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RESPIRATION OF CUCUMBER FRUITS ASSOCIATED WITH PHYSIOLOGICAL INJURY AT CHILLING TEMPERATURES[1]

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Phyiological injury to certain plant materials when subjected to temperatures below about 10° C, but above their freezing point, has been noted by many investigators (1, 7, 8, 10, 11, 14, 16, 19, 20, 21, 22 and 23). Numerous plants of tropical and subtropical origin are susceptible to such injury and include many horticultural crops now grown in temperate climates. This physiological disorder is often referred to as chilling injury and should not be confused with injury due to freezing. Temperatures in the range from 0 to 10° C will be referred to as chilling temperatures. The symptoms of this disorder include surface pitting, internal browning, increased susceptibility to decay, and in the case of some fruits, failure to ripen.

Although chilling injury is an interesting and important problem in plant physiology, very little is known regarding processes affected by chilling temperatures or possibly explanations of the primary injury. Conflicting reports relative to the respiratory responses of various cold-sensitive plant materials emphasize the need for a thorough investigation of the problem. Jones (5) reported a larger temperature coefficient for CO_2 production of papaya fruits between 7.2 and 10° C than for other temperature ranges above or below that of 10 to 7.2° C. This indicated to him a marked break in the nature of the processes having to do with CO_2 production of the papaya below 10° C. Gane (3), however, presents data showing only slight deviations in the temperature coefficients for CO_2 production of bananas at temperatures of 0, 5, 12.5, and 20° C. Platenius (15) concluded that neither the respiratory rates nor the respiratory quotients of cold-sensitive crops (including cucumbers) held at 0.5° C showed deviations from the results of those held at 10 or 24° C that would suggest an abnormal rate or course of respiration associated with chilling injury. However, Mack and Janer (8) reported a threefold increase in the rate of CO_2 production of cucumbers during a 3-week period in the temperature range of 2.2 to 3.3° C. They also reported a low initial respiratory quotient (0.45) for cucumbers held in this temperature range. Both papers mentioned above are in general agreement regarding the response of cucumber fruits held at temperatures of 10° C or above; i.e., the rate of CO_2 production decreased with time, and the respiratory quotients were near unity.

The purpose of the present investigation was to study the physiology of chilling injury to cucumbers by a comparative evaluation of the rates of CO_2 production and the respiratory quotients of fruits held at various constant chilling and non-chilling temperatures, and the rate of CO_2 production at 25° C subsequent to various chilling treatments.

MATERIALS AND METHODS

Cucumber fruits (*Cucumis sativus* L.) of the Cubit variety, ranging in length from 12 to 18 cm, were obtained from vigorous plants and handled carefully to minimize mechanical injury. To facilitate comparisons between tests, all experimental fruits, except where otherwise stated, were harvested in the morning. Comparable samples were selected and placed in respiratory chambers at constant temperature between 11:00 A.M. and 2:00 P.M. of the same day. The first determination was usually made the next morning, allowing sufficient time to establish temperature and gaseous equilibrium.

Respiratory responses were determined by three methods. Carbon dioxide production was measured by the method of Claypool and Keefer (2) in which an air stream of known flow is equilibrated with a buffered bromthymol blue solution and the percentage CO_2 estimated colorimetrically. The method was modified in that the water-saturated air passing over the fruit was not freed of CO_2 and the indicator solution was renewed for each determination. It was found that about one liter of air must be bubbled through the 10 ml of the indicator solution to establish equilibrium. To correct for the CO_2 content of the air entering the fruit chamber, the air was assumed to contain 0.03 % CO_2 at all times. The rate

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of air flow was regulated by capillary flowmeters so that the CO_2 concentration around the fruit was within the range of 0.3 to 1.0 %.

The second method was a static (closed) system similar to that described by Magness and Diehl (9) and later refined by Haller and Rose (4) and Platenius (15). This method facilitates the simultaneous determination of O_2 consumption and CO_2 evolution. Preceding each determination a 5-hour period was allowed for the establishment of equilibrium between the rate of production of CO_2 by the respiring fruits and the rate of absorption by the alkali. The fruit chambers remained closed between determinations, but were aerated with a water-saturated air stream.

