RESPIRATION OF APPLE TWIGS IN RELATION TO WINTER HARDINESS[1]

W. A. De Long,[2] J. H. Beaumont,[3] and J. J. Willaman[4]

(WITH SEVEN FIGURES)

The cold resistance of woody plants has hitherto been studied mainly from the static viewpoint. The problem has been attacked, in most cases, either by means of chemical analysis of whole ground tissues of hardy and tender plants or by microscopic analysis of the tissue structure. A minor amount of study has been directed towards microchemical determination of the distribution of food reserves within the tissues and the estimation of the seasonal changes in such food reserves. Some attention has also been devoted to measuring the amount of colloidal constituents and their degree of dispersion.

Life phenomena are, however, dynamic in character, and one of the most fundamental properties of living matter is its reaction to stimuli, or irritability. The fact of irritability may be demonstrated by the application of the most diverse agents, as anaesthetics, electrical shock, and changes in temperature. One of the most delicate means of showing that stimulation is so produced is by measurement of the changes which occur in respiration as evidenced by the rate of consumption of oxygen or of evolution of carbon dioxide. The present writers conceived the idea that respiration might throw some light on the phenomenon of winter hardiness in apple twigs. The present paper is a report of their findings.

It seems best to proceed directly with the description of the experiments, and to mention pertinent literature in connection with the discussion of our results.

Methods and apparatus

CO_2 production

The low temperatures desired were obtained by means of an ice-cream storage cabinet of the type described previously by us (**3**). Because of certain difficulties in temperature control encountered in the application of the air-bath method previously described, an oil bath was substituted in the second year of the work on the present problem. To this end the individual chambers of the cabinet were supplied with galvanized cans about one and

[1] Published with the approval of the Director as Paper no. 922, Journal Series, Minn. Agr. Exp. Station.

[2] Now at Acadia Univ., Wolfville, Nova Scotia.

[3] Now at North Carolina Agricultural Experiment Station, Raleigh.

[4] Now with Röhm and Haas Co., Bristol, Pa.

one-half inches smaller in diameter than the chambers themselves and of such a height as to permit of the insertion into the top of the chamber of a cover carrying the necessary controls. These cans were filled with petroleum distillate. A brass pump of the type ordinarily used in water thermostats was placed in each chamber to maintain circulation. Heat was supplied as necessary by 110 volt, 125 watt electric heaters of the knife-blade type. The heaters were activated by means of the Harvey thermo-regulator relay system previously described (**3,** fig. 4). The temperature of the oil baths was thereby maintained constant within a range of about 0.1° C.

In all of the work presented here continuous aspiration was used, so as to avoid the effects of accumulated CO_2 previously emphasized (**14**). The carbon dioxide evolved was collected in absorption cells of the coil type already described (**3,** fig. 6), and absorbed in approximately 0.2 N standard NaOH. The semi-closed gas circuit system of circulation activated by an electric blower, previously described (**3,** p. 494) was used in the preliminary work of the season of 1926–7. Later, a one-way flow with compressed air was used.

In the case of the latter method the ingoing air was washed by passing through two consecutive absorption towers filled with 40 per cent. NaOH solution. These towers were fitted with absorption coils similar to those used in the measurement of the carbon dioxide evolved, with the exception that the central tube was lengthened at the lower end to the extent of about six inches in order that more pressure might be exerted upon the coil without forcing the air out at the bottom rather than up through the coil in the normal manner. This increase in length necessitated the use of larger containers for the coils, that is, about 1000 cc. The rate of flow was about 18 liters per hour through the washing tower, or 6 liters per hour for each of the three sets of absorption coils. The volume of the respiratory cells used being approximately 650 cc. when empty, or 450–550 cc. when filled with twigs, the rate of flow used assured a complete renewal of the gases in the system once every 10–15 minutes. For a carbon dioxide production of 100 mg. per kilogram-hour, which was about the maximum found in the present studies, the concentration of CO_2 in the atmosphere surrounding the twigs would not be in excess of 0.2 per cent.

After passing the washing towers, the air was led through a calcium chloride drying tower to prevent the freezing out of moisture at the low temperature points in the system. It was then passed over the samples of twigs and conducted thence to the absorption coils, two of which were placed in series in order to ensure complete removal of the evolved carbon dioxide from the outgoing gases. So far as possible all connections between the washing towers and the absorption cells were of copper tubing, the necessary rubber tubing joints being made as short as conveniently possible. The effi-

ciency of the washing towers, and the tightness of the joints were tested at frequent intervals during the course of the work. This was done by running the empty apparatus exactly as when in use for periods of 20 hours or more and determining the amount of carbon dioxide in the absorption cells at the end of that time. The average of all such blank runs was 0.04 mg. carbon dioxide per hour, which is well within the experimental error of the titration procedure for intervals of 10 hours or less.

The twigs were placed in copper respiratory cells similar to those of glass previously described by us (**3,** fig. 2) with the exception that the inlet tube was not coiled but simply passed down one side of the cell, across the bottom, and up the other to near the top, where it entered. The temperature within these cells was checked with a thermograph and found to be the same as that of the external bath, thus showing that the ingoing air was cooled to the desired extent during its passage through the straight inlet tube. As in the case of the glass containers, the outgoing gases were drawn off near the bottom of the cell. The top was closed with a heavy rubber stopper.

The samples usually consisted of 30 one-year twigs. These were placed in the respiratory cells as soon as possible after removal from the tree, the interval from tree to cell being about 30 minutes on the average and rarely more than 35 minutes. The cells were then immersed in the oil bath of the desired temperature, connected to the washing towers and absorption cells, and aspiration commenced immediately. Before titration, the carbonate in the absorption cells was precipitated with 10 per cent. $BaCl_2$ solution and the residual NaOH immediately titrated with approximately 0.1 N standard solution of HCl, using phenolphthalein. The results are uniformly expressed on the basis of the number of mg. of CO_2 produced per kilogram (fresh weight) of twigs per hour.

Chemical analysis of twigs

The twigs were ground in a motor-driven pencil sharpener immediately after removal from the designated storage chamber. The material, as soon as ground, was thoroughly mixed by shaking, and 25-gram samples withdrawn for analysis. These samples were weighed as quickly as possible on a balance accurate to 0.05 gram and immediately dropped into sufficient boiling 95 per cent. alcohol containing 0.2 gram calcium carbonate to make a final concentration of approximately 80 per cent. Heating was continued for one-half to three-quarters of an hour. The ground wood was subsequently extracted with alcohol in a Landsiedl extractor until a negative Molisch test was obtained. After making to volume the alcohol was removed from an aliquot by distillation under reduced pressure at a temperature of 40–50° C. After clearing with neutral lead acetate and removing the excess

lead with potassium oxalate, total and reducing sugars were determined on the aqueous filtrate. The QUISUMBING-THOMAS reduction method (**11**) was used and the reduced copper was estimated by permanganate titration after addition of saturated acid ferric sulphate solution according to the official methods of the Association of Official Agricultural Chemists. Acid-hydrolysable "starch" was determined on the alcohol-insoluble residue using the official method, except that reduction was carried out as indicated above.

