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Concerning the influence of temperature treatment on the carbohydrate metabolism, the respiration and the morphological development of the tulip

I – III

BY

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Sur l'influence de la température sur le métabolisme des hydrates de carbones, la respiration et le développement morphologique de la tulipe.

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

(Communicated at the meeting of June 27, 1936).

INTRODUCTION.

From the researches of Prof. BLAAUW and others (3, 4, 6, 7, 8, 9 and 10) and of Prof. VAN SLOGTEREN and BEIJER (1 and 2) it has been clearly proved that the temperature affects in a complicated way the velocity of the morphological development. In this development two phases can be distinguished. In the first phase the formation of stem, leaves and flower predominates; in the second phase the extension of the organs formed takes place. With the tulip, the young plant already begins to form its first leaves in the bulb before the lifting-time early in July. After the lifting it continues doing so. The most rapid course of this process is at 17—20° C. With the application both of higher as well as of lower temperatures, the velocity of the formation diminishes. Thus the velocity at 13° C. is about as great as at 23° C.; at 9° C. it is between the velocity at 25.5° C. and 28° C. Through this, lots which have been treated quite differently will be in the same stage of development. Notwithstanding the great morphological conformity there exists however a large difference physiologically, as the plants after the same after-treatment behave quite differently. Forced into bloom under the same conditions, one lot can yield good flowers, whereas the other fails entirely. Hence it follows, that when asked if one can proceed with the cooling of the lot, one may not depend exclusively on the morphological development stage.

Fig. 1 gives a clear impression of the influence of the temperature on the second stage, viz; the extension of the plant. This figure applies to a parcel of tulips of the variety of Le Nôtre of the season 1934—1935. After having been lifted all the bulbs were first placed in a temperature of 20° C. At stage VI being reached, at which stage all the organs except the pistil had been formed, the parcel was divided on 17th August. One part remained in 20° C., the remainder of the bulbs were transferred to 17, 13, 9 or 5° C.

Figure 1 shows that up to planting on 9th October, the stretching is always fastest at 13° C. During all that time the temperature optimum for stretching is therefore at 13° C. The optimum is clearly lower here than the optimum for the organic formation. One cannot see from the figure where the optimum lies after planting, because the lots mentioned here were all planted in the open and therefore only underwent one temperature treatment. From the fact however, that in January and later, the plants in the hot-house at 13° C. grew less rapidly than at 15° C., at 20° C. again quicker than at 15° C. etc., it appears that the temperature optimum in the meantime had shifted to a higher temperature.

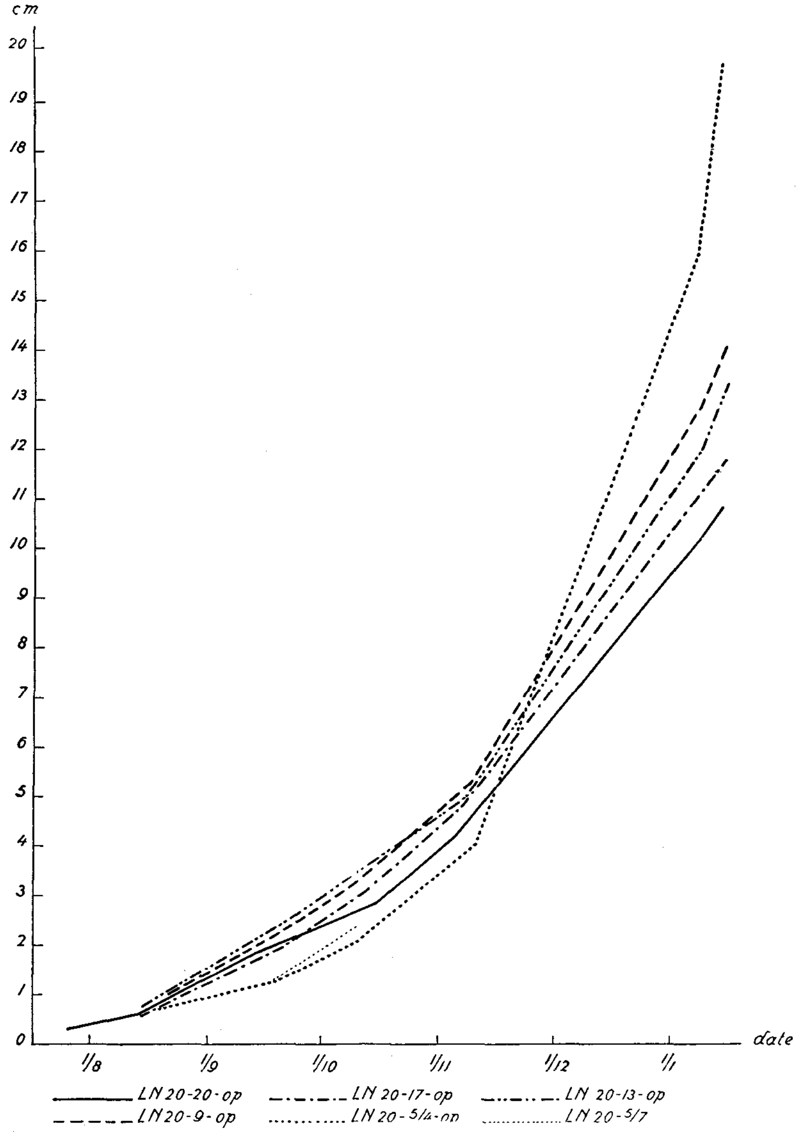

Fig. 1. Le Nôtre. Season 1934—1935. Length of the plant in cm.

One is inclined to infer from the above, that the quickest development is always reached by keeping the bulbs at those temperatures through which at every time the quickest extension takes place. This is however not the case, primarily because the temperature not only has a momentary influence, but can also have after-effects. This is already apparent from fig. 1. Although the lots planted in the open were in the same temperature, all of the plants did not however grow as rapidly. After some time the stretching is quicker according to the degree of cooling of the bulbs before planting. On the 9th January, probably earlier, the state is as follows, the plant has become longer in proportion to the summer treatment being cooler.

That after-effect also reveals itself in the velocity by which the optimum shifting takes place. The cooler the bulbs have lain, the quicker the optimum rises to a higher temperature. It already appears from the following, that a parcel of bulbs which has had a warm treatment during the summer and for instance was placed early in December in the hot-house at 20° C. stretches only a little quicker than in the much colder outside temperature, while the extension of a lot, which was cooled during the summer, at 20° C. stretched much faster than in the open. This proves that the temperature optimum for stretching is much lower in the former case than in the latter.

Due to the after-effect, the bulbs have to be kept in a cooler place during the summer than in accordance with their optimum in that period so as to obtain the quickest and earliest flowering. The retard in extension, caused thereby is amply recovered later on through the after-effect of the temperature.

In this sense HARTSEMA, LUYTEN and BLAAUW (6) are right when they call the temperatures optimal which are discussed on pages 31—35, and graphically represented in fig. 10. In their totality i.e. paying attention to the ultimate result they effect the quickest growth. It is however not correct that during the summer months the young plant extends itself quickest at 9° C. or even at 5° C. In this period the optimum is at about 13° C. Therefore it would have been better not to speak of optimal temperatures but of an optimal treatment.

We have thus seen that the optimum for the stretching, commencing at about 13° C. shifts to higher temperatures in the course of the season. It is self-evident to assume that this shifting is due to particular factors which at first check the growth at a higher temperature. The extension is namely not only dependent on the temperature but also on the quantity of food available in the bulb, for as long as the young plant cannot assimilate it is entirely dependent on the bulb.

