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Frost Hardiness Studies on Cabbage Grown under Controlled Conditions¹

Hubertus Kohn and J. Levitt

University of Missouri, Columbia, Missouri

So many factors have been found to vary directly with frost hardiness, and for each of these there are so many exceptions, that it is difficult to know which are causally related and which are not (2). Some may not be causally related to frost hardiness but may merely be produced by the same environmental conditions. It may be possible to recognize these by growing and hardening the plants under a variety of environmental conditions. Others may be causally related at 1 stage of hardening but not at another. It should be possible to identify these by hardening in stages.

Unfortunately, it is difficult if not impossible to distinguish these factors from each other either in the case of field grown or greenhouse grown plants; since the environmental conditions cannot be controlled. Furthermore, greenhouse grown plants cannot be hardened to their maximum ability and, therefore, the different stages of hardening cannot be compared. For instance, the maximum hardening at +3° of greenhouse grown cabbage seedlings resulted in killing points of -7 to -10°.

Both of these problems can be overcome by growing and hardening the plants in controlled growth chambers. There it is possible to A) control both day and night temperature, as well as light, so as

to give the best type of growth before hardening, and B) to harden the plants in stages by exposure successively to 5°, 0°, and -3°. Cabbage plants were found able to survive freezing at -20°. The purpose of the present investigation was, therefore, to find out 1) whether growth and hardening under such controlled conditions can help separate factors causally related to hardiness from those not causally related, and 2) at which stage of hardening each causally related factor is important.

Materials and Methods

The cabbage variety Early Jersey Wakefield was used in the first part of these experiments but it soon became evident that this variety is not genetically pure, since growth under controlled conditions led to a high degree of variability in size from seedling to seedling. A genetically selected variety (Badger Market) was therefore used in later experiments. The seedlings of this variety were highly uniform.

The plants were grown from seed in 3-inch pots in growth chambers. From the time of sowing until hardening (5-6 weeks), they were exposed to a 12-hour day temperature of 25° and a 12-hour night temperature of 15°, with an illumination of 2000 to 2200 ft-c and a relative humidity of 85 ± 5%. Temperatures were constant to ± 2°. Four compartments were used, with daylengths of 8, 12, 18, and 24 hours, respectively. At the end of 5 to 6 weeks, each plant had some 7 to 9 leaves. They were then har-

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dened progressively in the same chamber as follows: 1) One week at +5° day and night, 2) a second week at +5° day, 0° night, 3) a third week at 0° continuously, 4) a fourth week (or longer) at -3° continuously (no light). When lights were used (first 3 weeks) during hardening, the illumination was decreased to 1000 ft-c. The temperatures in all cases were determined at the undersurface of the leaves by means of a thermocouple and recorder. After a preliminary trial run, the experiment was performed twice. A fourth experiment was performed using only 8 and 18-hour photoperiods, and more prolonged hardening (i.e., 2 weeks) at each temperature.

Plants were removed weekly for frost-killing and other determinations. The pots were supplied a complete nutrient solution once a week during growth but not during hardening, and watered as needed. For each measurement, a single leaf of the same number (in order of development) was removed from each of about 6 plants. Thus, in the 1963 experiment, leaf number 4 was used for succulence and percent dry matter measurements. Yield was calculated in this experiment from total fresh weight of the above ground portion of the seedling, assuming that all the rest of the plant had the same percent dry weight as leaf number 4. In the last experiment, total above ground dry matter was determined directly and this gave the same general result. Frost killing points (2) were determined, using the same numbered leaf (in order of development) for all tests, or leaves of 2 adjacent positions where necessary. Osmotic potentials were determined plasmolytically (3), on petioles of leaves of the same number adjacent to these. For succulence determinations, leaf area was measured planimetrically.

Since the results were basically the same in all 3 series, only 1 series will be given in its entirety.

Results

Relation of Photoperiod to Frost Hardiness.

