PREVENTION OF CHILLING INJURY IN CUCUMBER LEAVES

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SUMMARY

Discs of cucumber leaves were kept at 2 °C in the dark. When, subsequently, transferred to a temperature of 24 °C they develop symptoms of damage. The degree of injury depends on the length of the period at 2 °C. An interruption of the treatment at 2 °C by a short period at 24 °C reverses the damage induced by the cold treatment. The degree of reversal depends on the length of the cold period before the interruption and on the length of the interruption at 24 °C.

The damage to plant tissues brought about by temperatures below freezing point are, directly or indirectly, the result of ice formation in the cell or in the intercellular spaces. Many species of plants, however, are injured by low temperatures above 0 °C. This is called chilling injury, whereas damage caused by internal ice formation is called frost injury. The external signs of chilling injury are decreasing turgor, wilting, with eventual desiccation of leaves and death of the plant. Physiological disturbances which occur after chilling are sharp reduction of photosynthesis, decreasing chlorophyll content, excessive increase of respiration, increased viscosity of the protoplasm, etc. The primary cause of these disturbances is unknown. In the present experiments the chilling of cucumber leaves has been studied.

Leaf discs of 6 mm diameter were floated on Hoagland solution or distilled water in Petri dishes. The Petri dishes were closed and placed at 2 °C in the dark. After different times of exposure to this temperature, the discs were placed on a 2% sugar solution at 24 °C under 4 Philips fluorescent tubes (TLF 40 W, colour 33). The light intensity was about 6000 Lux. After at least 3 days under these conditions external signs of damage became visible. These signs of damage were: first local, and later total discoloration of the leaf discs, due to decomposition of chlorohyll and loss of turgor. For one experiment discs were taken from about 40 plants. For each treatment at least 80 discs were used, the effect was assessed by determining the percentage of injured discs. The standard deviation was about 10%.

Leaf discs which remained at 2 °C in the dark did not show harmful after-effects when placed at 24 °C, even after 72 hours cooling. A longer period at 2 °C caused symptoms of damage to become visible when the discs were placed at 24 °C. *Fig. 1* gives the curve relating the percentage of discs showing injury to the time of cooling. When plotted on probability paper this relationship is a straight line. The cooling time needed to reach the 50% point (LD 50) was considered to be typical for the effect of the cold in these experiments.

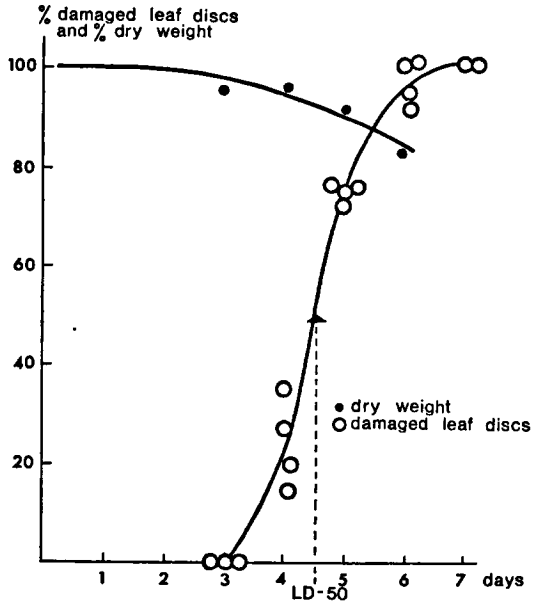

Fig. 1. Damage to Cucumis leaf discs after a 2 °C treatment in the dark for various periods of time.

The dry weight of the discs decreases only slightly during cooling *(fig. 1)*, the loss at the LD 50 point being only about 8 %.

It did not seem likely that the damage was caused by starvation of the discs. Determinations of the sugar and starch content during cooling have shown that, at the LD 50 point, the decrease in soluble sugars was nil or only slight, whereas the decrease in the starch content was 40 %.

During chilling the discs did not show any signs of damage up to a chilling time of 6–8 days. Injury symptoms appeared only at 24 °C in discs which had been chilled for more than 3 days. It was assumed that, during cooling, some harmful factor accumulated in the discs and that this factor had to reach a critical level to damage the cells when these were transferred to 24 °C. The question arose as to whether the time at which this critical level is reached could be postponed by an interruption of the cold treatment. This would be indicated by a corresponding increase in the time taken to reach the LD 50 point.

Fig. 2 shows that interruption of the cold treatment after 40 hours by a temperature of 24 °C for 4 hours did delay the LD 50 point by 43 hours. This suggests that at the higher temperature the effect of the previous cold treatment is completely removed and implies that chilling injury could be prevented by an interruption of the low temperature treatment at daily short intervals. Under certain conditions this proved to be so; leaf discs could be kept at 2 °C during 12–14 days, without any harmful after-effect if they were transferred to 24 °C for 4 hours each day. To achieve this result the discs had to be floated on a sugar solution, probably to prevent exhaustion of the carbohydrate reserve during the high temperature intervals, and the discs had to remain in the dark.