From the research of PINKHOF (11) it appears, that the temperature influences the chemical composition. Cooling during the summer causes a decomposition of the starch and an increase of the quantity of non-reducing sugars. Presumably then there is a parallelism between the changes in the carbohydrate percentage and the internal development.

The research commenced by PINKHOF at the instigation of Prof. VAN SLOGTEREN has been gone into further and in this and a following publication, the principal results of the research of the influence of the temperature during the stretching phase on the carbohydrate metabolism will be set forth. In the latter publication too, the influence which temperature has on the respiration of the bulbs will be discussed. In a third publication, by means of data obtained so far, we shall try to find an explanation for the influence of the temperature treatment on the velocity of the morphological development.

THE CARBOHYDRATE METABOLISM.

Strictly taken, it is perhaps necessary to be acquainted with the entire metabolism of the bulbs in order to attain the object in view. As however this consists of about 80 % of carbohydrates, it seemed desirable to commence the investigation on this group of substances and to investigate how far a satisfactory solution can be obtained through this.

The following carbohydrates were determined: the reducing and the non-reducing sugars and the starch. The method according to which these substances were determined is the same as described by PINKHOF (11).

In the season of 1934—1935 moreover the quantity of sucrose was determined by means of invertase according to COOKE's method (5).

All these substances were expressed in per cents of the dry weight.

All the determinations were performed by Miss A. L. C. VAN WAVEREN. My grateful thanks to her for the work done in connection with this, which she carried out with precision.

The tulip varieties of Murillo and Le Nôtre will be discussed which in the seasons of 1929—1930, 1934—1935 resp., were subjected to various temperature treatments. With Murillo at first only the bulb was examined, from 7th October, moreover the young plants too. However, the bulb will only be discussed here. With Le Nôtre, bulb and plant were as a rule analysed collectively; with a couple of parcels individually.

Murillo. Season 1929—1930.

The research commenced on 6th May, i.e. about two months before lifting. On 1st July nearly all the bulbs were lifted; the remainder 10th July (Table I). Up to July the whole parcel was therefore in the open under the same temperature conditions, after 1st July a division took place. Table I also shows the temperatures applied after the lifting.

TABLE I.

MURILLO. Season 1929—1930. Survey of the temperature treatment applied.

Lot	Lifting date	Treatment					Planting-date
		1/7—22/7	22/7—1/8	1/8—17/9	17/9—5/11	5/11-end of the determin.	
M 3 w 17—9—9	1—7—'29	17	9	9	9	9	17—9—'29
M3w17—9—9/17	1—7—'29	17	9	9	9	17	17—9—'29
M 4 w 17—9—9	1—7—'29	17	17	9	9	9	17—9—'29
M 17—17—op	1—7—'29	17	17	17	in the open	in the open	17—9—'29
M 17—17—17	1—7—'29	17	17	17	17	17	17—9—'29
M 17—17	1—7—'29	17	17	17	17	17	unplanted
M 21—13—op	1—7—'29	21	21	13	in the open.	in the open	17—9—'29
M non—non—op	10—7—'29	non—treatment			in the open	in the open	19—9—'29

The various lots have been named according to their treatment. The first figure after the letter M (= Murillo) indicates the temperature during the pre-treatment. This pre-treatment almost coincides with the period of the formation of the organs. The second figure indicates the temperature of the after-treatment. The third figure refers to the temperature when the bulbs were planted. If the bulbs were planted in the open, the letters op are used. In case it is necessary for the sake of distinction, the duration of the treatment is indicated in weeks e.g. M 3 w 17—9—9. Should the temperature during a period vary, then the initial and final temperatures are stated e.g. M 3w 17—9—9/17. The lot mentioned last on the table was stored in an unheated room and did not receive any controlled treatment. From 10—20 July the temperature here was near 30° C., from 20—30 July the temperature gradually dropped from 30° C. to 18/19° C. and then kept about 17/18° C. till the planting time.

Reducing sugars.

Table II and fig. 2 show the percentage of reducing sugars of each lot. Till about November the bulbs contained very little of these sugars. After 6th May the concentration diminished rapidly, was almost constant then till 1st July. Then a rise and fall ensued after which finally a continually quicker rising commenced. During the period between lifting and planting, there is a difference between the various lots, which can hardly be attributed to the temperature applied. This is probably due to variability and errors in the determinations.

Also after the planting, one notices little difference at first, but after the middle of December, two groups can be distinguished. The concentration of the bulbs planted at 9° C. and 17° C. increases more considerably than the parcels planted in the open. As the outside temperature was much lower than 9° C. in that period, it is self-evident that the temperature now plays a predominant part. The higher it is, the quicker the reducing-sugars increase. Yet the after-effect of the temperature applied in summer makes itself felt. This is evident from the fact that the sugar percentage of M 3w 17—9—9/17, the temperature of which had been advanced from 9° C. to 17° C. on the 5th November had already greatly increased on 31st December whereas M 17—17—17 which had also been planted at 17° C. was only able to do so after 1st January. This lot could only then increase at 17° C. its concentration of reducing-sugars more rapidly than the bulbs planted at 9° C.

The preceding treatment in summer and autumn therefore influences the date when the bulb is capable of increasing its percentage of reducing-sugars at a fairly high temperature.

That the planting, as was to be expected, exercises a great influence too, is apparent from the unplanted M 17—17 bulbs which could not at all increase their concentration.

From 6th May until the lifting a great fall takes place (Table II and fig. 3). On discussing the percentage of starch it will appear that this

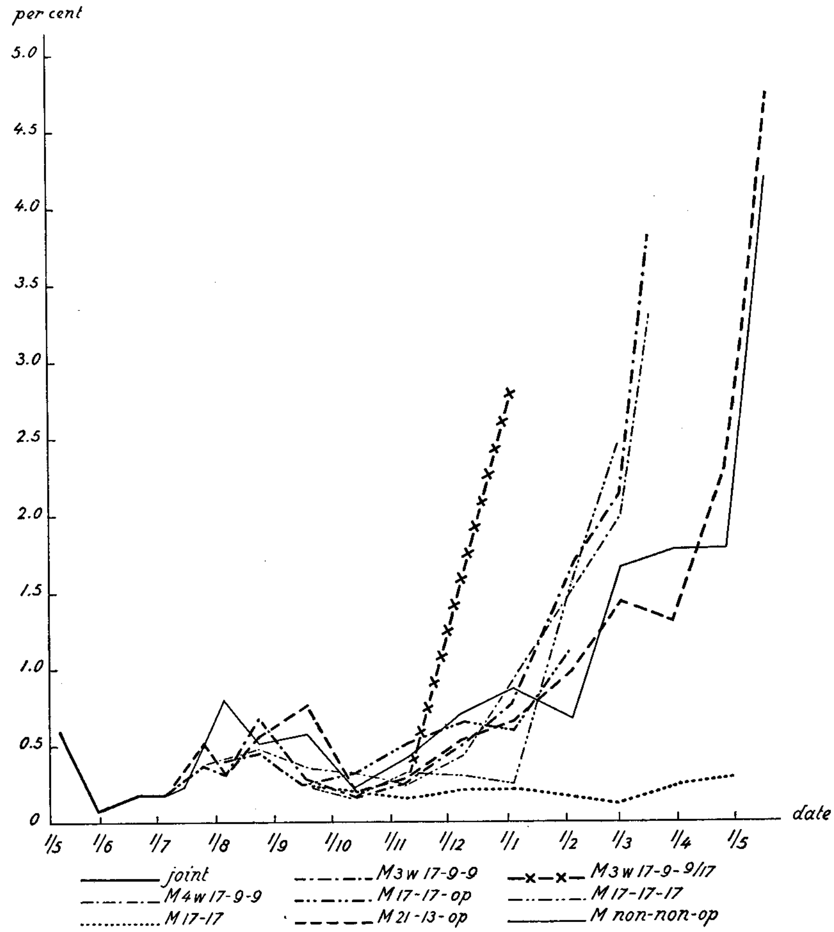

Fig. 2. Murillo. Season 1929—1930. Reducing sugars in per cents of the dry weight.