The photoperiod has frequently been found to have a pronounced effect on frost hardiness (2). But these results have been obtained mainly with trees. Those trees that are induced to grow by exposure to an artificial long photoperiod during the fall fail to harden. The normal short photoperiod, on the contrary, leads to cessation of growth and to frost hardening. In the case of cabbage there are no such effects either on growth or on hardiness (fig 1). The longest day (continuous light) actually leads to a maximum hardiness among the 4 photoperiods both before hardening and after 1 week of hardening. After the second week of hardening, however, the relation is reversed and the 8-hour plants become the most hardy. Identical results were obtained with longer hardening periods (fig 2). The main effect of photoperiod on cabbage is simply a quantitative one, for the relative yields

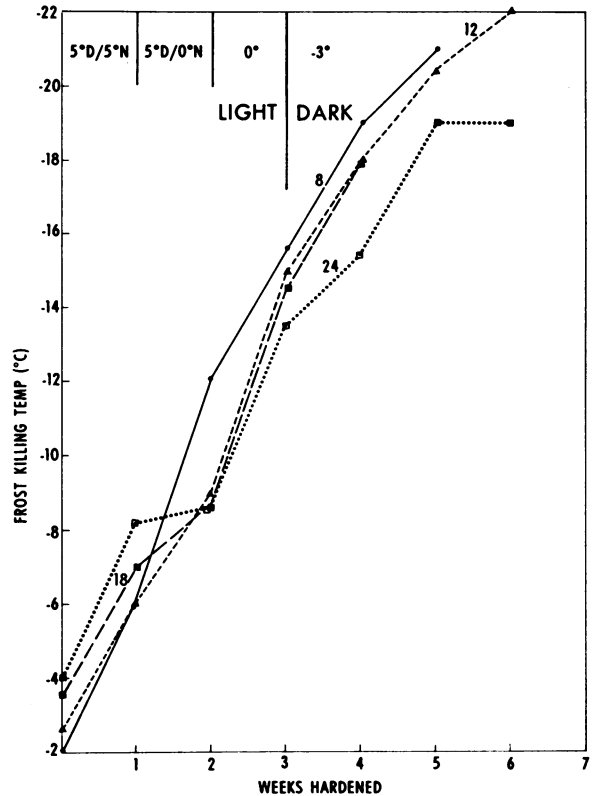


FIG. 1. Increase in frost hardiness of cabbage seedlings during 6-week hardening period. Plants grown at 8, 12, 18, and 24-hour photoperiods, during 6 weeks before hardening (2000-2200 ft-c) and during the first 3 weeks of hardening (1000 ft-c).

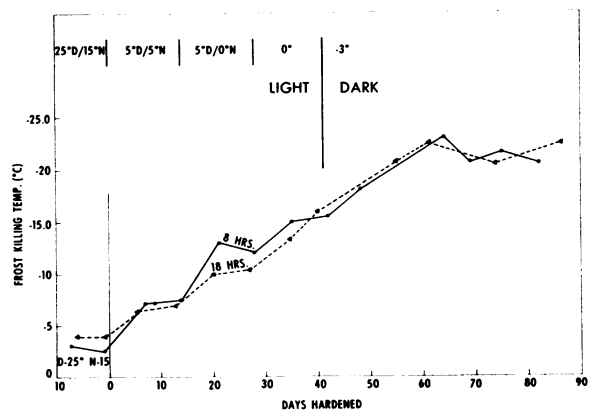


FIG. 2. Increase in frost hardiness when subjected to more than 1 week at each hardening temperature.

agree almost perfectly with the relative daylengths (table I). This would seem to indicate that the main photoperiodic effect is due to the differences in the daily length of time the plants are able to photosynthesize. The successively lower temperatures are necessary for maximum hardening: for longer peri-

Table I. *Relation of Yield of Dry Matter to Photoperiod*

Photoperiod	Relative amount of light	Relative yield
8	33	33
12	50	46
18	75	85
24	100	100

ods at each hardening temperature do not substitute for the drops in temperature (fig 2).

