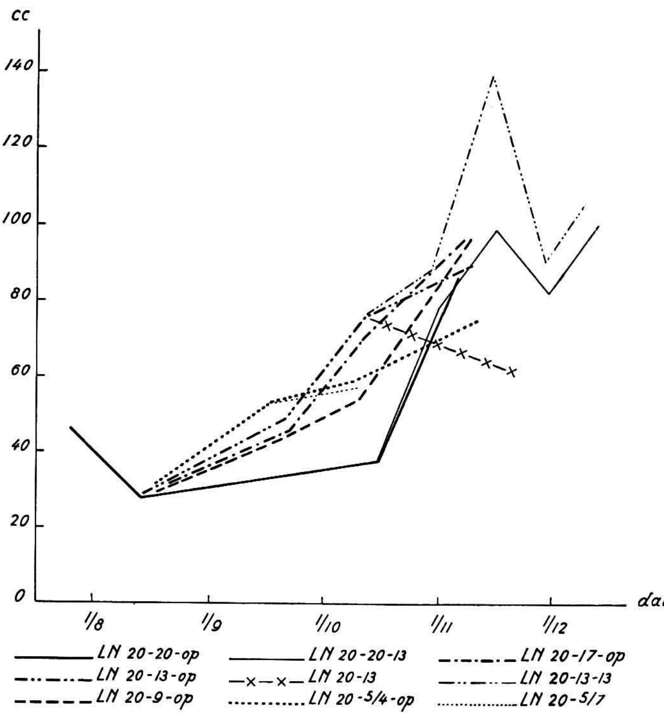

Fig. 6. Le Nôtre. Season 1934—1935. Carbon dioxide liberation (in cc) per K.G. dry weight/hour, calculated on the basis of 20° C.

After the calculation too, differences still continue to exist between the lots, so that the carbon dioxide liberation must be affected by the temperature treatment. After planting, most of the differences between bulbs planted in the open have disappeared, only LN 20-5/4-op has a slighter carbon dioxide production. Between LN 20-20-13 and LN 20-20-op there is hardly any difference up to the beginning of November; LN 20-13-13 however respires a little stronger than LN 20-13-op.

The oxygen consumption.

From Table III and fig. 7 it appears that the consumption of oxygen in outline agrees with the carbon dioxide liberation. Immediately after being transferred about 17th August the oxygen absorption exhibits a few irregularities. It is namely greater at 17° C. than at 20° C., and at 5° C. greater than at 9° C. This was already pointed out when discussing the carbon dioxide emission.

The oxygen quantities of LN 20-13-op and LN 20-13 form a flowing line. This course is more probable than the form of the carbon dioxide

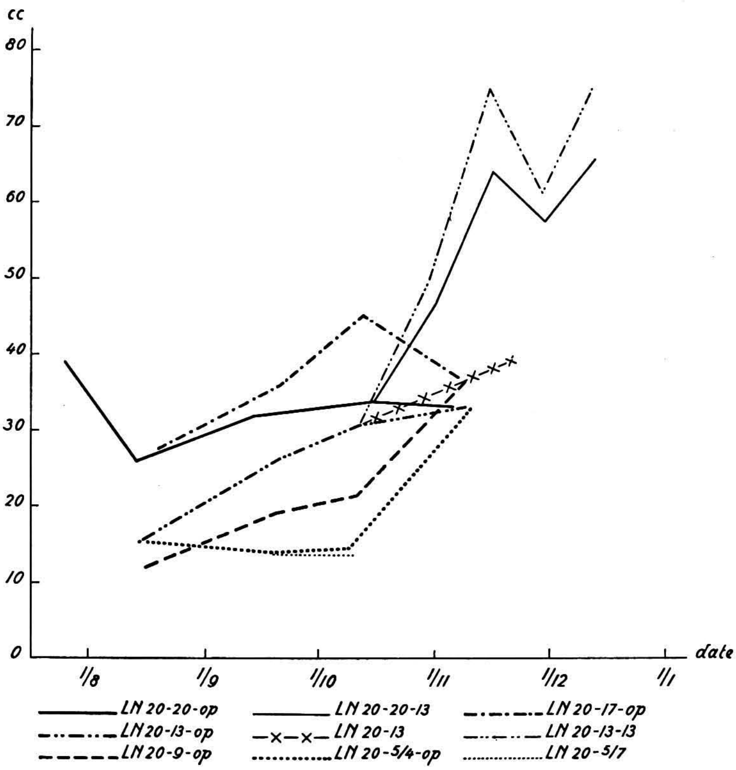

Fig. 7. Le Nôtre. Season 1934—1935. Oxygen consumption (in cc) at the temperatures applied, per K.G. dry weight/hour.