RESPIRATION OF EXCISED TWIGS

In all cases in this paper the term "respiration" should be considered as synonymous with "CO_2 output," or "CO_2 evolution." On account of the very great experimental difficulties encountered by WILLAMAN and BEAUMONT (**13**) in their preliminary study of the respiration of apple twigs at low temperatures, in which measurement of the CO_2 production of twigs while still attached to the tree was attempted, it was decided to use only excised twigs in the present investigation. Such being the case it became of importance to determine to what extent the wounding of the twigs in their removal from the tree affected the subsequent CO_2 production. To this end uniform samples of twigs were collected at different times, divided into two portions, one of which was aspirated as collected while the twigs of the second portion were first cut in two, thus giving three cut surfaces per twig in place of one. Assuming that the effect of the second cut was practically identical with that of the first, one-half the difference between the amounts of CO_2 evolved by the first and second portions of the sample would be equal to the amount liberated from the cut surfaces and produced as the result of wound stimulation. This proved to be a relatively small and fairly constant fraction of the total, namely, 5–8 per cent. as shown by the data presented in table I.

EFFECT OF CHANGE OF TEMPERATURE ON THE RESPIRATION RATE.—When excised twigs are held at a constant temperature the rate of CO_2 evolution becomes fairly constant after 20 or 30 hours, but after 150 or 200 hours a steady decline is noticed. In this paper, however, we will deal with time periods of less than 30 hours, and in this case we do not have an equilibrium rate.

Of the first two samples of twigs investigated, the one (A) was held at $+6°$ C. and the other (B) at $-2°$ C., and it was found that while the respiratory level of (B) became relatively constant after the first few hours of aspiration, that of (A) continued to show a very appreciable falling off in rate even after 200 hours. It was then decided to reverse the temperatures of the two samples in order to determine the effect of small temperature changes upon the intensity of respiration at this low level. The result

TABLE I

EFFECT ON CARBON DIOXIDE OUTPUT OF CUTTING TWIGS

VARIETY	NO. OF CUTS	DATE	TOTAL CO_2	CO_2 PER KG.	DIFFERENCE	TOTAL CO_2, DUE TO ONE CUT
			mg.	*mg.*	*per cent.*	*per cent.*
Duchess	One	Feb. 29	194	916		
	Two		197	1019	+11.2	5.6
Florence	One	March 4	195	1269		
	Two		250	1446	+14.0	7.0
Hibernal	One	March 14	134	789		
	Two		152	919	+16.5	8.25
Hibernal	One	March 24	409	2601		
	Two		449	2948	+13.4	6.7
Hibernal*	One	March 31	58	378		
	Two		66	416	+10.2	5.1
				Mean difference (per cent.).....		6.53 ± 0.26

* Aspiration at −10° C. All the preceding determinations were made at +6° C.

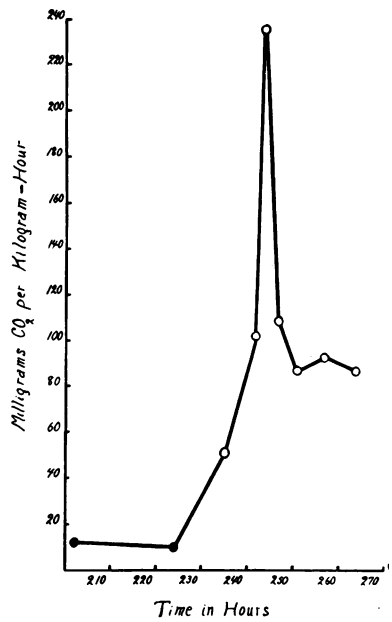

FIG. 1. Output of CO_2 by apple twigs at 6° C. after storage at −2° C. Solid dots represent measurements at −2° C., and circles those at 6° C.

of changing sample (B) from $-2°$ C. to $+6°$ C. is represented graphically in fig. 1, a conclusive demonstration that small upward variations in temperature within this range were capable of greatly accelerating this respiratory rate. On the other hand, changing the temperature of sample (A) from $+6°$ C. to $-2°$ C. merely caused the rate of respiration to fall to a level practically identical with that previously exhibited by sample (B) at that temperature.

These results were repeatedly confirmed. Fig. 2 contains representative curves of respiration over periods of 90 hours, when the temperature of

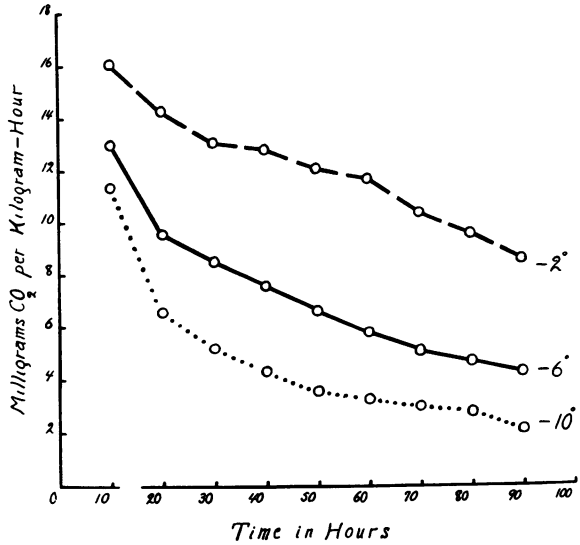

FIG. 2. Output of CO_2 by apple twigs at three lower temperatures after storage above 0° C.

the respiration period is *lower* than that of the previous period. It will be seen that there is an immediate falling off in CO_2 output, with a tendency to assume a level after some time. On the contrary, when the temperature of the experimental period is *higher* than that of the previous period, the CO_2 production always rises to a peak within a few hours, and then declines to a more or less constant value. Furthermore, the lower the previous temperature, the greater is the peak production. This is illustrated in fig. 3.

If this phenomenon obtains generally, it is obvious that, in attempting to determine the respiratory rate of plant material, cognizance must be taken of its previous temperature history. We thought that one way of determining whether this is the case would be to take samples at intervals during the winter, noting the temperatures during the previous 24 hours, and measuring the rate of CO_2 production at 6° C., which temperature would be higher than that of the orchard for all samples.

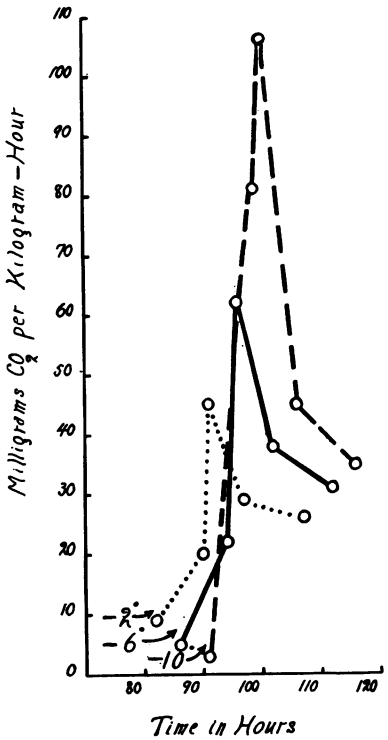

FIG. 3. Output of CO_2 by apple twigs at 6° C. after storage at −10° C., −6° C., and −2° C., respectively, for about 90 hours.