Non-reducing sugars.

diminishing of concentration is attended with an increase of starch. It is one of the phenomenons of the ripening process of the bulbs by which the sugars formed are stored as reserve food in the form of starch.

After the lifting the reversed process takes place viz; the decomposition of the stored starch. This decomposition or conversion into more useful carbohydrates is greatly dependent on the temperature. As soon as this is decreased, the percentage of non-reducing sugars increases. The lots M 3 w 17—9—9, M 3w 17—9—9/17, M 4w 17—9—9 and M 21—13—op already do this before being planted, as soon as they have been put in 9° resp. 13° C. M 17—17—op and M non—non—op immediately after being planted in the lower outside temperature. M 17—17—17 which was planted at 17° C. increased its concentration much slower. The unplanted

M 17—17 bulbs were still much tardier. Besides the lowering of temperature, the planting therefore also promotes the raising of the concentration.

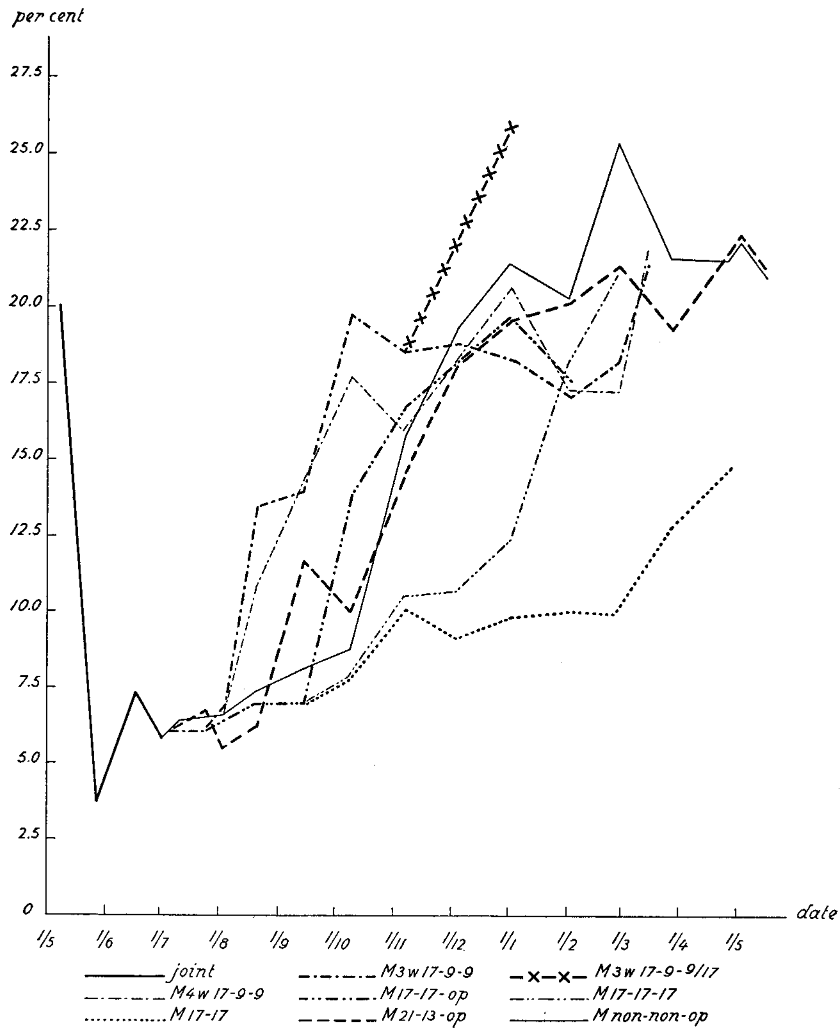

Fig. 3. Murillo. Season 1929—1930. Non-reducing sugars in per cents of the dry weight.

Early in December the lines of parcels planted at 9° C. and in the open intersected; their concentrations were therefore now almost equal. During the first period after this intersection the concentration at 9° is somewhat lower than in the colder outside temperature; after that the first mentioned parcels again rise to the percentage of the bulbs planted in the open. It is open to doubt whether this difference, as during the summer treatment is really based on the fact that the percentage of these sugars is lower at a higher temperature, as a comparison between M 3 w 17—9—9 and M 3 w 17—9—9/17 shows us. On 31st December the latter lot contained much more non-reducing sugars than the former lot. If the determination of M 3 w 17—9—9/17 is correct then in this

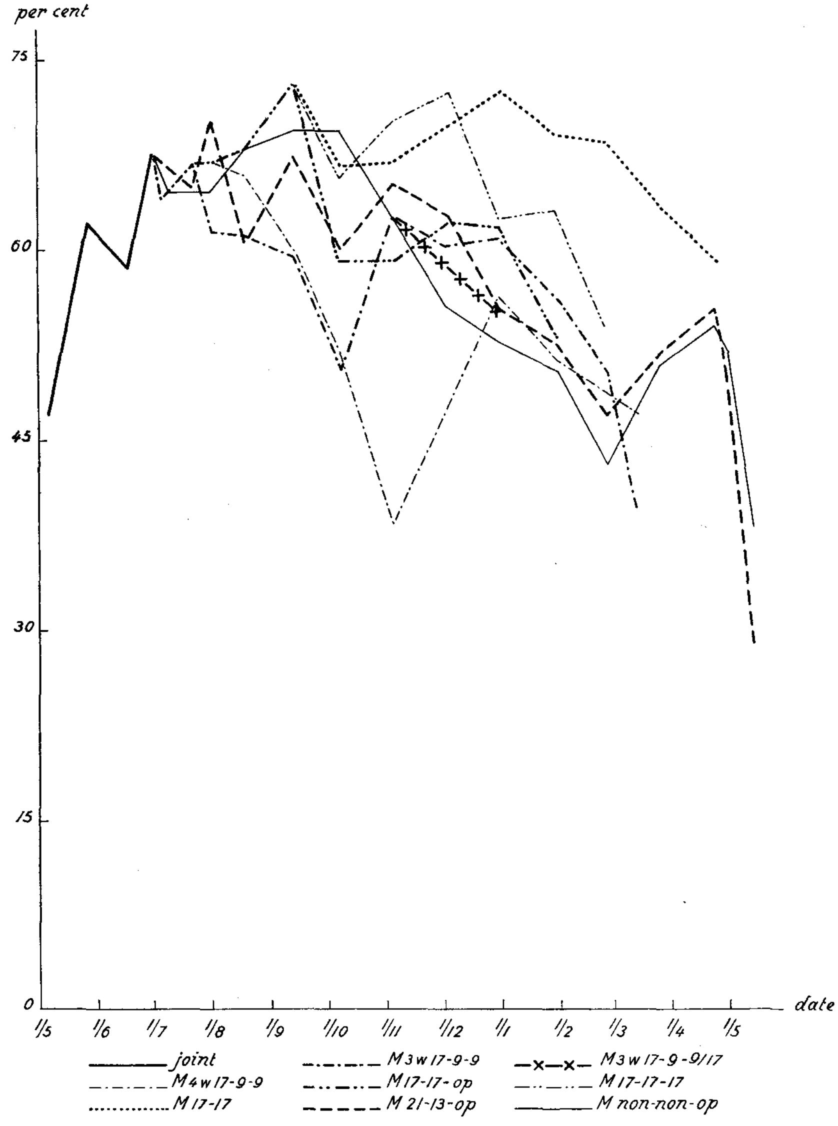

Fig. 4. Murillo. Season 1929—1930. Starch in per cents of the dry weight.

case the higher temperature must have promoted the forming of non-reducing sugars. This contrast will be discussed elsewhere, but already it may be observed that the bulbs planted at 9° C. were in a much further advanced stage on 31st January and 26th February and had a smaller dry weight than the bulbs planted in the open. Both groups are therefore not quite comparable.