The third method of measuring respiration was the "indirect method" of Warburg technique as described by Umbreit et al (18), by which the gas exchange (O_2 uptake and CO_2 production) of tissue slices was studied.

Results and Discussion

Effect of Continuous Exposure to Constant Temperatures: The magnitude and drift of respiration rates of cucumber fruits at non-chilling temperatures were compared with those at chilling temperatures. During the 1950 and 1951 seasons, six or eight samples of 14 cucumbers each were studied at each of the following temperatures: 0, 5, 10, 13, 15, 20, 25, and 30° C. Except on week-ends, when only a morning reading was taken, determinations were made twice daily by the colorimetric method, as described above. Morning and afternoon rates were averaged to arrive at the rate for a given sample on a given day. These values for the various samples were then averaged according to the days after harvest and are plotted at noon of the appropriate day in figure 1. The termination of each curve indicates the storage life, in days, of the fruits at that temperature; i.e., the number of days at a given temperature before the fruits reached an unusable condition. The coefficients of variability for the average rates were less than 5 %, except for the initial values and a few near the end of the storage period, when decay organisms may have contributed to the variability, thus indicating good agreement between samples obtained throughout the two seasons.

The continually declining rate of respiration displayed by the fruits held at non-chilling temperatures (13 to 30° C, inclusive) is taken as the normal pattern of respiration for unchilled cucumbers. The increasing rate near the end of the storage period at 15° C is probably due to decay organisms that were not detected before they contributed to the observed respiratory rate. These data are in general agreement, where comparisons are possible, with the rates reported by Platenius (15) and by Mack and Janer (8).

Cucumber fruits held at 0 and 5° C, and, to a lesser degree, those held at 10° C, deviated from the pattern of the unchilled fruits by displaying an increasing rate of respiration during part of their storage life. There was a rate increase from the third to eighth day at 5° C and from the first to the fourth day at 0° C, followed by a plateau at the respective