A series of 64 samples was taken from Dec. 21, 1927, to April 15, 1928. After placing in the respiration chamber the CO_2 was swept out continuously and measured every one or two hours until the peak was passed. The data will be presented in two groups. The first involves 8 samples taken during a period of unusual change in temperature. They are presented in fig. 4. The maximum rate of CO_2 production is plotted for each sample. These curves are clearly the mirror images of each other. This means, of course, that the lower the temperature previous to the period at 6° C., the greater is the CO_2 production at that temperature. The second group includes all the samples secured during the whole winter period. The results were analyzed statistically.

The biometrical constants determined are summarized in table II. In this table the letters A, B, C, D, E, F, G, and R have the following significance:

A = the temperature in the orchard at the time of collection.

B = the maximum temperature attained on the day of collection.

C = the minimum temperature attained on the day of collection.

D = the rate of carbon dioxide production (in milligrams per kilogram-hour) during the first hour of aspiration.

E = the maximum rate of carbon dioxide production attained (milligrams per kilogram-hour).

F = the total amount of carbon dioxide produced in the first twenty-four hours of aspiration (milligrams per kilogram).

G = the rate in milligrams per kilogram-hour for the second and third hours of aspiration.

R = the total amount (F) of carbon dioxide produced in twenty-four hours minus the amount produced in the first hour (D) in milligrams per kilogram.

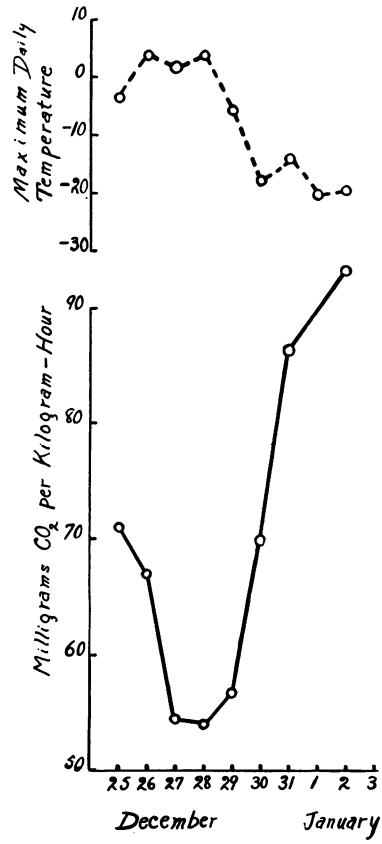

FIG. 4. Effect of orchard temperatures on rate of CO_2 output by apple twigs.

It should be noted that in the case of the temperature values a uniform increment of thirty degrees was added. By this means all temperature readings were raised to positive values.

In the calculation of the biometrical constants reported the following formulae were used:

$$\text{Mean} = S(X)/N.$$

$$\text{Standard deviation} = \sqrt{S(X)^2/N - \overline{X}^2}.$$

$$\text{Coefficient of correlation} = \frac{S(XY)/N - \overline{X}\,\overline{Y}}{s_X s_Y}.$$

Coefficient of correlation of a part to the remainder of the whole,* or

$$r_{XY} = \frac{S(XY) - S(X)^2/N - \overline{X}\,\overline{Y}}{s_X s_Y}.$$

(Note: This was used only in the case of the correlation coefficient for DR.)

$$\text{Probable error or mean} = 0.6745 \cdot s_X \sqrt{N}.$$

$$\text{Probable error of correlation coefficient} = 0.6745 \cdot (1 - r^2)/\sqrt{N}.$$

In these formulae the letters have the significance: S = summation, X = the values of one variable, Y = the values of the other variable, N = the number of observation, r = the coefficient, s = the standard deviation, $\overline{X}$ and $\overline{Y}$ the means of the respective variables.

TABLE II

RELATION BETWEEN CO_2 OUTPUT AND PREVIOUS ENVIRONMENTAL CONDITIONS. FOR EXPLANATION OF SYMBOLS SEE TEXT

	MEAN	STANDARD DEVIATION	CORRELATION COEFFICIENT
A	23.127	7.144	AD ..... − 0.049 ± 0.084
			AE ..... − 0.458 ± 0.067
			AF ..... − 0.424 ± 0.069
B	27.195	7.342	BD ..... − 0.078 ± 0.084
			BE ..... − 0.432 ± 0.069
			BF ..... − 0.399 ± 0.071
C	19.469	7.458	CD ..... − 0.121 ± 0.083
			CE ..... − 0.525 ± 0.061
			CF ..... − 0.490 ± 0.064
D	36.420 ± 1.252	14.764	DE ..... + 0.751 ± 0.037
E	53.456 ± 1.140	13.528	GE ..... + 0.857 ± 0.022
F	1175.453 ± 24.351	288.809	DR ..... + 0.745 ± 0.038

* The writers are indebted for permission to make use of this formula to the originator, Dr. J. A. HARRIS, late Professor of Botany, University of Minnesota.

The results obtained support the conclusion that the rate of respiration is proportional to the increase in temperature to which the twigs are subjected when they are brought from the orchard to the respiration chamber. In addition, another very interesting and important relation is revealed. Thus it is found that a very good correlation exists between the rate of carbon dioxide production in the first few hours of aspiration and the maximum carbon dioxide production attained. The existence of this correlation indicates that in the comparison of the respiration of hardy and tender varieties of apple it is necessary to make determinations during only the first four or five hours.

Of the three temperature values, the minimum gives a better correlation with both the initial and maximum rates, and also with the total production of carbon dioxide, than either the temperature at the time of sampling or the maximum temperature of the day. This was to be expected from the observations above on the influence of extent of elevation of temperature upon carbon dioxide evolution. This is further supported by the fact that the sampling temperature, which was lower than the daily maximum (since the collections were made in the morning), shows a better correlation with both the maximum rate and the total for 24 hours than does the maximum. It is noteworthy also that the correlation of all temperatures with the *maximum rate* of carbon dioxide production is higher than with the initial rate or the total in twenty-four hours. In other words, so far as the effect of temperature is concerned the maximum rate is the most significant measurement. However, as shown by r_{DE} there is a significant relation of the initial to the maximum rate, a relation which it is not unlikely a refinement of technique would show to be more pronounced than is here indicated. Thus, under the experimental conditions adopted, the measurement of the initial rate has been the least accurate of any of the observations made, by reason of the facts that the initial hour was, as a rule, the shortest time interval over which measurements were made, that the amount of carbon dioxide evolved during the first hour was usually less than that given off at any subsequent hour under observation, and that an hour or two was required to bring the twigs to 6° C. In addition, the maximum rate measured is at best only an approximation, especially when the rise and fall in carbon dioxide production is rapid. Early experience showed that a maximum rate could be expected to occur in the vicinity of the 8th to 10th hours. Accordingly, readings were taken more frequently up to this point than later; nevertheless, no claim can be made that the true peak of the curve was more than approximately located.