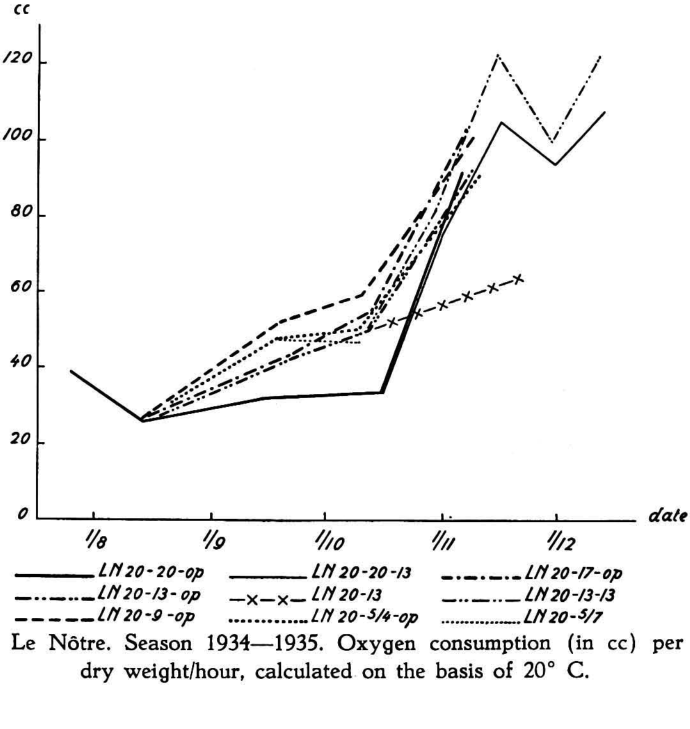

Fig. 8. Le Nôtre. Season 1934—1935. Oxygen consumption (in cc) per K.G. dry weight/hour, calculated on the basis of 20° C.

curve. The determination of carbon dioxide on 12th October presumably turned out too high, which was perhaps caused by the air-pump having stopped during the night. The respiration of the bulbs planted in the open is almost equal early in November. Of the lots planted at 13° C. LN 20-13-13 absorbs a little more oxygen than LN 20-20-13.

After being calculated on the basis of 20° C. (Table III and fig. 8) differences still exist in the oxygen utilization between the lots, which mainly correspond to the differences discussed in the carbon dioxide liberation.

LITERATURE CITED.

1. ALGERA, L. Concerning the influence of temperature treatment on the carbohydrate metabolism, the respiration and the morphological development of the tulip. I. Proc. Royal Acad. Amsterdam **39**, 846—855 (1936).
2. COOKE, D. Untersuchungen über den Kohlehydratumsatz in den Blättern der Küchenzwiebel (Allium Cepa). Planta **8**, 522—526 (1929).
3. PINKHOF, M. Untersuchungen über die Umfallkrankheit der Tulpen. Rec. Trav. bot. néerl. **26**, 135—288 (1929).

Lisse, May 1936. *Laboratory for Bulbresearch.*

Microbiology. — *A sampling-Apparatus for Aeroplankton.* By M. A. VAN OVEREEM. (Communicated by Prof. L. G. M. BAAS BECKING).

(Communicated at the meeting of September 26, 1936).

The classical research of LOUIS PASTEUR (8) upon spontaneous generation has convinced us that the air carries life, be it in latent or in vegetative form. This floating condition of life has been named, by H. MOLISCH (7), the aeroplankton.

Aeroplankton has been investigated from several angles and by different methods, it is only recently, however, that the airplane has come to our aid in the study of this curious transit of living forms, for transit it is, with metabolism reduced, from one part of our planet to the other.

F. C. MEIER and C. A. LINDBERGH (6), B. E. PROCTOR (9*a* and *b*), E. C. STAKMAN (13), A. W. STEVENS (14) and L. BERLAND (2*a* and *b*) investigated, by means of various apparatus, the germ-content of the atmosphere with the aid of the airplane.

They either submitted the material to direct microscopic examination or cultured bacteria, fungi, yeasts and actinomycetes from it.

Microbiological investigation of the atmosphere by other means has been carried out either from a general biological point of view (of which the work of PASTEUR is certainly the prototype) or in the interest of hygiene, phytopathology or the study of allergic phenomena.