It is also of importance that M 3 w 17—9—9/17 notwithstanding its higher concentration at 17° C. showed a much quicker increase than

M 17—17—17. A similar rapid increase with M 17—17—17 only appeared after 1st January. In this case too, the temperature applied in summer makes itself felt. The dates at which an advance of the temperature causes an increase of the non-reducing sugars is therefore through previous cooling shifted to an earlier date.

It cannot be explained to what factors this change in behaviour is due. Perhaps the formation of the non-reducing sugars is controlled in summer by a process which elapses quicker at lower temperatures and some time after planting by a process which is more rapid according to the temperature being higher, therefore a changing of the limiting factors.

Starch.

Table II and fig. 4 show the increase of starch before lifting which has already been discussed. The behaviour after lifting principally corresponds to what can be expected from the behaviour of the non-reducing sugars. For instance the percentage of starch is lower on 13 September in proportion to the bulbs containing more non-reducing sugars on that day. In broad outlines the curves for starch and non-reducing sugars are each others reflection.

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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 24th 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 9th 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 temperature 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

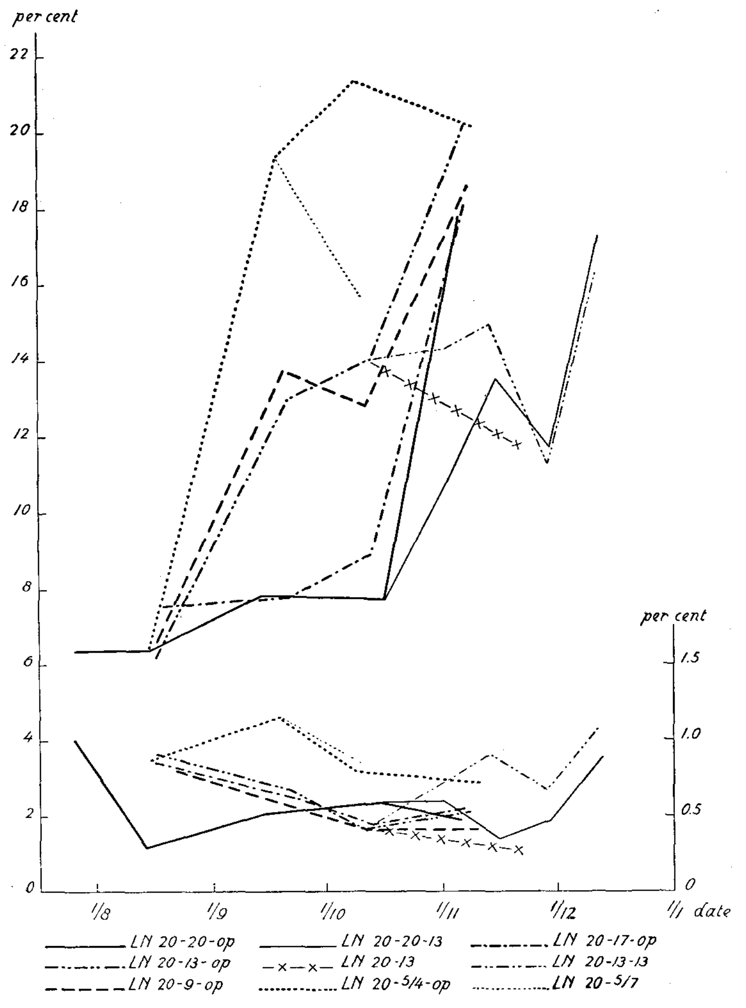

Fig. 1. Le Nôtre. Season 1934—1935. Reducing sugars (below; ordinate to the right) and non-reducing sugars (above; ordinate to the left) in per cents of the dry weight.

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.

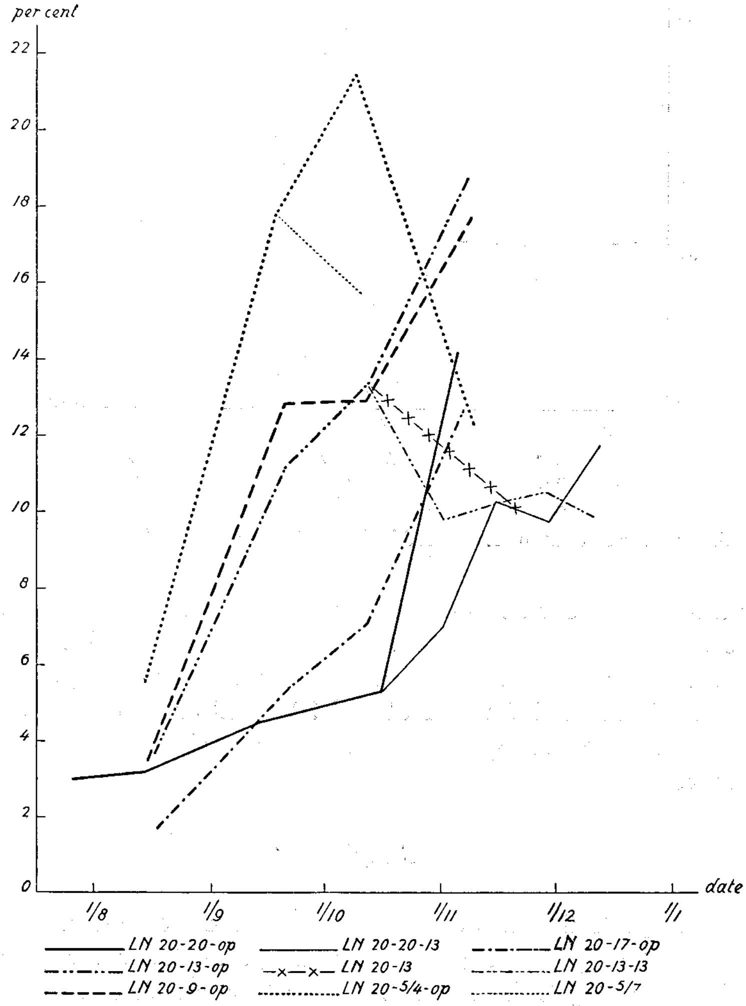

Fig. 2. Le Nôtre. Season 1934—1935. Sucrose in per cents of the dry weight.

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.

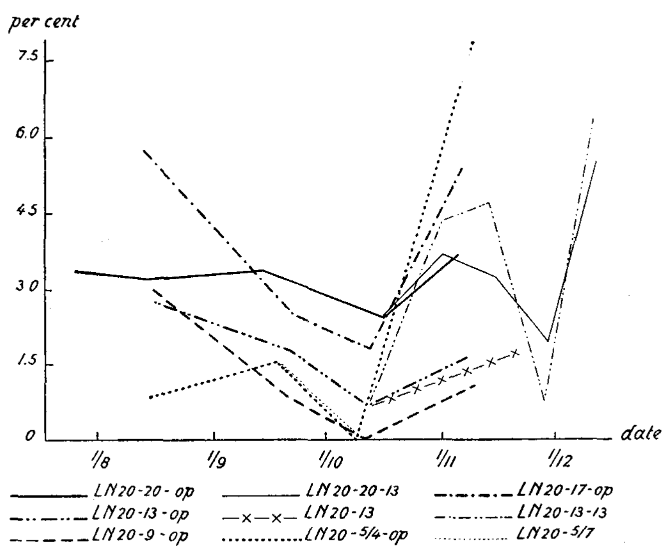

Fig. 3. Le Nôtre. Season 1934—1935. Inulin in per cents of the dry weight.