It is our opinion, therefore, that the actual correlation between the initial rate and the maximum attained is considerably higher than that

actually found, and that a refined technique might make possible the use of a shorter observational period, possibly as low as four hours.

Before offering an explanation of the above results, it might be desirable to summarize them in concise form: (1) When twigs are brought from a lower to a higher temperature for the measurement of CO_2 production, the latter rises to a peak and then declines to a more or less constant value. (2) The lower the previous temperature, the greater is the peak value. (3) The latter principle holds not only for excised twigs in storage, but for twigs on the trees.

Two possible explanations of the above phenomena occur to us. They may be stated as follows: (1) When plant tissues are subjected to the action of low temperatures very considerable changes occur in the tissue-water equilibrium. It is a generally accepted fact that at temperatures below 0° C. ice crystals are formed, not within the individual cells, but in the adjoining intercellular spaces. This withdrawal of water from living tissues must occasion far-reaching disturbances of cellular chemical equilibria and of physical organization. It is accordingly suggested that the apparent increase in carbon dioxide production observed to follow the elevation in temperature of apple twigs from levels below to levels above 0° C. is primarily the result of a temporary stimulation of carbon dioxide production resulting from that derangement of the tissue-water equilibrium. Examples of such low temperature effects upon chemical equilibria within living tissues are the well-known accumulation of sugar in potato tubers, and the increased production of lactic acid in the living muscle of the frog at $-2°$ to $-3°$ C. The postulated stimulation of carbon dioxide production in apple twigs may then be explained as the result of the increased effective concentration of respiratory substrate within the cells following withdrawal of water to the intercellular spaces. Another contributing factor may be increased hydrogen-ion concentration following accumulation of carbon dioxide.

The observed differences between twigs from hardy and from tender varieties of the apple may, on the basis of the above hypothesis, be related directly to the possession by the former of hydrophilic colloids in greater amount, or of more intense imbibitional capacity. On this assumption the water loss from the cells of hardy twigs would be less, and, consequently, the disturbance of normal cellular equilibria correspondingly less, than in the cells of the tender twigs. That is to say, the hardy twigs are more stable or less irritable than twigs of more tender varieties. Further, the return of water to the cells and the resumption of more or less normal metabolism would be more rapid in the case of the former than in that of the latter, a factor conceivably of importance in determining the survival of tissues under rigorous climatic conditions.

(2) Another possibility is that we are dealing here simply with the solution of CO_2 in the twig sap. This gas is increasingly soluble in aqueous media at decreasing temperatures. Therefore, the peak of CO_2 evolution represents the greater solubility of the gas at the previous lower temperature than at the temperature of measurement. Conversely, when measurement is made at a lower temperature than that of the storage, the resultant time curve represents the establishment of an equilibrium between the solubility of CO_2 in the tissue and its diffusion into the outer atmosphere. Evidence favoring this conception is offered in the article following this (**15**). It consists in measuring the dissolved CO_2 of twigs by submerging the latter in boiling alcohol in a closed system and distilling out the CO_2 under vacuum. The CO_2 is completely removed in an hour or so, and it is demonstrable that the amount of the dissolved gas varies inversely with the temperature, and that this is roughly of the same magnitude as that contained in the peak of the curves in this paper. This method had not been devised at the time the measurements in this paper were made; hence, it is utilized here merely as a possible explanation of the observed facts.

Relation of CO_2 production to winter hardiness

A number of varieties of apple known to be of varying degrees of hardiness have been compared in respect to the evolution of CO_2 at 6° C. Only samples collected on the same day and so far as possible subjected to identical conditions subsequent to removal from the tree have been compared directly. Twigs obtained from several different sources and possessing correspondingly variant histories as noted below were utilized.

On January 19, 1928, samples of one-year twigs of Hibernal, Duchess, Charlamoff, McIntosh, Haralson and Jonathan were collected at the University Fruit Breeding Farm. These twigs were taken between the hours of 11 A. M. and 2 P. M., tied in bundles, and kept at a temperature as near as possible to that obtaining in the orchard on that date until received at University Farm, when they were placed in a cold storage room in which the temperature was maintained at 0° C. $\pm$ 2°. Samples were taken for measurement at intervals during the period January 21–31. That the respiratory power of the material did not vary greatly during this period is indicated by the results obtained with Hibernal (table III). The results obtained in the comparison of these different varieties are presented in fig. 5, the curves in which are based either upon single determinations or the average of two or more determinations, as follows:

Beaumont and Hildreth have devised a system of evaluating the amount of winter injury in apple twigs, by the degree of browning in certain specific regions of the twigs. By means of this they have been able to

TABLE III

CONSTANCY OF CARBON DIOXIDE EVOLUTION BY HIBERNAL TWIGS COLLECTED ON JANUARY 19TH, 1928

DATE ANALYZED	NUMBER OF TWIGS	WEIGHT OF TWIGS	CARBON DIOXIDE PER KILOGRAM-HOUR AT 6° C.						
			AT 30 MIN.	AT 3 HRS.	AT 7 HRS.	AT 10 HRS.	AT 15 HRS.	AT 20 HRS.	AT 30 HRS.
			mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Jan. 24.....	30	137 g.	22	32	39	41	40	39.5	38.0
Jan. 26.....	40	154 g.	22.5	31	39	41.5	42.5	42.7	.....
Jan. 31.....	30	135 g.	24	36	38.5	39	39.2	38.5	36.5

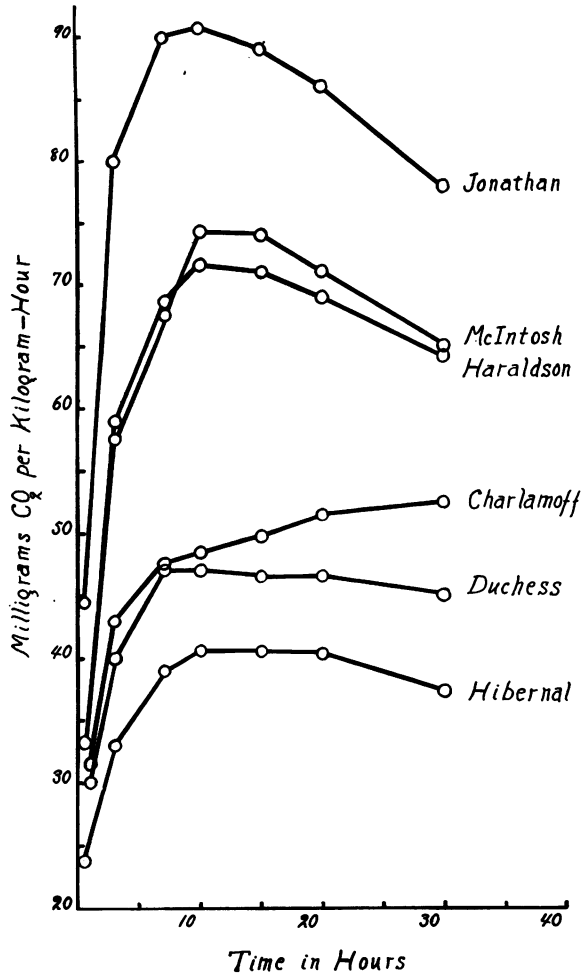

FIG. 5. CO_2 output of 6 varieties of apple twigs of varying degrees of winter hardiness.

