

Direct Relation Between Growth and Frost Hardening in Cabbage Leaves¹

Wesley Cox and J. Levitt

Department of Botany, University of Missouri, Columbia, Missouri 65201

Received February 12, 1969.

Abstract. Potted cabbage plants were grown in growth chambers at 25° day and 15° night and hardened successively at 5, 0, and -3°. Leaf growth was determined by measuring leaf area, hardness by freezing at a series of temperatures and determining percent survival. Leaf growth increased progressively with leaf number, reaching a maximum rate of growth and final area in the tenth and eleventh leaves when the plants become potbound. Leaf growth continued at hardening temperatures of 5 or 0°, the Q_{10} being 2.0 to 2.5. Ability to harden also increased with leaf number, paralleling the growth rate of the leaves just before hardening as well as the growth rate and the total growth during hardening. The above results were similar whether prolonged (several weeks) or brief (24 hr) hardening was utilized.

It has long been known that plants achieve their maximum frost hardness when growth ceases in the fall, and that these same plants possess little hardness when growing in the spring (3). Results have led to the conclusion that, in general, frost hardness and growth are inversely related. Thus, the hardening process itself occurs only at low temperatures which retard growth. The following investigation has revealed an unexpected direct relation between growth rate of cabbage leaves and their ability to harden.

Materials and Methods

Cabbage plants (var. Badger Market) were grown from seed in growth chambers. Each plant was raised in a 3 inch pot of vermiculite and received 150 ml Hoagland's solution twice a week and tap water on all other days. The unhardened plants were exposed to 25° day (12 hr) and 15° night (12 hr) and an illumination of 2200 to 2400 ft-c for an 18 hr photoperiod up to the date of hardening, for a 12 hr photoperiod thereafter. The group of plants to be hardened were removed from the above set and subjected to the following successive temperature regimes starting at the age of 6 weeks from sowing: A) 2 weeks at 5° D/N, B) 2 weeks at 5° D/0° N, C) 2 weeks at 0° D/N, D) 3 to 6 weeks at -3° D/N. Light conditions were changed to a 12 hr photoperiod of 1000 to 1100 ft-c for the first 3 temperature regimes, complete darkness for the final -3° regime.

Three series of plants were used: 1 for measurements of growth and the other 2 for measurements of frost resistance.

The growth series consisted of 12 plants. Leaf areas were measured by periodic tracings of leaf outlines on paper. The areas were measured by means of a planimeter. The leaves were numbered from the base upward, starting with the first true leaf as number 3. At the age of 6 weeks, these 12 plants were divided into 2 groups, 6 plants being left unhardened and 6 hardened by the above schedule. Growth curves were plotted from the average area measurements for leaves of each level. Growth rates were determined from the slopes of these curves. The plants became potbound by about the end of 6 weeks, and this must have limited the growth rate.

Starting at the age of 5 weeks, frost resistance was measured by freezing individual leaves and determining the temperature that killed 50% of the leaf (2). In 1 series of 72 plants, measurements were made every 6 to 7 days throughout the hardening period; in the other series of 48 plants, measurements were made daily, but only during the first week of hardening at 5°.

In these 2 series, frost resistance measurements were made on a different group of 6 plants at each date. The growth measurements, on the other hand, were made on another group of 6 plants which was used throughout the experiment. The experiment has since been repeated using the same plants for both the growth and hardness measurements, with identical results. The standard error of the leaf measurements was $\pm 1\%$. The freezing temperatures were measured by thermocouples inserted into leaf petioles and read on the recorder to 0.1°. Since the accuracy of the killing temperatures did not justify this precision, values were expressed to the nearest 0.5°.

Results

Unhardened Plants. Leaf growth increased progressively with leaf number, reaching a maximum

¹ This investigation was supported by a grant from the National Science Foundation (NSF GB 6828).