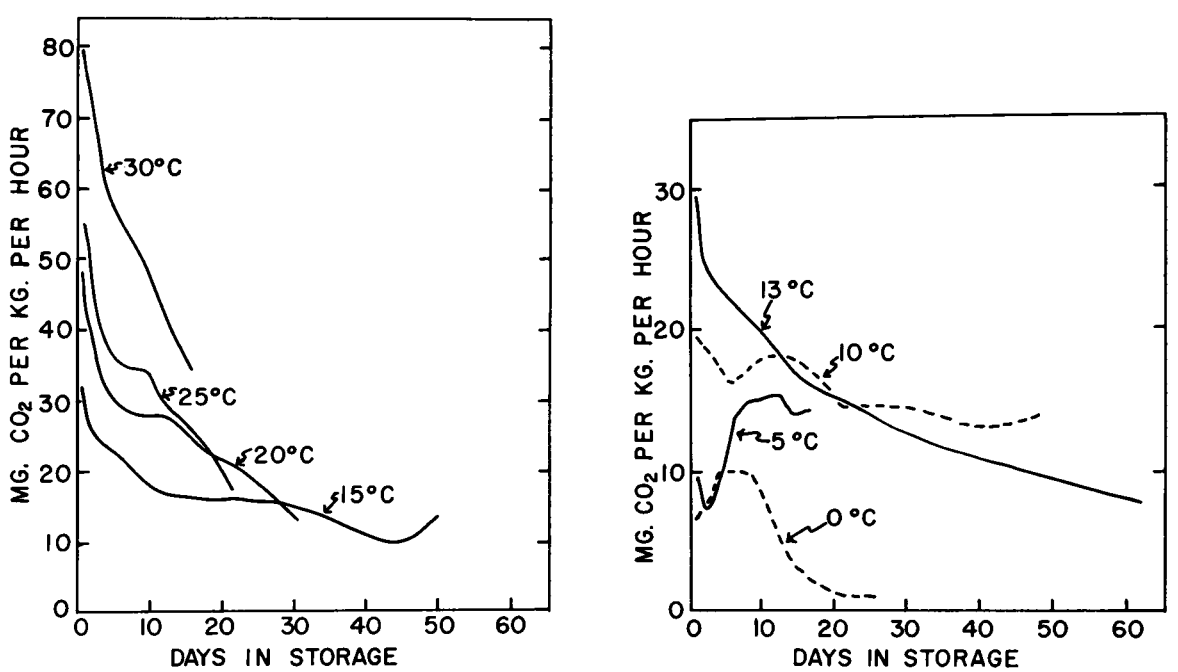

Fig. 1. Av. rates of CO_2 production of cucumber fruits during their entire storage life at various constant temperatures. *Left*—Non-chilling temperatures of 15, 20, 25, and 30° C. *Right*—Chilling temperatures of 0, 5, and 10° C and the non-chilling temperature of 13° C for comparison.

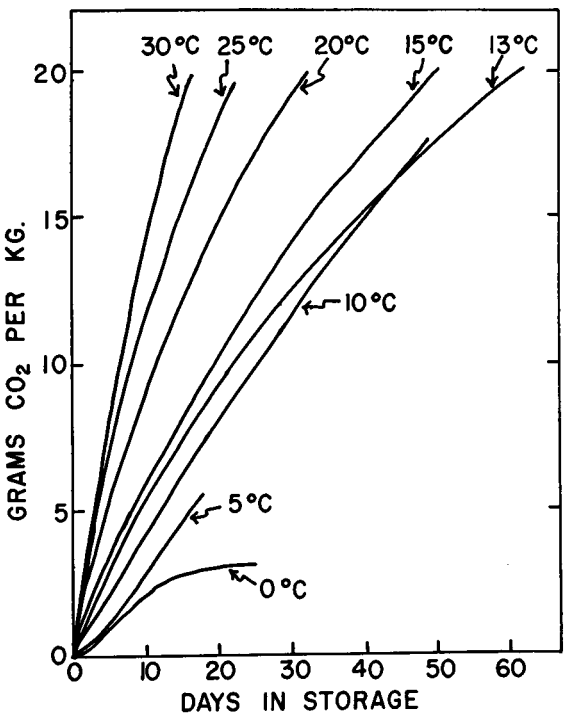

FIG. 2. The cumulative CO_2 production of cucumbers during their entire storage life at various constant temperatures.

levels and then a decline. Mack and Janer (8) reported an increasing respiration rate for cucumbers at chilling temperatures, agreeing in general with that reported here. However, Platenius (15) did not observe this phenomenon, probably because of the infrequency of his determinations. The increasing rate of respiration reported here for cucumber fruits held at chilling temperatures was correlated with the time of onset and development of chilling injury as measured by the degree of surface pitting and deterioration rate of fruit when transferred to 25° C after various exposures to low temperatures. The descriptive data of these holding tests is to be published elsewhere.

It has been suggested by several investigators that cumulative CO_2 production may be a measure of the storage life of harvested material. This assumes that a given amount of respirable substrate is available for respiration and that the product reaches the end of its useful life when this substrate is exhausted. Accordingly, the cumulative CO_2 production was calculated by multiplying the average rate by 24 and summating these daily values for the entire storage period at each temperature (fig 2). The average rate at midnight (read graphically) was used because all lots were set up near noon and this value was thought to approximate most closely the average daily rate. In figure 2 it can be seen that the fruits held at non-chilling temperatures (13 to 30° C, inclusive) produced approximately 20 gm CO_2/kg of fruit during their entire storage life. However, the fruits held at chilling temperatures produced less than 20 gm CO_2/kg, fruit held at 10° C producing slightly