TABLE IV

DETERMINATIONS USED IN FIG. 5

VARIETY	NO. SAMPLES	DATE DETERMINED	AVERAGE WEIGHT OF SAMPLES
Haralson	3	Jan. 24, 26, and 31	129
Duchess	1	Jan. 21	126
Charlamoff	2	Jan. 21 and 24	131
McIntosh	2	Jan. 27 and 29	114
Haralson	3	Jan. 26, 27 and 29	91
Jonathan	1	Jan. 31	136

assign definite numerical hardiness values by which the varieties may be compared. Table V contains their values.* The lowest values indicate least browning and hence greatest hardiness.

It will be seen that the order of magnitude of CO_2 production in fig. 5 is the same as the order of hardiness for these 6 varieties. Thus the hardiest varieties evolve the least CO_2 under the conditions of measurement, and the tenderest evolve the most. Not only is the serial order the same, but the quantitative relations are rather regular; thus the hardiness values of the 6 varieties in fig. 5 from bottom to top are 6, 8, 10, 12, 16, and 24.

TABLE V

BEAUMONT AND HILDRETH'S HARDINESS VALUES FOR APPLE VARIETIES

VARIETY	AVERAGE BROWNING FOR 4 YEARS— 1924–1928	AVERAGE BROWNING FOR 3 YEARS— 1924–1927
Dolgo	2.0	2.3
Hibernal	7.0	6.0
Oldenburg (Duchess)	7.0	7.7
Charlamoff	.....	10.0
Patten	11.8	11.0
Haralson	14.5	12.0
Wealthy	15.5	12.7
Anisim	.....	14.0
McIntosh	21.2	15.7
Minnehaha	20.5	17.0
Fameuse	22.2	16.3
Cortland	.....	18.3
Salome	.....	19.3
Delicious	25.0	20.0
Wolf River	26.8	21.0
Winesap	29.0	22.0
Jonathan	28.5	23.7
Ben Davis	28.5	24.0
Sugar Loaf	.....	24.7
King David	.....	25.7
Lansingburg	.....	25.7
Yellow Belleflower	.....	30.3
Tompkins King	.....	36.3

These results are contrary to the preliminary measurements of WILLAMAN and BEAUMONT on Charlamoff and Delicious (**13**), in which the hardier seemed to emit more CO_2 than the tender. The explanation is that at the time those measurements were made we did not realize the very great effect

* Although these values have not been hitherto published, a description of the method of arriving at the values is given by B. H. WILSON in Sci. Agr. **10**: 598–606. 1930.

of the previous temperature. The two varieties were measured alternately and no attention paid to the temperature previous to each measurement. We can see now that this fact could completely jeopardize any varietal differences.

Since examination showed that considerable winter injury had already occurred in certain of the samples reported on above, as indicated by the "browning test," particularly in McIntosh, Haralson, and Jonathan, it appeared possible that the increased CO_2 production shown by these varieties over that of the more hardy sorts might be the result of this winter injury rather than due to any inherent difference in respiratory power. Accordingly, samples of Red Duchess and Delicious, in which practically no frost injury could be detected, were obtained from the tubbed breeding stock in the cold storage cellar at the Fruit-breeding Farm. These samples were taken on February 10th, held in the cold cellar of the Horticultural Build-

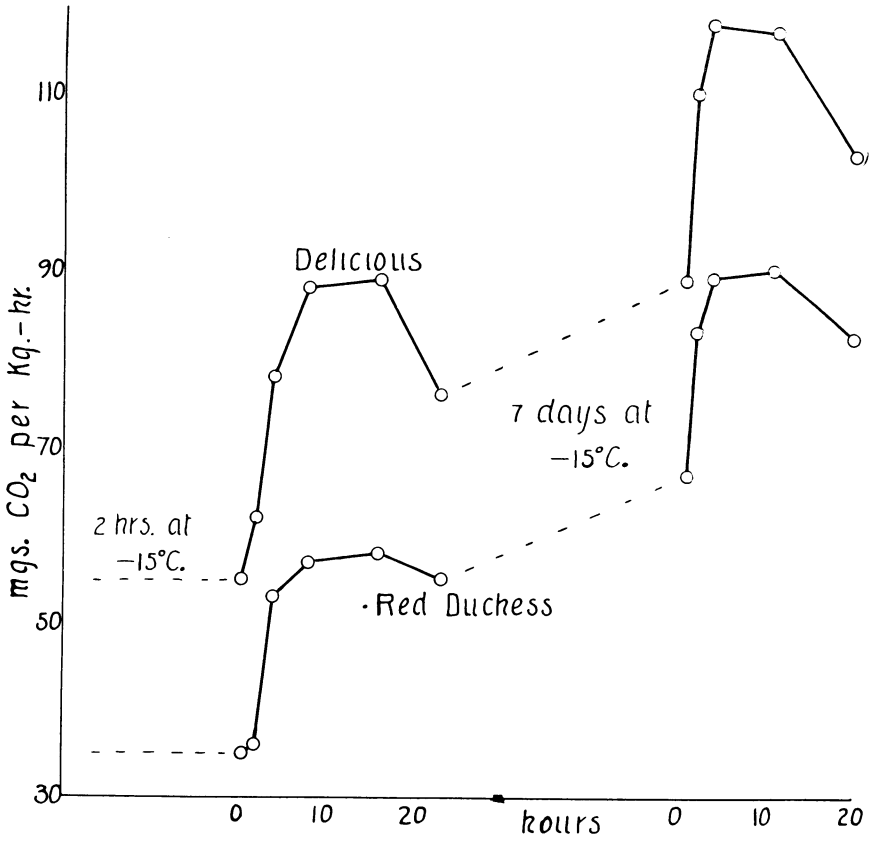

FIG. 6. Accumulation of CO_2 in apple twigs stored at −15° C. These curves also illustrate a varietal difference due to hardiness, Duchess being hardier.

ing at University Farm over-night at a temperature of about 5° C., and then, on the morning of February 11th, subjected to a temperature of – 15° C. for two hours before aspiration was commenced. The temperature changes occurring subsequent to removal of these twigs from the long-continued even temperature of the storage cellar at the Fruit Farm probably account for the relative high rate of carbon dioxide production found in both these samples. No more of this material was available. Consequently, after twenty-four hours' aspiration the twigs were replaced in the storage room at – 15° C., where they were held for seven days. At the end of this time they were again aspirated at 6° C. for a second twenty-four hour period. The results obtained are shown in fig. 6. It will be noted that the respiratory level has been raised by holding at – 15° C. in the case of both varieties, but that the *relative* difference between the hardy Duchess and the tender Delicious has not been materially altered. It is therefore considered that the differences shown in fig. 6, as well as in fig. 7, are due to inherent varietal qualities rather than to low temperature injury.