Relation of Kind of Growth to Frost Hardiness. To the man of experience, many morphological characteristics are obviously related to frost hardiness. Over a period of years, for instance, it has been observed that when cabbage seedlings are grown in the greenhouse, their ability to harden can always be predicted by their appearance. When the plants are spindly and the leaves are dark green, thin, and long-petioled, they are tender and show a minimum ability to harden. When the plants are stocky and the leaves are thick, light green, covered with bloom and short-petioled, they possess maximum hardening ability. These 2 kinds of growth correspond exactly to the 8-hour and 24-hour plants respectively (the 12- and 18-hour plants falling between the extremes). The relative hardiness of these 2 groups is in agreement with the above expectation, but only until the end of 1 week's hardening. After this, the relation is reversed. The morphological characters considered here can be related to cabbage hardiness, only when this is moderate.

Relation of Water Content and Dry Matter to Frost Hardiness. Frost hardiness has frequently been correlated directly with dry matter content or inversely with moisture content (2). Thus, trees supposedly must ripen or mature (i. e., lose water) before hardening, and succulent plants are usually unable to harden, though many exceptions exist. Marked differences were found between these quantities in the plants grown at different photoperiods (fig 3). Succulence, percent dry matter, and yield of dry matter all increased with the photoperiod.

This means, of course, that the succulence (measured by weight per unit area) was in this case due to both an increase in water and dry matter per unit leaf surface, but that the latter increase was greater. When succulence is inversely related to hardiness, it is associated with greater water content (2).

Frost hardiness is directly related to all 3 of these measurements, but only up to the end of 1 week's hardening. After further hardening, the relation is reversed (fig 3).

Osmotic Potential of Cell Sap and Frost Hardiness. Many investigators have shown a pronounced parallel between sugar content or the consequent osmotic potential of the cell sap, and frost hardiness, though some striking exceptions occur (2). This parallel is obvious in all 4 photoperiods during the first 3 weeks of hardening (fig 4 a-d). When a more gradual hardening was used (fig 2), osmotic potential paralleled hardiness during the first 6 weeks. In all cases, the parallel continued only for part or all of the hardening period in the light. During the succeeding weeks of hardening in the dark, frost hardiness continued to rise just as rapidly, but osmotic potential either dropped or remained essentially constant. The correlation for all 4 photoperiods suggests that osmotic potential is a factor in frost hardiness, but this can be claimed only for the first half of the hardening period. Thus, though hardiness rose steadily, there were 2 obvious stages: a first or light stage at $+5^{\circ}$ to 0° during which osmotic potential rose, and a second or dark stage at -3° during which osmotic potential did not rise.

Discussion

The main effect of the photoperiod on the growth of the cabbage plants (unlike the effect on trees) seems to be due to its control of the length of time the plant can carry out photosynthesis (table I). This explains the correlation with yield and percent dry matter. Even succulence is also proportional to photosynthate accumulated, since the increased succulence was accompanied by an even greater deposit of dry matter than of water. The fact that these characteristics were directly correlated with hardiness only up to the end of the first week of hardening suggests that the amount of photosynthate accumulated before hardening may control the small amount of hardiness that develops during this period. This conclusion is supported by the correlation with osmotic potential (i. e., sugar content). The increase in osmotic potential during the first week of hardening may be due primarily to hydrolysis of the previously accumulated starch to sugar. After the first week of hardening, however, the correlation between hardiness and total photosynthate before hardening no longer holds, though osmotic potential continues to parallel hardiness. The 8-hour plants, that originally had the lowest hardiness and least amount of photosynthate (and also of sugars judging by osmotic potential), now