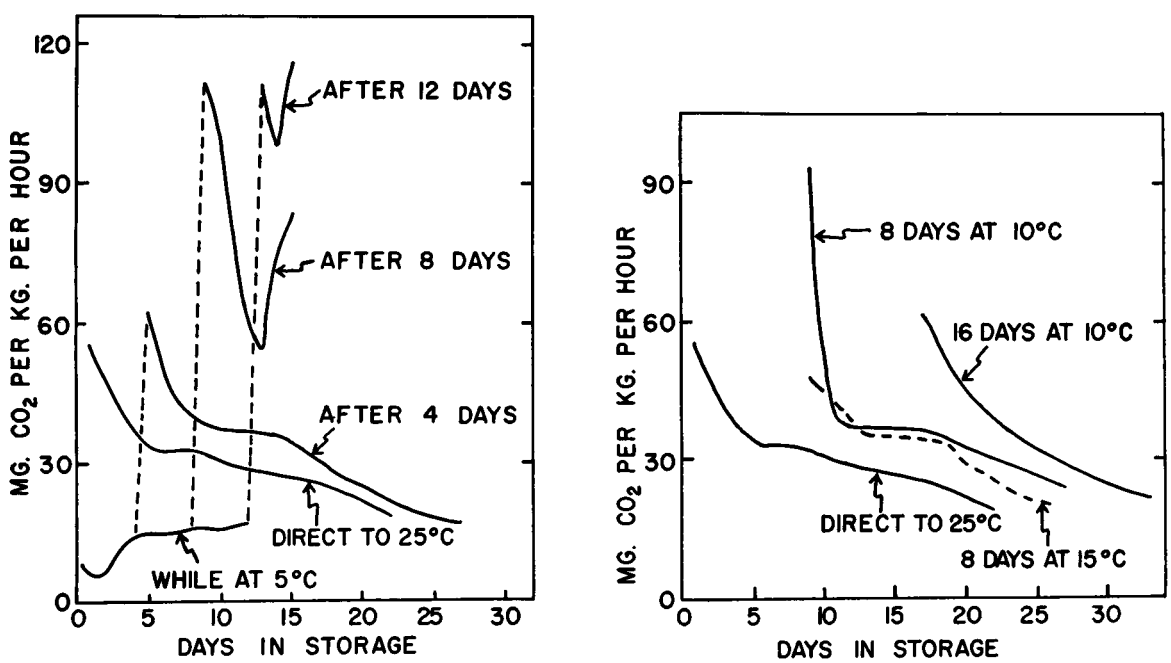

FIG. 3. Effect on respiratory rate of transferring cucumber fruits from a lower temperature to 25° C. *Left*—The effect of prior holding at 5° C. *Right*—The effect of prior holding at 10 and at 15° C.

less, and those at 5 and 0° C producing considerably less. Fruits held at non-chilling temperatures and transferred to 25° C also produced 20 gm CO_2/kg during their entire storage life, whereas fruits exposed to chilling temperatures and transferred to 25° C produced less CO_2, the amount depending upon the severity of the chilling.

Data presented by other investigators indicate that other harvested plant materials such as apples (6, 17) and bananas (3) produce approximately the same amount of CO_2 per kilogram. The calculated CO_2 production for these crops closely approximate that of cucumbers in the present study (20 gm CO_2/kg). It would be of interest to determine whether or not this is generally true for most harvested materials, and, if so, the physiological significance.

Effect of Chilling Treatments upon Subsequent Respiration at a Non-chilling Temperature: The rate of CO_2 production of cucumber fruits was determined for the following exposure periods and temperatures: 2 and 8 days at 0° C; 4, 8, and 12 days at 5° C; 8 and 16 days at 10° C; and 8 days at 15° C. After these exposures the samples were transferred to the constant non-chilling temperature of 25° C. The rates and trends at the low temperatures were similar to those reported in figure 1. A 24-hour period was allowed after transfer to 25° C to permit the establishment of temperature and CO_2 equilibrium between the tissue and the air stream.