Further data respecting the relative rates of carbon dioxide production in hardy and tender varieties were obtained on materials of a more restricted range in hardiness, none of which showed appreciable injury. Such was the case for the comparison of Patten with Duchess of Oldenberg, and Florence with Hibernal, the former variety in each instance being the less hardy. Samples were taken on 4 different dates for the first pair and on 5 for the second. The data for the various samples were averaged, and

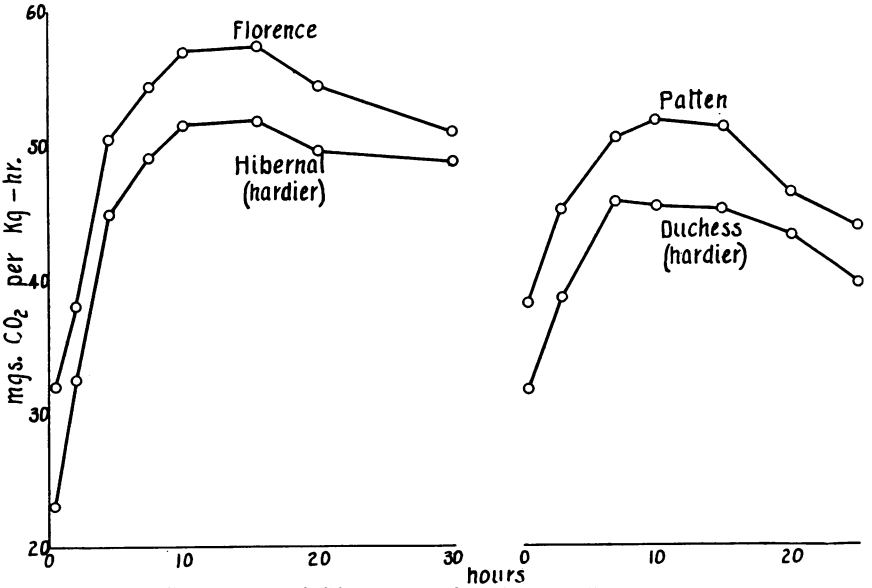

FIG. 7. Comparison of CO_2 output of varieties differing in hardiness.

are presented in fig. 7. The mean difference with its probable error, the standard deviation, and the deviation from a random distribution were calculated for these data, and found to be as follows:

TABLE VI

STATISTICAL STUDY OF DIFFERENCES BETWEEN NON-HARDY AND HARDY APPLES

VARIETIES	MEAN DIFFERENCE	STANDARD DEVIATION OF DIFFERENCE	DEVIATION FROM RANDOM DISTRIBUTION
Florence–Hibernal	$\pm$ 5.4903 $\pm$ 0.6494	5.3605	+ 9.5 $\pm$ 1.8777
Patten–Oldenburg	$\pm$ 5.9425 $\pm$ 0.4088	4.0408	+ 18.0 $\pm$ 2.2483

For the first two varieties the mean difference is thus seen to be more than eight times its probable error, while the deviation from a random distribution is more than five times as great as the probable error. In the case of Patten and Duchess the differences are even more significant, the corresponding values being nearly fifteen and more than eight times their probable errors, respectively.

We were able to make still another comparison of varieties during the winter of 1928–9. Eleven varieties were secured at the University of Minnesota Fruit Breeding Farm on Dec. 28, stored at – 15° C., and analyzed during the next few days. The data secured are given in table VII. Before placing in the respiration chamber the twigs in series A were kept at 20–23° C. Those in series B were placed in the chamber directly from the storage chamber. Because of the small number of samples it was not considered feasible to calculate the ordinary coefficient of correlation, hence that by rank was used, according to the formula $r = 1 - 6\ (SD^2_k)/n^3 - n$.

Although in series A the agreement between hardiness and CO_2 production is only fair, that in series B is very good, and they both indicate the same relation in the samples of the previous winter. We therefore feel justified in concluding in general that the hardier an apple variety, the lower its intensity of CO_2 production under the experimental conditions described.

The question immediately arises as to whether the solubility of CO_2 in the twig sap can be invoked here also in explanation of these facts. Evidence is presented in the following paper by WILLAMAN and BROWN (15) that such may be the case: these varieties differ in their content of dissolved CO_2 in the same order as their hardiness, and in quantities comparable to the peaks in CO_2 curves shown above.

TABLE VII

CO_2 OUTPUT OF APPLE TWIGS AT 6° C. DURING FIRST SIX HOURS

VARIETY	HARDINESS VALUE*	SERIAL ORDER OF HARDINESS	A		B	
			THAWED BEFORE PLACING IN THERMOSTAT**		NOT THAWED	
			CO_2 PER KG.	SERIAL ORDER	CO_2 PER KG.	SERIAL ORDER
			mg.		*mg.*	
Hibernal	6	1	88	3	106	2
Duchess	8	2	80	1	98	1
Patten	11	3	105	8	133	4
Haralson	12	4	97	7	146	7
Wealthy	13	5	90	5	138	6
McIntosh	16	6	91	6	137	5
Fameuse	16	7	88	4	126	3
Wolf River	21	8	81	2	154	8
Jonathan	24	9	128	10	175	9
Sugar Loaf	25	10	119	9	212	10
Lyman Perfect***			93		.....	

* BEAUMONT and HILDRETH.

** Kept in laboratory for 30 minutes.

*** Not listed by BEAUMONT and HILDRETH.

Coefficient of correlation (by rank) between tenderness and CO_2 output:—

$r_A = +0.478 \pm 0.172$

$r_B = +0.818 \pm 0.074$

CHANGES IN COMPOSITION OF EXCISED TWIGS

It seemed desirable to determine to what extent chemical changes were occurring in the twigs under the experimental conditions used. Analytical results on a number of samples under various storage conditions are given in table VIII. The averages of the changes in composition at each temperature were calculated to a basis of 10 day periods, and are given in table IX.

No striking changes in composition occurred. Nevertheless, certain tendencies are apparent, which may be summarized as follows: (1) The moisture losses are not great. (2) With aspiration, there is a slight gain in starch and a loss in sugars. (3) Without aspiration there is not much change in starch, and a gain in sugars. (4) In other words, the accumulation of CO_2 in the air around the twigs, and hence its presumed accumulation in the tissues, causes an accumulation of sugar in the tissue, either because of reduced respiration, or because of a changed equilibrium between starch and sugar.