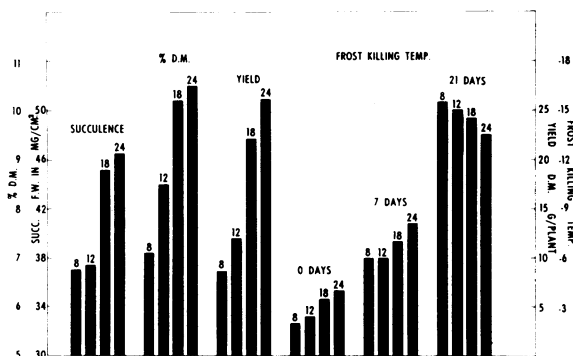


FIG. 3. Variation of succulence, percent dry matter, yield, and frost killing temperature with photoperiod.

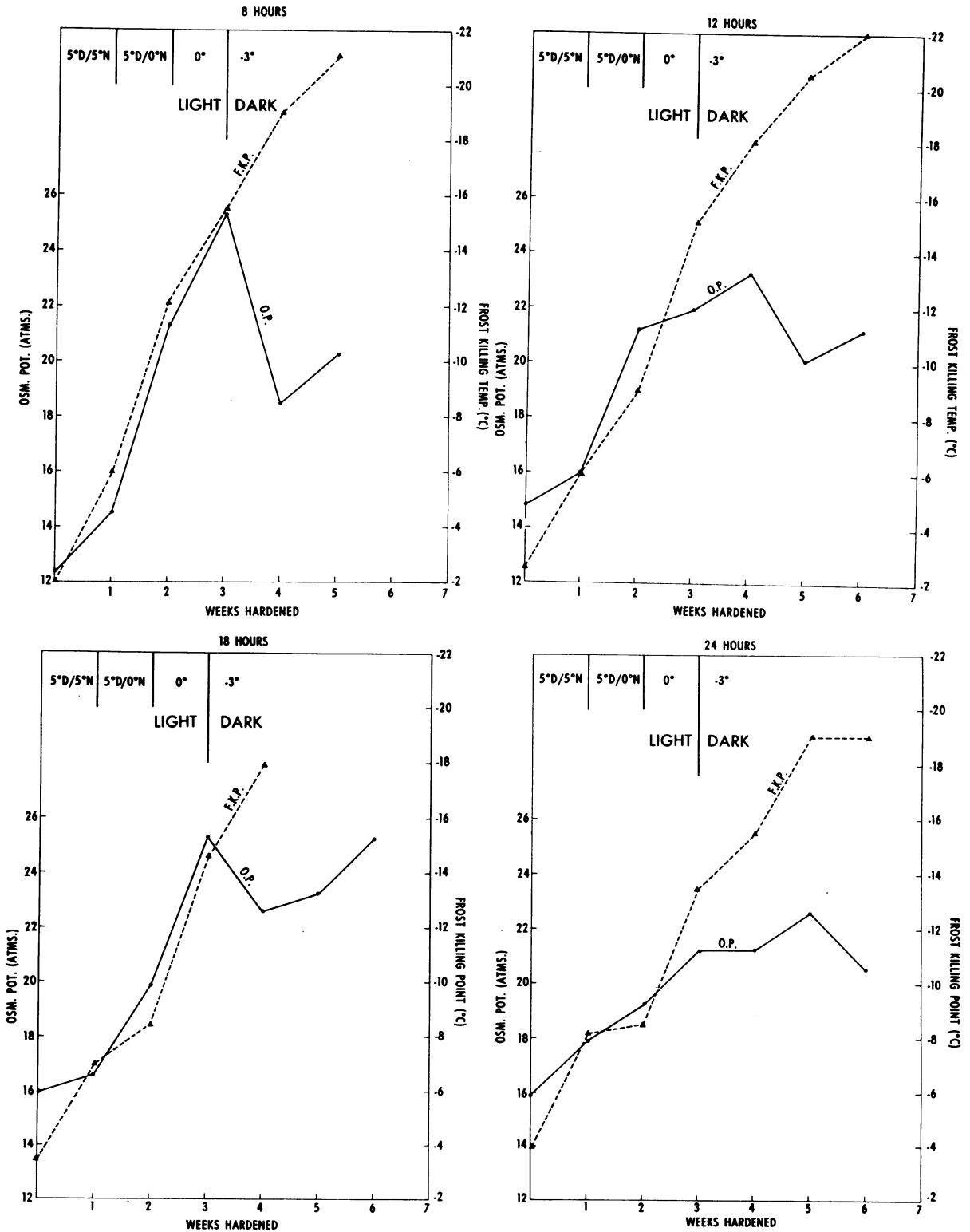


FIG. 4. Variation of osmotic potential and frost killing point with hardening period (a-d).

have the highest hardiness and osmotic potential. Since they had little if any reserve carbohydrate, this can only mean that they were able to accumulate photosynthate more rapidly than the other plants at the low temperature. This may be related to their higher chlorophyll content per cell, for though the leaves were much thinner than those of the other treatments, they were also a darker green. At the higher (nonhardening) temperatures, even if the 8-hour plants photosynthesized more rapidly than the others, the rate of respiration during the long (16-hour) night might be sufficient to use up so large a fraction as to leave the net photosynthate below that of the other daylengths. At the low temperatures, particularly starting with the second week when the night temperature was 0° , the respiratory loss would be negligible compared to the photosynthetic gain (1). Further evidence that the increase in osmotic potential during the second and third weeks of hardening was due to photosynthesis and not simply a hydrolysis of starch to sugar, is the abrupt end to the rise in osmotic potential when the temperature was dropped to -3° , for the lights were then turned off day and night in order to avoid loss of water from the leaves that could not be replaced from the frozen soil.

From these results, 3 successively developing factors appear to parallel the development of frost hardiness.

1) The amount of original photosynthate before hardening. This parallels both the daylength and frost hardiness up to the end of the first week of hardening ($5^{\circ}\text{day}/5^{\circ}\text{night}$). The small increase in osmotic potential may be due to a hydrolysis of accumulated starch to sugars.