Figure 3 illustrates several points regarding the effect of previous treatment on the respiration rate of the fruits at 25° C. Severe chilling (8 days at 0° and 12 days at 5° C) resulted in the response typified in figure 3 (left) by 12-day exposure to 5° C, i.e., a high initial respiration rate at 25° C, which remains at a high level. An 8-day exposure to a temperature of 5° C, which is considered to be moderately injurious, resulted in high initial respiration rates which decreased rapidly and then increased. The increase during the latter portion of the storage life is probably a result of undetected decay organisms. Treatments which usually do not cause symptom development (2 days at 0° C, 4 days at 5° C, and 8 and 16 days at 10° C), illustrated by the 4-day exposure to 5° C and by the 8- and 16-day exposures to 10° C in figure 3 resulted in respiration rates slightly greater than those of fruits exposed directly to 25° C. The rate of respiration decreased with time to about 20 mg CO_2/kg per hour at the end of storage. Fruits transferred to 25° C after 8 days at 15° C respired at a slower rate than those exposed directly to this temperature. Therefore, the initial respiration rate at 25° C (24 hours after transfer), and the subsequent drift with time, may be used as a rough index to the chilling injury sustained by the fruit.

Respiratory Quotients Determined by the Static Method: The simultaneous determination of the volume of oxygen utilized and CO_2 produced by intact cucumber fruits was made periodically at 0, 5, and 15° C during a 13-day period. The resulting respiratory quotients serve to indicate probable substrates of respiration as well as deviations from the normal respiratory process. Duplicate samples of 12 freshly harvested fruits were set up at each temperature at the same time and this procedure was repeated until 6 samples per temperature had been studied.

The good agreement between the average magnitude and drift of CO_2 production determined by the static and colorimetric methods is shown in figure 4. The coefficient of variability of less than 5 % for all static-method values indicates the uniformity within this method. The average respiratory quotient values are graphically presented in figure 5. The coefficient of variability was less than 4 % in all cases. At 15° C, the respiratory quotient was essentially unity throughout the experimental period, a condition which may be interpreted as indicating that complete oxidation of carbohydrate (hexose sugars) occurred. At 5° C the values were less than unity, with a general trend toward unity as the storage period progressed. However, at 0° C the respiratory quotient was less than unity for the first 7 days and then rose abruptly above unity. It is unfortunate that the determinations were not continued a few more days in order to study further the response at the lower temperatures, particularly to observe whether the trend

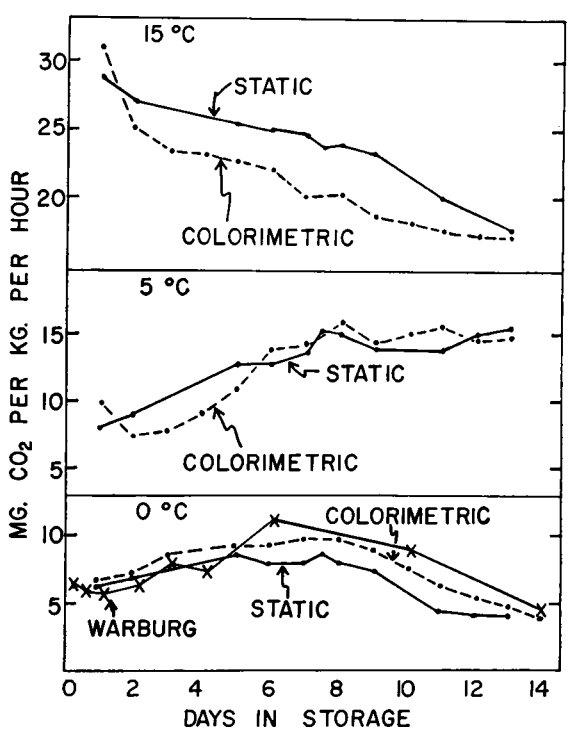

Fig. 4. Av. rates of CO_2 production of cucumbers determined by the colorimetric and static methods on intact field-grown fruits at 0, 5, and 15° C, and determined by the use of the Warburg apparatus on tissue slices of greenhouse-grown fruit at 0° C.