TABLE VIII

CHANGES IN COMPOSITION OF EXCISED TWIGS DURING STORAGE AT LOW TEMPERATURES

DATE	MOISTURE	REDUCING SUGARS		TOTAL SUGARS		ACID-HYDROLYZABLE "STARCH"		REMARKS
		FRESH	DRY	FRESH	DRY	FRESH	DRY	
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	
Feb. 3	46.0	1.52	2.82	5.47	10.93	10.8	20.0	At time of collection
	40.7	2.12	3.58	6.67	11.26	12.0	20.2	After 18 days without aspiration at −10°
	46.4	1.28	2.39	4.40	8.22	11.5	21.5	After 21 days aspiration at −2°
	41.2	1.32	2.25	4.51	7.67	12.6	21.4	After 21 days aspiration at −6°
	45.4	1.31	2.46	4.27	7.83	12.0	21.5	After 21 days aspiration at −10°
Feb. 26	43.9	1.26	2.25	4.19	7.47	12.3	21.9	At time of collection
	42.3	1.43	2.48	4.80	8.33	12.7	22.0	After 6 days without aspiration at −6°
	42.7	1.40	2.45	4.88	8.53	12.6	22.1	After 6 days without aspiration at −10°
	43.6	0.99	1.76	3.55	6.30	12.5	22.2	After 30 days aspiration at −2°
	41.9	1.28	2.20	4.58	7.88	13.0	22.4	After 30 days aspiration at −6°
	44.5	1.14	2.06	3.97	7.16	12.0	21.7	After 30 days aspiration at −10°

TABLE VIII—(*Continued*)

Date	Moisture	Reducing sugars		Total sugars		Acid-hydrolyzable "starch"		Remarks
		Fresh	Dry	Fresh	Dry	Fresh	Dry	
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	
March 29	47.9	1.28	2.46	4.43	8.51	11.1	21.4	At time of collection
	45.2	1.46	2.66	5.30	9.90	11.5	21.1	After 4 days without aspiration at −2°
	46.8	1.43	2.69	4.93	9.28	11.5	21.6	After 4 days without aspiration at −10°
	45.2	1.15	2.10	4.68	8.55	12.0	22.0	After 8 days aspiration at −2°
	47.0	1.17	2.21	4.27	8.06	11.6	22.0	After 8 days aspiration at −10°
April 6	48.9	1.12	2.19	4.05	7.93	11.5	22.5	At time of collection
	44.6	1.58	2.85	5.97	10.78	11.1	20.1	After 8 days without aspiration at −2°
	47.2	1.08	2.05	4.68	8.86	10.8	20.5	After 8 days without aspiration at −10°
April 16	45.4	1.20	2.20	3.58	6.57	10.1	18.5	At time of collection
	50.0	1.89	3.79	7.17	14.36	11.2	22.4	After 13 days without aspiration at −2°
	49.5	1.22	2.42	4.64	9.20	10.8	21.5	After 13 days without aspiration at −10°

TABLE IX

CHANGE IN COMPOSITION OF APPLE TWIGS PER 10 DAY PERIODS UNDER VARIOUS STORAGE CONDITIONS

CONSTITUENTS	DRY MATTER BASIS					
	WITH ASPIRATION			WITHOUT ASPIRATION		
	− 2° C.	− 6° C.	− 10° C.	− 2° C.	− 6° C.	− 10° C.
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Starch	+ 0.54	+ 0.44	+ 0.47	− 0.30	+ 0.11	+ 0.16
Reducing sugars	− 0.27	− 0.15	− 0.19	+ 0.85	+ 0.38	+ 0.26
Total sugars	− 0.43	− 0.53	− 0.69	+ 3.01	+ 0.86	+ 1.46
Moisture	− 1.50	− 1.50	− 0.43	− 2.70	− 2.50	− 1.20

These results cannot be emphasized strongly, because of the limited number of samples and the rather small differences in composition. Nevertheless, they are suggestive and are included here partly to augment the very sparse published analyses of apple twigs.

Discussion

It has been shown that storage of apple twigs at lower temperatures, followed by their elevation to a higher temperature, gives rise temporarily to a considerable increase in rate of CO_2 output. Furthermore, the rate at the higher temperature is greater, the lower the previous temperature of storage. The same facts obtain when twigs are not taken from storage, but measured immediately after taking from the tree. It has also been shown that the hardier varieties respond to such variations in temperature to a less degree than the tenderer. We have shown in another paper that the CO_2 dissolved in the twig tissue can probably account for most of this temporary flush, and that the quantity of this dissolved CO_2 is proportional to the tenderness of the variety.

These findings agree with many of the observations found in the literature of plant respiration. In 1885 DEHÉRAIN and MAQUENNE (**2**) first called attention to the CO_2 dissolved in plant sap and to its possible effect on respiration measurements. Later MAQUENNE (**6**) attempted to remove this CO_2 by evacuation, but was only partly successful. SIMON (**12**) has measured the CO_2 production of excised twigs of beech, red oak, basswood, and horse chestnut. The twigs were brought from various outdoor temperatures to 22.5° C. for measurement, and SIMON noted that the lower the previous temperature, the greater was the production of CO_2 during

the early part of the measurement period. This effect was so pronounced that he extended his measurements over a period of seven days in order to avoid that temporary flush.

Increasing the temperature within the range of normal growth has frequently been observed to produce a temporary increase in CO_2 evolution. A very definite effect of this nature was early noted by MÜLLER-THURGAU (**8**) in potato tubers, and has since been reported for many other types of tissue, as, for example, etiolated shoots of *Vicia faba* (PALLADIN, **10**), gladiolus bulb (ZALESKI, **16**), and possibly sweet potato tubers (JOHNSTONE, **5**) and bananas (OLNEY, **9**).

In the case of the sweet potatoes the tubers were stored at 15–20° C. for some time and then brought to 25° C. for CO_2 measurement. The curves show a peak at about the tenth day, followed by a decline to a constant level for another 20 or 30 days. The author says, "The slight increase in respiration shown by the curves on the fourth to seventh days is undoubtedly due to transferring the sweet potatoes to the higher temperature for respiration determination, but cured sweet potatoes do not respond so readily to such a change as uncured." This statement does not, of course, explain the temporary flush of CO_2. We cannot readily see how this peak could be due to dissolved CO_2 accumulated at the lower temperature, because it requires seven to ten days for dissipation. In the case of the bananas the respiration curves have a shape very similar to those of apple twigs; but again the peak comes during the second to fourth days. It is possible that in each case we are dealing with the slow evolution of dissolved CO_2, and that the thickness of the tissue, its wateriness and its solvent power for CO_2 determine the time required for an equilibrium to be established at a higher temperature. It might be mentioned here that, when twigs are submerged in boiling alcohol and evacuated, an hour and a half is required for complete removal of the dissolved CO_2; hence 5 to 10 days might be a plausible period for this purpose in a tissue like sweet-potato tubers, with only the normal circumstances for diffusion of the gas.