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.

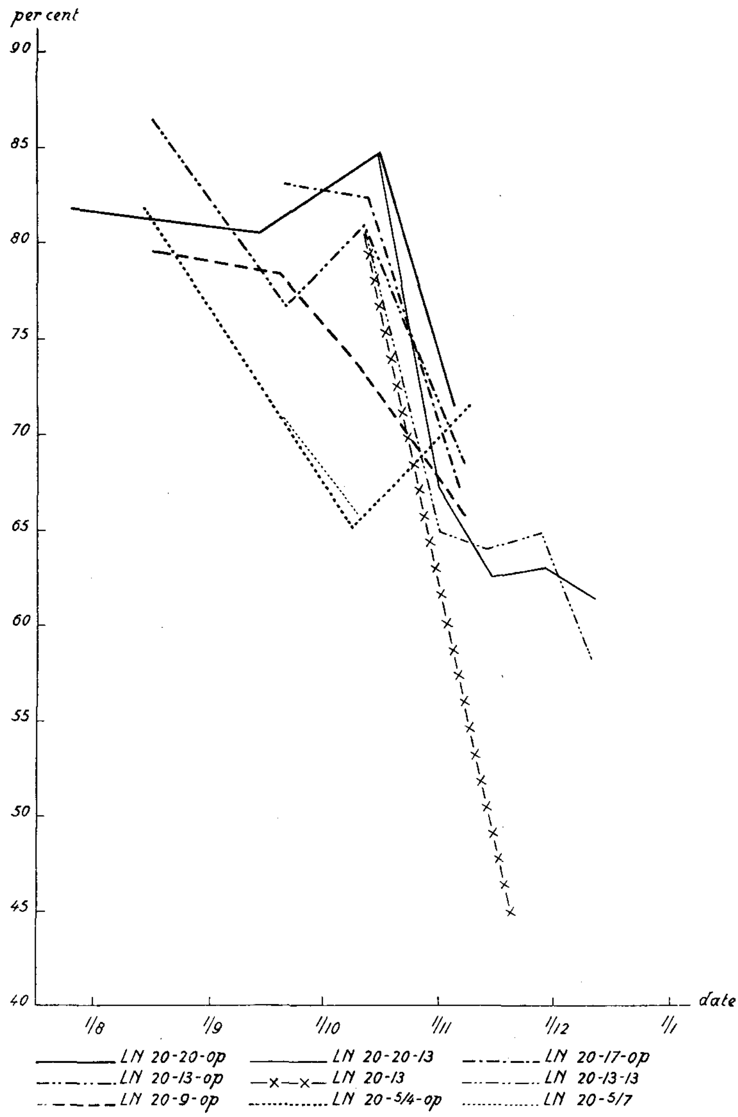

Fig. 4. Le Nôtre. Season 1934—1935. Starch in per cents of the dry weight.

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

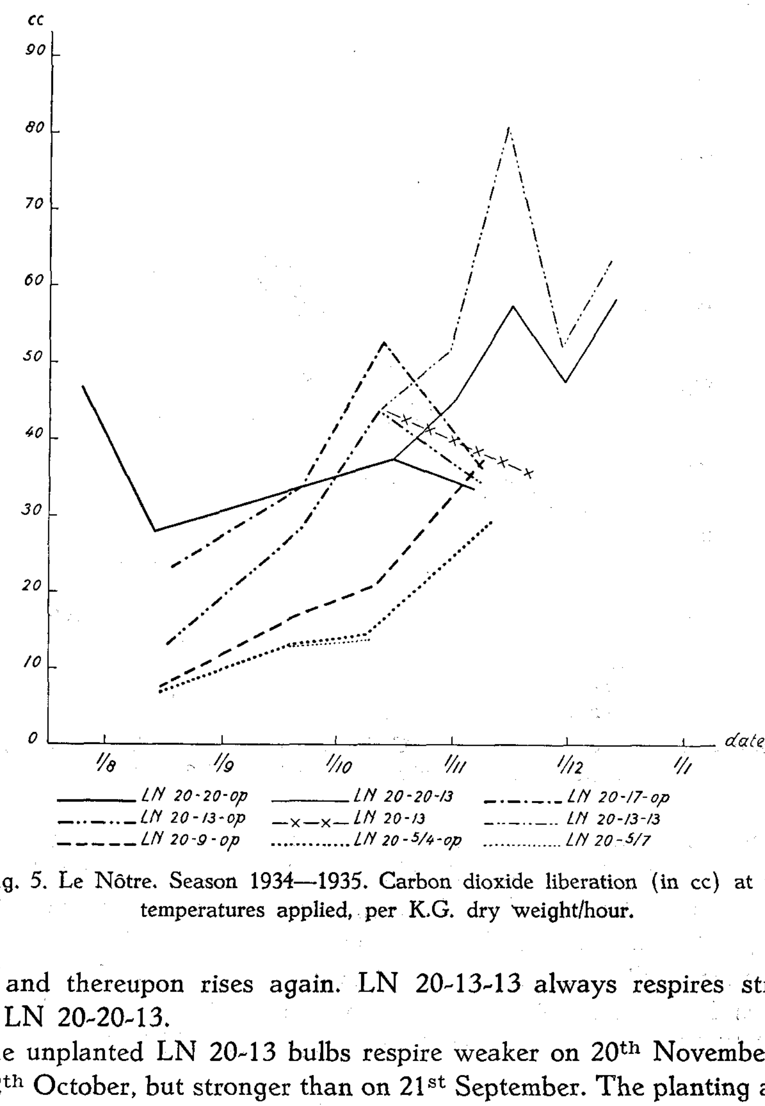

Fig. 5. Le Nôtre. Season 1934—1935. Carbon dioxide liberation (in cc) at the temperatures applied, per K.G. dry weight/hour.

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.

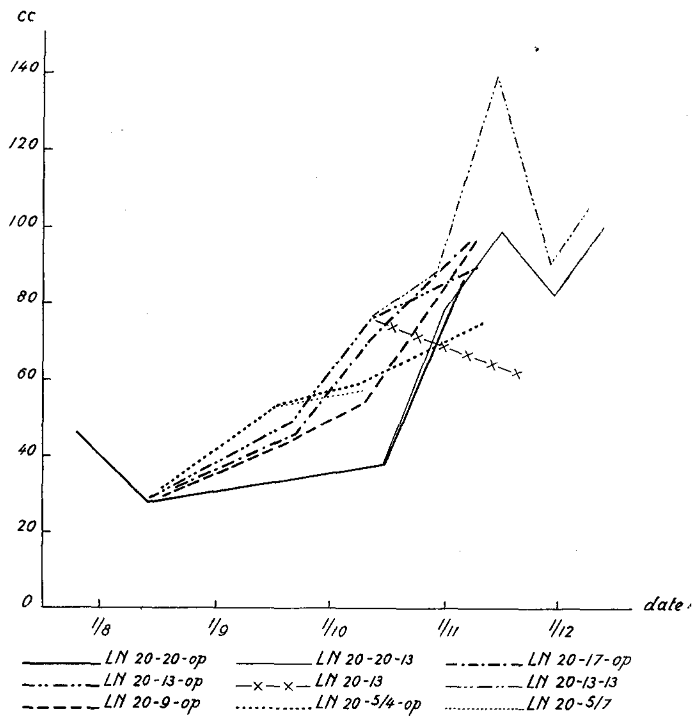

Fig. 6. Le Nôtre. Season 1934—1935. Carbon dioxide liberation (in cc) per K.G. dry weight/hour, calculated on the basis of 20° C.