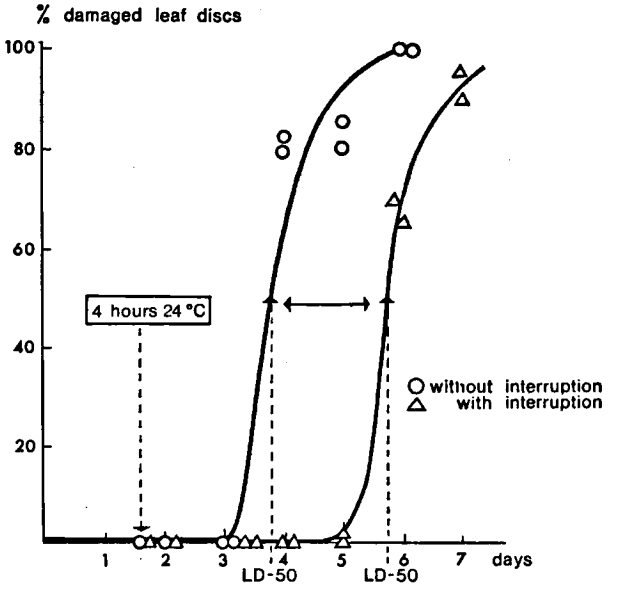

Fig. 2. The effect of interrupting the cold period by a short high temperature treatment on the chilling injury of Cucumis leaf discs.

If discs were chilled in the light of fluorescent tubes, a short treatment at 24 °C could not prevent damage.

Further experiments showed that the degree of recovery was not independent of the length of the chilling period and the length of the treatment at 24 °C. To assess recovery, the delay of the LD 50 point obtained by the treatment at 24 °C was expressed as a percentage of the time from the start of cooling until the high-temperature period was reached. When these times were equal recovery was 100%, i.e. the effect of the cooling period had disappeared. At the 50% recovery level the treatment at 24 °C had resulted in a delay of the LD 50 point of only half of the length of the previous cooling period. *Table 1* gives the recovery by a treatment at 24 °C after various cooling periods and *table 2* shows the effect of the length of the treatment at 24 °C on recovery.

Table 1. Effect of treatment at 24 °C for 60 minutes

previous treatment at 2 °C	delay of LD 50 as compared to controle	percentage recovery
48 hours	54 hours	121%
72 hours	47 hours	65%
96 hours	47 hours	49%

In the second experiment of table 2 the cold treatment has affected the plant much more than in the first. The difference in recovery caused by an interruption at 24 °C of 45 minutes and by one of 60 minutes is evident from both experiments.

Table 2. Effect of the length of an interruption with a temperature of 24 °C of the cold treatment in three different experiments.

Length of previous treatment of 2 °C	Length of interruption with 24 °C					
	45 minutes		60 minutes		90 minutes	
	delay of LD 50	recovery %	delay of LD 50	recovery %	delay of LD 50	recovery %
72 hours	6 hours	8%	58 hours	51%	67 hours	94%
72 hours	0 hours	0%	29 hours	40%	– –	–
48 hours	40 hours	83%	– –	–	– –	–

Chilling injury is well known from fruits that are stored or transported under low temperature conditions. Owing to its economic importance much research has been carried out on this type of chilling injury.

PLANK (1941) assumes that the damage to fruits in cold storage is due to an accumulation of particular intermediates of metabolism. SMITH (1947) found serious damage in plums that had been stored at 31 °F (–1 °C) for 5 weeks. If, however, after 15–20 days, the cold treatment was interrupted by a temperature of 65 °F (18 °C) for 2 days, chilling injury was greatly reduced when the low temperature treatment was resumed. In terms of a "toxic substance theory" this would mean that during the short high temperature period the toxic substance disappears. A correlation between the degree of chilling injury and the accumulation of any toxic substance has not yet been found. (PENTZER & HEINZE 1964).

The effect of the short interruption of the cold treatment of cucumber leaf discs in the present experiments, strongly resembles the results of Smith's experiments with fruits. It is highly improbable that the processes caused by low temperatures in fruits and in cucumber leaf discs are identical, but there is a parallelism in the results that points to the existence of a common pattern in the chilling injury to both tissue types.

From the present experiments two facts are clear:

1. the length of the interruption with a higher temperature determines the degree of recovery.
2. with equal length of the high temperature treatment, the length of the previous cold treatment determines the degree of recovery.

These facts point to a gradual increase of some metabolic change in the course of cooling. The results of Smith allow a similar conclusion to be drawn. In his experiments after a cold period of 35 days the chilling injury was 100%. A higher temperature after 15–20 days reduced the chilling injury to zero. Treatment at the higher temperature after a cold period of 25 days, however, resulted only in a slight reduction of the damage, so it appears that after too long a chilling period the still invisible damage becomes irreversible.

REFERENCES

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