Respiratory activity in winter cereals has been more recently studied by GOVOROV (**4**) and by MARTIN (**7**). The former found that in winter wheat and rye the amount of CO_2 liberated was 300 and 800 mg. per hour per kilo of dry weight at 3° C. He also found that spring wheat and spring rye, under the same conditions, gave off considerably larger quantities of carbon dioxide, namely, 1800 and 1700 mg. respectively. It should be noted that the roots of these plants were removed prior to the determination of the respiratory rate, and also that during the twelve hours previous to this operation, the plants had been maintained at 0° C. The stimulation resulting from the wounding incident to the removal of the roots, and from the rise in temperature, may therefore be assumed to be, in part at

least, responsible for the fact that these results are considerably higher than those reported by MARTIN for similar material. The latter has determined the respiration of a number of varieties of winter and spring wheat and of rye at still lower temperatures. His results may be summarized as follows:

	Milligrams CO_2 per kilogram of dry weight per hour		
	At 0°	At −5°	At −10°
Wheat	182.5–259.3	141.3–188.6	50.3–127.0
Rye	516.9	165.8	70.8

In accordance with the results of GOVOROV, the hardier varieties were found to evolve less carbon dioxide than those less resistant to low temperatures, particularly at −10°. The relation between wheat and rye at 0° is also similar to that found by GOVOROV.

It should be noted that MARTIN used the same plants for the determination of the respiratory activity at the different temperatures and proceeded from the higher to the lower levels, allowing 12 hours for accommodation to the new temperature. This is important, since there is much evidence that the direction in which the temperature alteration occurs may cause appreciable differences in the results obtained at the new level.

Although the balance of the evidence is in favor of the view that increasing the temperature of plants brings about stimulation of the rate of respiration, there is, however, some contrary evidence. ZIEGENBEIN (**17**), for example, found no stimulation in respiration on raising the temperature of seedlings of vetch and lupine from 15–20 to 30° C. He used the continuous aspiration method and apparently ample time was allowed for the temperature equilibrum of the tissues with the surrounding atmosphere to become established at the different temperatures.

BLANC (**1**), also, has concluded that, in the case of etiolated shoots of *Vicia faba,* bean seedlings, and young leaves of rye, the respiratory transition is gradual from one temperature to another and without stimulative effects due either to rising or falling temperature.

Nevertheless, we are confident that, when plant tissue is brought from a lower to a higher temperature for the measurement of its CO_2 production, the resultant curve will typically not assume a level until after a preliminary peak has been passed. We further believe that this peak is largely explainable by the lesser solubility of the CO_2 at the higher temperature; and that the CO_2 of the peak of the curve represents that which was produced by respiration at some time previous to that of the measurement.

Summary and conclusions

1. It has been shown that wound respiration in excised apple twigs is a small and relatively constant fraction of the total respiration.

2. The rate of CO_2 production as measured at any temperature is conditioned by the previous temperature environment of the twigs. If the previous temperature has been higher, a constant level of CO_2 emission is gradually assumed. If the previous temperature has been lower, there is a peak of CO_2 evolution for several hours, after which a level is gradually attained.

3. The lower the previous temperature, the higher is the peak amount of CO_2. This was proved both by twigs kept in storage before measuring the CO_2 output, and by twigs taken directly from the orchard at various temperatures throughout the winter.

4. It is suggested that this temporary excess of CO_2 represents that which was produced during the previous period but which remained dissolved in the twig sap, and which diffuses out of the twigs at the higher temperatures because it then becomes less soluble.

5. Hardier varieties of apples show a lower peak of CO_2 evolution than do the tenderer. In fact, the order of increasing tenderness among 11 varieties coincides almost exactly to that of increasing CO_2 during the peak.

6. Twigs stored at $-2°$ C., $-6°$ C., and $-10°$ C. for periods up to 30 days with and without aspiration of the surrounding air did not show striking changes in chemical composition. However, without aspiration there seems to be slight gain in sugar; and with aspiration there seems to be a slight gain in starch and loss in sugars.

7. It is emphasized that in the measurement of the respiratory rate of any plant tissue the effect of dissolved CO_2, and hence the previous temperature history of the material, must be kept in mind.

MINNESOTA AGRICULTURAL EXPERIMENT STATION,
ST. PAUL, MINNESOTA.

LITERATURE CITED

1. BLANC, L. Recherches expérimentales sur l'influence des variations de température sur la respiration des plantes. Rev. Gén. Bot. **28**: 65–79. 1916.
2. DEHÉRAIN, P., and MAQUENNE, L. Sur la respiration des feuilles à l'obscurité. Acide carbonique reteuu par les feuilles. Compt. Rend. Acad. Sci. Paris. **101**: 887–889. 1885.
3. BEAUMONT, J. H., WILLAMAN, J. J., and DE LONG, W. A. Notes on apparatus for low temperature respiration studies. Plant Physiol. **2**: 487–495. 1927.

4. GOVOROV, L. I. The diverse characters of winter and spring forms of cereals in connection with the problem of hardiness in winter crops. Bull. Appl. Bot. & Plant Breeding (Trans. title) **13**: 525–559. 1922–3.
5. JOHNSTONE, G. R. Physiological study of two varieties of *Ipomoea batatas*. Bot. Gaz. **80**: 145–167. 1925.
6. MAQUENNE, L. Sur la respiration des feuilles. Compt. Rend. Acad. Sci. Paris. **119**: 100–102. 1894.
7. MARTIN, J. H. Comparative studies of winter hardiness in wheat. Jour. Agr. Res. **35**: 493–535. 1927.
8. MÜLLER-THURGAU, H. Über Zuckeranhäufung in Pflanzentheilen in Folge niederer Temperatur. Land. Jahrb. **11**: 751–828. 1882.
9. OLNEY, A. J. Temperature and respiration of ripening bananas. Bot. Gaz. **82**: 415–426. 1926.
10. PALLADIN, W. Influence des changements de température sur la respiration des plantes. Rev. Gén. Bot. **11**: 241–257. 1889.
11. QUISUMBING, F. A., and THOMAS, A. W. Conditions affecting the quantitative determination of reducing sugars by Fehling solution. Elimination of certain errors involved in current methods. Jour. Amer. Chem. Soc. **43**: 1503–1526. 1921.
12. SIMON, S. Untersuchungen über das Verhalten einiger Wachstumfunktionen sowie der Atmungstätigkeit der Laubhölzer während der Ruheperiode. Jahrb. wiss. Bot. **43**: 1–48. 1906.
13. WILLAMAN, J. J., and BEAUMONT, J. H. Preliminary report on the respiration of apple twigs during the winter. Proc. Amer. Soc. Hort. Sci. **1924**: 99–104. 1924.
14. ————, and ————. The effect of accumulated carbon dioxide on plant respiration. Plant Physiol. **3**: 45–59. 1928.
15. ————, and BROWN, W. R. The carbon dioxide dissolved in plant sap and its effect on respiration measurements. Plant Physiol. **5**: 535–542. 1930.
16. ZALENSKI, W. Zur Frage über die Wirkung von Reizen auf die Athmung per Pflanzen. Bot. Centralb. **95**: 251–252. 1904. (Abs. from Russian original.)
17. ZIEGENBEIN, E. Untersuchungen über den Stoffwechsel und die Athmung keimender Kartoffelknollen sowie anderer Pflanzen. Jahrb. wiss. Bot. **25**: 563–606. 1893.