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

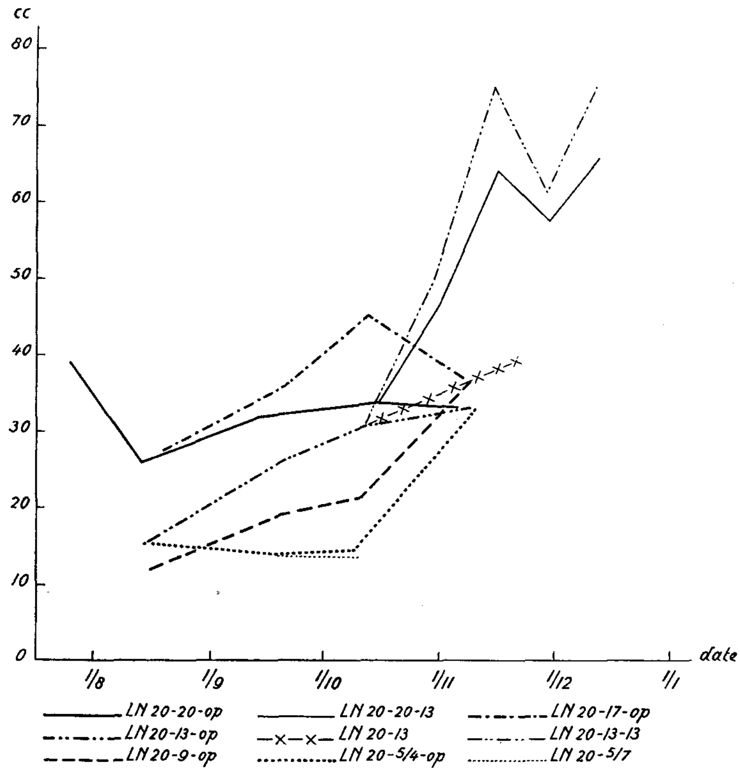

Fig. 7. Le Nôtre. Season 1934—1935. Oxygen consumption (in cc) at the temperatures applied, per K.G. dry weight/hour.

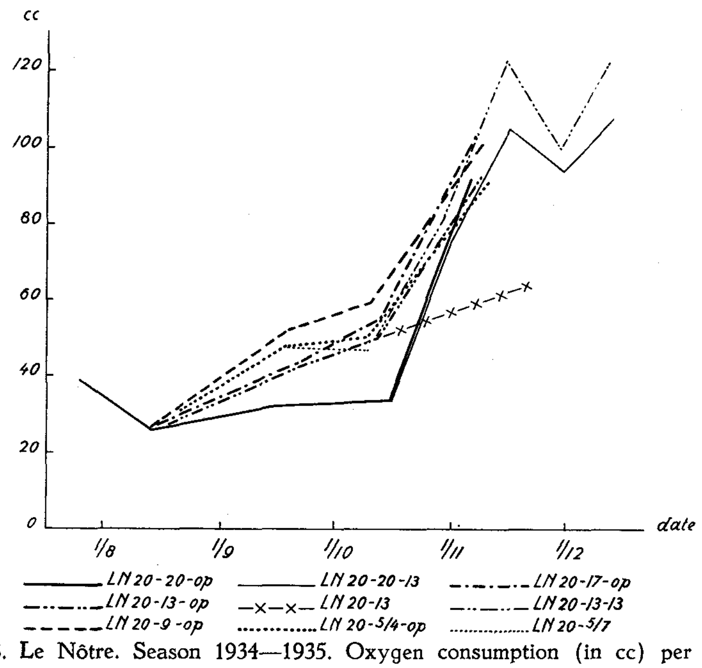

Fig. 8. Le Nôtre. Season 1934—1935. Oxygen consumption (in cc) per K.G. dry weight/hour, calculated on the basis of 20° C.

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.

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

(Communicated at the meeting of October 31, 1936).

DISCUSSION OF THE RESULTS.

The respiration and the carbohydrate metabolism.

Table III and figures 6 and 8 of the preceding publication (1) show that the respiration also after being calculated on the basis of 20° C. is dissimilar in the various lots. Is this to be explained by the assumption that the applied temperature influences the forming or activation of the respiratory enzymes or can the differences discovered be attributed to the difference in the sugar percentage. It has been proved that the concentration of the reducing sugars is little changed by the temperature treatment. It is more obvious to ascertain whether there exists a relationship between the non-reducing sugars and the respiration.

If the intensity of the respiration was exclusively controlled by the percentage of these sugars, then there would have to be a strict proportion between them. The quotient:

$$\frac{\text{carbon dioxide emission (oxygen consumption) per K.G. dry weight/hour}}{\text{quantity non-reducing sugars per K.G. dry weight}}$$

should then on the different dates not only be continually equal in the same lot but also in all the lots individually.

Table III (1) and the figures 1 and 2 show how far this is correct. From 26th July till 17th August the value of the quotient declines. After the division the lines of LN 20-20-op, LN 20-13-op and LN 20-9-op at first drop a little and then gradually rise. The deviations of the horizontal line are however slight so that in this period there is a fairly good proportion between the respiration and the non-reducing sugars. With LN 20-5/4-op the value of the quotient continues declining up to planting, and at planting it is then considerably lower. With LN 20-17-op it continually keeps on rising up to planting. A month after the planting the differences have chiefly disappeared again.

With the lots, which were planted at 13° C., the value of the quotient increases rapidly at first after which a slower increase ensues and then drops again. The proportion is not so clear here.

As the non-reducing sugars chiefly consist of sucrose, the respiration was also calculated on the basis of this sugar. A proportion is however hardly perceptible here.

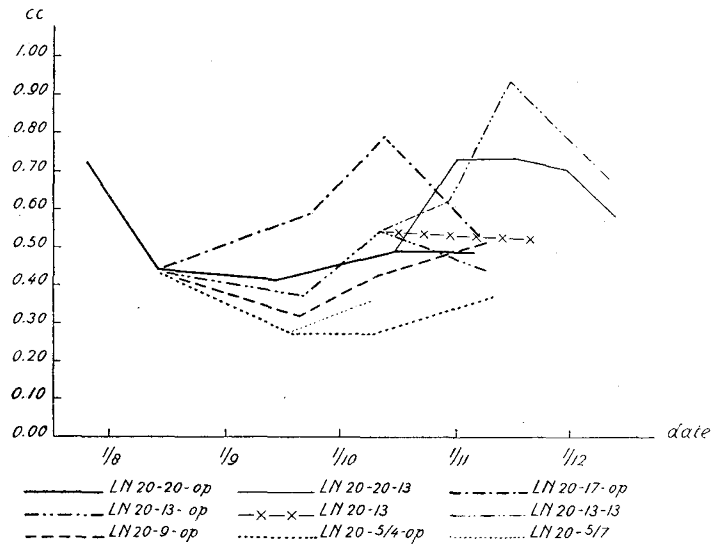

Fig. 1. Le Nôtre. Season 1934—1935. Carbon dioxide liberation (calculated on the basis of 20° C.) divided by the non-reducing sugars.

From the proportion found between the intensity of the respiration and the concentration of the non-reducing sugars, we can deduce something concerning the way in which the sugars discussed are formed.