2) The amount of photosynthate accumulated during exposure to hardening low temperatures. The increase in osmotic potential is due to a direct production of sugars by photosynthesis. This parallels the increase in hardening during the next 2 weeks ($5^{\circ}\text{day}/0^{\circ}\text{night}$ and $0^{\circ}\text{day}/0^{\circ}\text{night}$).

3) A factor unrelated to amount of photosynthate in general or sugar in particular. This factor seems to be related directly to exposure to low temperature rather than exposure to light. The increase in frost hardiness occurs at -3° in continuous darkness when no photosynthate accumulates:

and the lack of any further increase in osmotic potential proves that there is no hydrolysis of previously accumulated photosynthate to sugars.

Summary

Cabbage seedlings (var. Badger Market) were grown in growth chambers (25° day 15° night) and hardened for 6 weeks at successively lower temperatures from $+5^{\circ}$ to -3° . The maximum hardiness attained was a frost killing point of -20° , as opposed to a maximum of -7 to -10° for greenhouse grown plants hardened at $+3^{\circ}$.

The effects of photoperiod on growth of the cabbage plants were apparently chiefly if not solely due to their effect on total net photosynthate accumulated per day.

Frost hardiness was related directly to photoperiod (8, 12, 18, 24 hr) up to the end of the first week of hardening, but inversely from the end of the second week and for the next 2 to 4 weeks.

Growth characteristics such as dry matter and morphological characters usually correlated with hardiness were correlated only up to the end of the first week of hardening.

Osmotic potential was correlated with hardiness at all photoperiods up to the end of the 3 to 6 weeks of hardening at $+5$ to 0° , during which lights were used, but not during the succeeding 3 to 6 weeks of hardening at -3° in the dark.

Acknowledgments

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