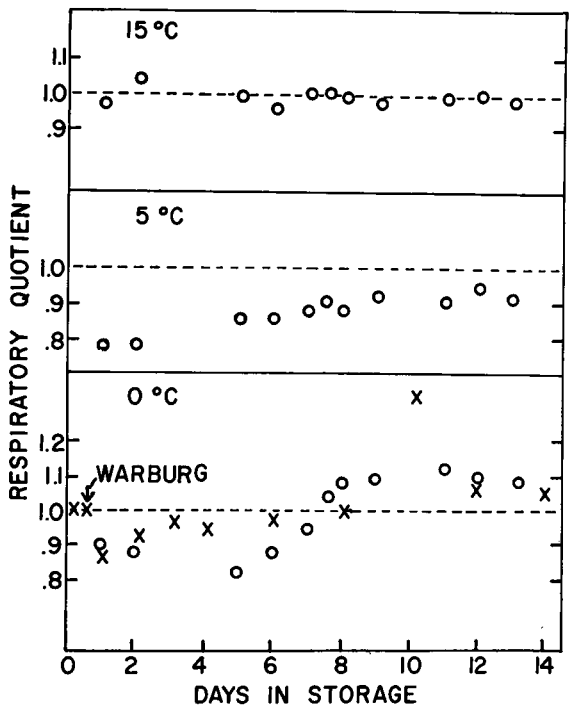

FIG. 5. Respiratory quotients of cucumbers determined by the static method on intact fruit at 0, 5, and 15° C, and by the Warburg apparatus on tissue slices at 0° C.

at 5° C continued or reacted similarly to that at 0° C by abruptly rising, and also whether the values at 0° C returned to unity or remained high.

RESPIRATORY QUOTIENTS DETERMINED BY THE WARBURG TECHNIQUE: The respiratory responses of cucumber tissue slices at 0° C were determined. The greenhouse-grown fruits used were obtained by air freight from Terre Haute, Indiana. A total of approximately 48 hours elapsed between harvesting and placing the fruits at constant temperature (0° C). Upon arrival the fruits used in this test were sorted into 12 samples of eight fruits each. Samples were removed for determination after 1 hour, 12 hours, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 14 days. Tissue slices from each of 5 fruits of each sample were used for respiratory determinations in the Warburg apparatus; the other 3 fruits of each sample were observed for symptom development.

To obtain rapid temperature equilibrium at the outset, the cucumber samples were placed in ice water and rapidly cooled to 0° C. After 1 hour the samples were removed from the ice water and one sample was selected at random for a determination in the Warburg apparatus. The others were immediately placed in respiration jars at 0° C, as previously described, and the CO_2 production of all the samples present was determined daily by the colorimetric method. Sections and Warburg flasks were prepared at 0° C and then placed in the Warburg bath at the same temperature. Each set of determinations extended over about a 6-hour period.

In several preliminary tests fruits grown in the greenhouse at Davis, California, were used to evaluate and standardize the techniques and conditions of the experiments. The relative activity of tissue from different regions of the cucumber fruit was determined at 0, 5, and 25° C. Samples consisting of slices taken near the stem end, the middle portion, and the blossom end of the fruit showed little or no difference in respiration rates. Tissue from different depths was tested by using 15 sections averaging 200 mg each, taken from slices from three areas of the same fruit, as follows: (a) the outer section, which included the epidermis and about 2 mm of tissue below it; (b) a middle section consisting of the next 3 mm of tissue; and (c) the internal section, which included the remainder of the tissue extending to the seed cavity. Wedge-shaped sections including tissue from all three areas were also used. On the basis of the respiration rate of the wedge-shaped sections as 100, the relative rate of the outer sections was about 160, that of the middle sections about 60, and that of the internal sections about 80. Several fruits were sampled during these tests, and the variation between fruits was primarily associated with maturity, younger fruit being more active.

The influence of light on the CO_2 production of the tissue was also observed during the preliminary tests. In general, the observed rate for wedge-shaped sections was about 20 % greater in the dark than in the indirect north-window light. The different rate was attributed to photosynthetic activity of the green tissue. Supporting evidence was found in the results with sections of different depths. The outer section, which contained considerable green color, gave about a 30 % greater rate in the dark than in the light, while the other two sections, containing essentially no chlorophyll, were not influenced by the light.