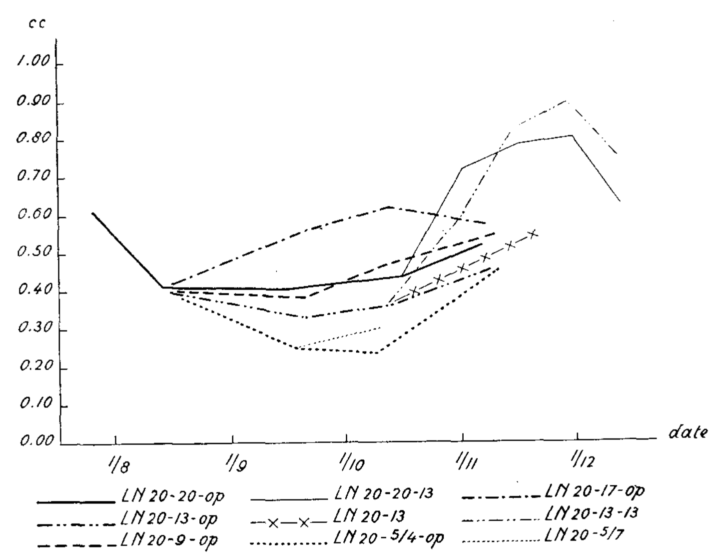

Fig. 2. Le Nôtre. Season 1934—1935. Oxygen consumption (calculated on the basis of 20° C.) divided by the non-reducing sugars.

Three views are possible with regard to their way of formation:

1°. The starch-decomposition takes place according to the scheme: starch → non-reducing sugars → reducing sugars.

2°. The reducing sugars are formed in another way than as indicated in 1°.; the non-reducing sugars are secondarily formed out of the reducing sugars.

3°. Both possibilities are combined.

As the concentration of the reducing sugars is very small up to some time after the planting and decreases rather than increases, the supposition expressed in 2°. leads to the presumption that the reducing sugars, which arise through unknown intermediate reactions are partly respired, and for the rest are converted into non-reducing sugars. According to this view, a proportion between the non-reducing sugars and the respiration is not very explicable. A proportion with the reducing sugars would then be more conceivable.

It is therefore more probable that the reducing sugars are formed via the non-reducing sugars. The greater the concentration of the latter sugars, the quicker the reducing sugars will be formed from them under otherwise equal conditions. Notwithstanding this fact, their concentration between 20° and 9° C. is however the same and at 5 (4)° C. only a little higher. A simple explanation for this equality is the assumption, that the reaction: non-reducing sugars → reducing sugars is so tardy that the reducing sugars formed are immediately respired. In these circumstances the respiratory process is thus limited by the quantity of respirable sugars available and its rate depends on the intensity with which these sugars are formed. As the rate of this formation calculated on the basis of equal temperature depends on the concentration of the non-reducing sugars, it is clear why the respiration, calculated on the basis of 20° C., in such a great measure is proportionate to this concentration. The low value of the quotient at 5 (4)° C. perhaps shows that the concentration is so high here that it no longer acts as a limiting factor for the respiration. Through this the percentage of reducing sugars too, may be somewhat higher. It is not yet explicable why the quotient with LN 20-17-op before the planting is so high.

As a matter of fact the proportion discovered is not absolute; just before and after planting especially at 13° C., the respiration increases more rapidly than in accordance with the concentration of the non-reducing sugars. This can be attributed to an acceleration of the reaction: non-reducing sugars→reducing sugars, e.g. by an increase or activating of the enzymes which control the rate of this process. There however exists a possibility too, that as the season advances, continually more reducing sugars are being formed by another way than via the non-reducing sugars. In other words the case put in 3° is becoming more and more actual. When in this way reducing sugars are formed faster and faster a point of time comes after which the respiratory enzymes are no longer able to utilize all of these sugars and from now on the percentage of reducing sugars begins to increase.

After the intensity of the respiration has been largely deduced from the quantity of the non-reducing sugars and the small percentage of reducing sugars has been attributed to respiration, the question is raised to what factors the increase of the non-reducing sugars in the cooled bulbs are due.

The temperature treatment and the non-reducing sugars.

Also on discussing the relationship between temperature treatment and the amount of non-reducing sugars, the respiration has to be considered. The increase of these sugars at a lower temperature can be explained in two ways. It can be a result of a quicker starch decomposition but also of a slower sugar utilization, so in our case of a slower respiration.

According to MÜLLER—THURGAU (3) the latter possibility has been realized with the potato.

The quantity of starch which at a certain date since 26th July has been converted into sugars, is equal to the increase of the sugars existing, increased with the quantity which till that time has been respired.

In table III (1) and fig. 3 the result of this calculation is summed up on a basis of 1 K.G. dry weight. The starch decomposition is quicker in proportion to the temperature being lower, except that it is slower at 9° than at 13° C. In the period from 17th August till 9th October, at 5 (4)° C. about twice as much starch is converted than at 20° C.

SNELL (4) also arrives at a similar conclusion with the potato. At the same time he refers to HOPKINS and SCHANDER, who also combat the view of MÜLLER—THURGAU.

The differences largely disappear after the planting. The bulbs which have received a warmer treatment overtake the more cooled bulbs through a more rapid starch decomposition.

Shortly after the planting too, the lowering of temperature promotes the sugar formation. This is evident if LN 20—13—op is compared with LN 20—13—13 and LN 20—20—op with LN 20—20—13. In both the cases the sugar formation at 13° C. is less rapid than in the open, although the average temperature here was 9° C. The difference between LN 20—13—13 and LN 20—13 shows that the planting is also of influence.

In general a chemical conversion takes place quicker at a higher than at a lower temperature. This gives rise to the assumption that through the effect of the temperature something changes in the bulb, through which just the reverse is brought about.

So in this way cooling would for instance be able to effect an increase or activation of the enzymes which control the reaction starch→sugars. By greater enzyme activity the retarding effect of a lower temperature would be more than compensated.

This explanation does not seem to me to be correct, for if this were the case, then the bulbs at planting would have to contain more enzyme

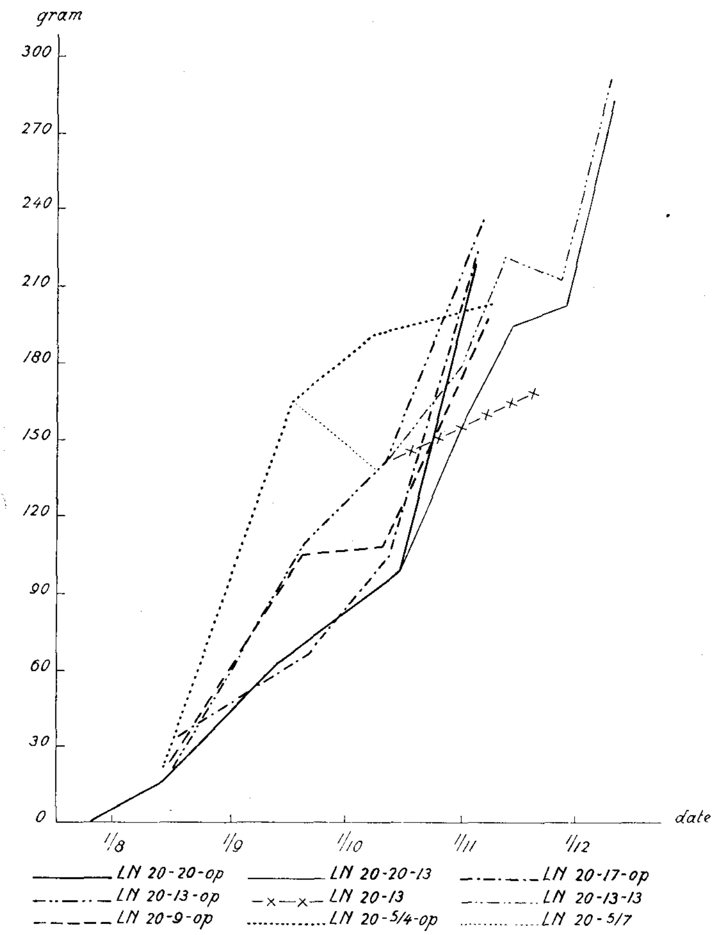

Fig. 3. Le Nôtre. Season 1934—1935. Sugar increase since 26th July + respired food per K.G. dry weight (in gr.).

in proportion to the cooling having been cooler. As all the lots planted in the open have been subjected here to the same temperature conditions, then the cooled bulbs should form sugar at the greatest rate. The reverse is however the case.

At planting LN 20—13—13 will contain just as many enzymes as LN 20—13—op and LN 20—20—13 as many as LN 20—20—op. The warmest planted bulbs should then form sugar quickest. Likewise LN 20—13—13 should be more rapid than LN 20—20—13. It is rather tardier, although the difference is not very great.

LN 20—5/7 in the period from 24[th] September till 9[th] October should also be quicker than LN 20—5/4—op. Instead of increasing, a part of the sugars is even re-formed into starch.

According to my point of view, the following explanation, is at least provisionally, more satisfactory. This explanation makes use of the principle of the moving equilibrium. This principle says, as far as it applies to the temperature, that with a process which takes place under evolution of heat, the entire quantity of substance formed increases by a lowering of the temperature. In other words the process only comes to an equilibrium at a higher concentration of the substance formed.

The analyses indeed show an influence of the temperature on the equilibrium concentration of the non-reducing sugars (Table II and fig. 1 of the preceding publication).

The bulbs of the season of 1934—1935 were lifted on 20th July 1934. Round about this date the outside temperature was about 20° C. From 20th July till 24th July the bulbs were in an unwarmed bulb house, where the temperature will also have been in the neighbourhood of 20° C. On the 24th of July the bulbs were transferred to a room where the temperature was regulated exactly at 20° C. On 26th July they had therefore been exposed to 20° C. for some time. The fact that on 17th August the percentage of non-reducing sugars is equal to that on 26th July indicates that an equilibrium has been reached. Only such a quantity of sugar is formed as is absorbed by the respiration. If the parcel is divided on 17th August and the bulbs transferred to 5, 9, 13 and 17° C. resp. remain at 20° C., the latter will be near to their equilibrium. The others are further removed from it, in proportion to their having been put in a lower temperature and as a reaction passes quicker in proportion to its being removed further from its equilibrium, the sugar formation, for this reason will take place more rapidly as the temperature is lower. This tendency is apparently stronger than the retarding effect of the fall of temperature.

It is not known whether the concentrations at planting on 9th October are equilibrium concentrations. The differences would perhaps have been still greater on the treatment being prolonged further.

The fact that all lots after having been planted in the open a month contain about the same amount of non-reducing sugars, proves that under the influence of the equal temperature, average 9° C., they tend to reaching the same equilibrium concentration. This equilibrium concentration according to the principle of the moving equilibrium is clearly lower than the concentration attained at 4° C. on 9th October.

Both the lots too planted at 13° C., notwithstanding their differences at the time of planting, tend to attain the same equilibrium concentration which, as was to be expected, is lower than with the bulbs planted in the open.

Finally, the behaviour of LN 20—5/7 can still be mentioned. Its concentration is lower on 9th October than on 24th September, apparently because the concentration at 5° C. had then already become greater than corresponded to the equilibrium at 7° C.

The question arises whether the principle of the moving equilibrium can really explain everything here. The hydrolytical splitting of starch into sugars is a process which is attended with a very small evolution of heat and therefore only a slight equilibrium shifting can be expected.

BĚLEHRÁDEK (2) however points out that the position of an equilibrium is also shifted a.o. by viscosity- and chemical changes of the medium.

The possibility exists, that the bulb, at a lower temperature has a different percentage of water than at a higher temperature. Table II of the preceding publication (1) shows that LN 20—5/4—op indeed contains a little more water than e.g. LN 20—20—op. But LN 20—9—op contains as much as LN 20—20—op, although at 9° C. there are far more sugars. In spite of the slight differences in the entire amount of water, yet at a lower temperature a greater part of the water can be "free", be available to dissolve the sugars in it and in this way to dilute the concentration through which the sugar formation takes place more easily.

It is not inconceivable that the temperature influences one or more of the mentioned factors and in this way indirectly affects the rate of the sugar formation.

Although it has clearly appeared that before and shortly after the planting the non-reducing sugars are formed more rapidly at a lower than at a higher temperature, the behaviour of M 3 w 17—9—9/17 shows with respect to M 3 w 17—9—9 that some time after the planting the sugar formation is on the other hand promoted by a higher temperature. Should this observation be correct, then this contrast is difficult to explain. Apparently during the course of the development an exchange of limiting factors takes place. At first the conversion is controlled by a process, which at a low temperature takes place quickest; after that by a process the rate of which increases by a rise of temperature.

Morphological development and carbohydrate metabolism.

Finally some remarks about the question which relationship there exists between the morphological development and the carbohydrate metabolism.

The quickest extension of the young plant is at about 13° C. in the period from 17th August until planting on 9th October. This low optimum can be explained by two factors:

1^{0}. The direct temperature influence through which in the range of 5 to 20° C. the growth is quicker in proportion to the bulbs being in a warmer place.

2^{0}. Through the greater store of non-reducing sugars at a lower temperature, the plant will have a tendency to grow more rapidly at a lower temperature. Presumably the reducing sugars here too are the direct source of food, but their forming is accelerated by a higher concentration of the non-reducing sugars.

Through the co-operation of both these factors, a curve is formed, the optimum of which is evidently at about 13° C.

In the introduction it has also been discussed, that the optimum shifts to higher temperatures in the course of the season. On the ground of the above, this is possible if the forming of the sugars is promoted by a higher temperature than is the case earlier in the season.

With the Murillo's of 1929—1930 the amount of reducing sugars during the first period after planting at 9° C. and in the open, is greater than at 17° C. (M 17—17—17). This may be partly due to the smaller concentration of the non-reducing sugars, and presumably partly to a stronger respiration at 17° C. After 1st January the reducing sugars of M 17—17—17 increase; even much faster than in the open with M 17—17—op. Round about this time there exists a possibility that the optimum is at a higher temperature.

The fact too, that previous cooling shifts the optimum quicker, can be connected with the carbohydrate metabolism. This already appears from the often discussed behaviour of M 3 w 17—9—9/17 with respect to M 17—17—17. The former lot can at 17° C. increase its percentage of reducing and non-reducing sugars quicker and is therefore already at an earlier date capable of growing faster at a higher temperature.

From this it appears that the "after-effect" of the cooling applied in summer exists in an increase of the capacity so as to form a sufficient amount of sugars from the stored starch at a higher temperature.

If the lots of 1929—1930, which in the middle of December contain about equal amounts of reducing and non-reducing sugars were to be forced into bloom at 20° C. or higher in about that period, it would appear that the rate of extension of the plants is very different. Judging from their equal concentration of sugars this was not to be expected. The differences in the rate of stretching are however understandable, when we think, that as a result of their dissimilar summer treatment they have an unequally strong capacity to form useful sugars sufficiently fast at a high temperature.

This however does not mean that the "after effect" exclusively exists in the possibility of a more rapid sugar production. No more than the whole developing process can only be explained by the carbohydrate metabolism. We only intended to show that certain quantitative differences in the carbohydrate metabolism can be connected with quantitative differences in the rate of the morphological development.

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