These preliminary tests suggested that the most reproducible results and the best estimate of the fruit respiration could be obtained by using 3 gms of tissue from slices taken near the middle of the fruit and containing a cross section of the fruit tissue, excluding the seed cavity. The slices were cut into 15 wedge-shaped sections, and it was determined experimentally that small variations in the size of the sections did not influence the observed respiratory rate. All determinations were made in the dark.

The rate of CO_2 evolution of the Indiana greenhouse-grown fruits during storage at 0° C, as determined by the colorimetric method, was in general agreement with previous results obtained with field-grown material. Furthermore, the rate of respiration of the tissue slices appeared to parallel that of intact field-grown fruits measured by the other two methods, as shown in figure 4 (Warburg). Each point represents an average of 5 samples, each from a different cucumber fruit. In preliminary tests, however, the rate for tissue slices was slightly higher at

5° C and considerably higher at 25° C than that for intact fruit. The variation in rates obtained from the 5 fruits sampled after various periods of exposures to 0° C was small for the first few days, but it became greater toward the end of the storage period.

The respiratory quotient for the tissue from each fruit was calculated by dividing the average rate of CO_2 production by the average rate of O_2 consumption. These values for the 5 fruits used at each sampling were averaged and are shown in figure 5. The coefficients of variability of these averages were less than 3 %, except for the value after 10 days in storage, which was 25 %. The wide deviation of the values obtained for the 10-day storage period makes this mean of doubtful use. The values for the first samplings at 0° C were unity, but the other values followed the general trend observed with the static method.

Low respiratory quotients observed for intact fruits and tissue slices at the chilling temperatures were observed during the onset and development of chilling injury as measured by rate of deterioration and symptom development at a higher temperature after chilling. These values indicate an altered course of metabolism in which protein utilization or some enzymatic disturbance may have occurred. However, without biochemical confirmation it cannot be stated whether protein or fat is being respired, whether some enzymatic disturbance has occurred which resulted in the utilization of O_2 other than as the final electron acceptor, or whether part of the carbon is diverted into some form other than CO_2 to give these low respiratory quotients.

Platenius (15) suggested that if suboxidation—the inability of the tissue to obtain or utilize sufficient O_2 as claimed by Nelson (12, 13)—was a factor in low-temperature injury, there should be marked deviation in the respiratory quotients of cold-sensitive crops at chilling temperatures, which he failed to find. The respiratory quotients of less than unity and the increased rate of respiration at chilling temperatures presented above indicate that chilling may be associated with an excessive, rather than with a limited, use of oxygen. Furthermore, the crops studied by Nelson (12) at low temperature and in the absence of oxygen at room temperature are ones not now considered cold-sensitive. Therefore, present information does not permit the conclusion that chilling injury is due to anaerobic respiration.

Peculiarities in the respiratory behavior associated with chilling injury indicate that the ultimate cause may be found in the cellular metabolism of the tissue. This anomalous respiratory behavior associated with chilling temperatures may in itself be a symptom of a more primary disturbance and may not be a contributing factor to the development of visual symptoms. An interesting approach to the fundamental cause of chilling injury might therefore be found in comparing the pathway of respiration in non-chilled and chilled plant materials.

Summary

The physiology of chilling injury evaluated by observing the respiratory responses of cucumbers to chilling and non-chilling temperatures indicates that the course and rate of metabolism was changed as a result of chilling injury. At non-chilling temperatures the rate of CO_2 production decreased with duration of storage, whereas at chilling temperatures the rate increased with time to a plateau that was followed by a decline. The increasing rate occurred at the same time as the onset and development of chilling injury, and the decline occurred at the time of general death of the tissue. The respiration rate and drift at a temperature of 25° C following chilling treatments could be used as a rough index to the severity of the treatment.

At all temperatures within the non-chilling range, cucumbers produced essentially the same total amount of CO_2 (20 gms/kg of fruit) during their storage life; but at chilling temperatures lesser amounts were produced.

The respiratory quotients of fruits held at a non-chilling temperature of 15° C were near unity, whereas at chilling temperatures the quotients were less than unity during the time of the onset and development of chilling injury. Quotients obtained with tissue slices at 0° C agree with those of intact fruit.

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