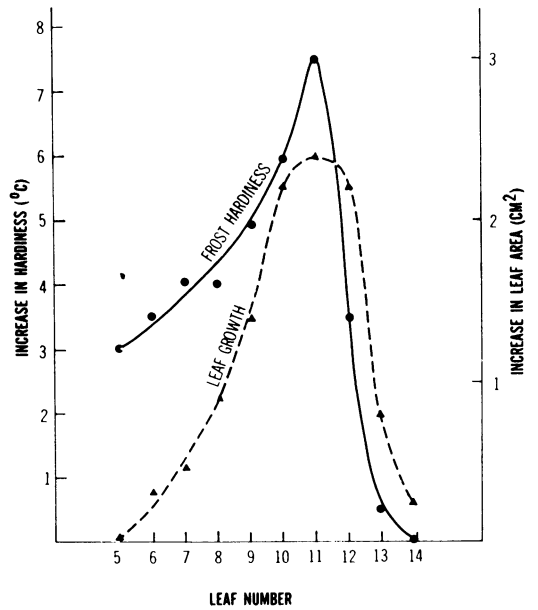
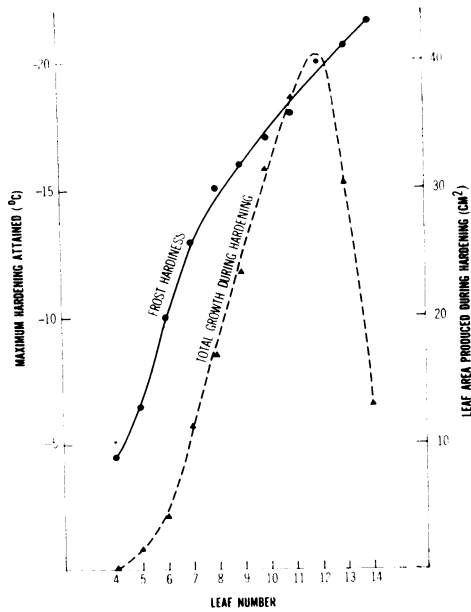
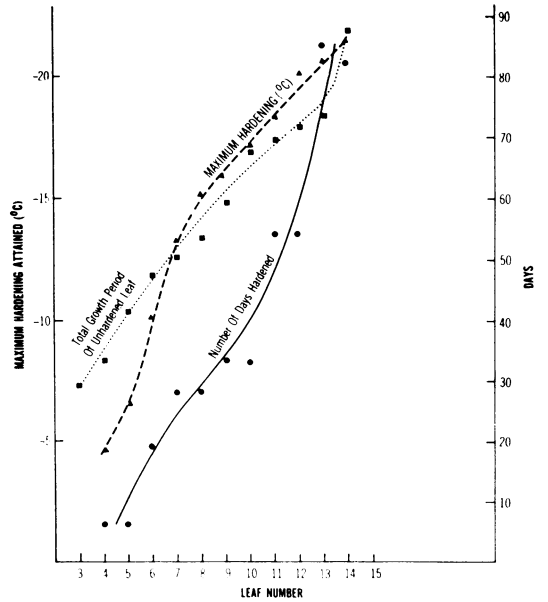
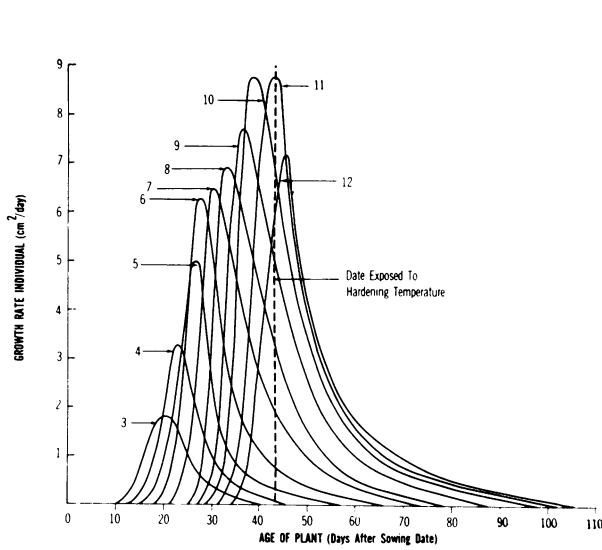


FIG. 1. (top left) Growth rate curves for cabbage leaves 3 to 12 at increasing levels of insertion on the plant. Stage of growth on transfer of half of plants to 5 ° for hardening is indicated by the line drawn at 43 days. Plants grown at temperatures of 25D/15N throughout growth period.

FIG. 2. (top right) Relation of maximum hardening achieved (▲----▲) to number of days at hardening temperature (●——●) and to total period of growth (■----■).

FIG. 3. (bottom left) Relation of maximum hardness developed (●——●) and of leaf growth produced during prolonged hardening, (△----△) to leaf number.

FIG. 4. (bottom right) Relation of increase in frost hardness (●——●) and leaf growth (▲----▲) during 24 hr hardening to leaf number.

final area, as well as the highest maximum rate of growth in the tenth and eleventh leaves (table I). This occurred just after the plants had become pot-bound. The growing period as well as the total life span of each leaf also increased progressively with leaf number, reaching a maximum in the fourteenth leaf and twentieth to twenty-fifth leaf respectively.

The time required for each leaf to reach its maximum growth rate remained constant up to the leaves with the maximum growth rate (Nos. 10 and 11—table I), though this rate increased with leaf number to 5×. This relationship can be seen by the increase with leaf number in the steepness of the growth curves (Fig. 1). Similarly, the total life span of the leaves only doubled with leaf number, whereas the maximum area attained increased to 7×. The increase in area with leaf number up to leaf 11,

therefore, depends primarily on a greater acceleration rate and only secondarily on a longer growth period. On the other hand, the leaves beyond leaf 11 begin to show a greater dependence on growth period, so that leaf 25 had the same maximum growth rate as leaf 5, yet grew to double the size of the latter, due to a growth period double that of leaf 5.

Hardened Plants—Prolonged Hardening. In all except the first-formed leaves, growth continued at the higher hardening temperatures (5° and 0°—table II), though at a reduced rate compared to the control plants left at the original growing temperature. Growth of all the measured leaves stopped at -3°. As in the case of the unhardened plants, the growth rate increased with leaf number. The low temperature exposed to during hardening, increased the longevity of the leaves very little (not more than 20%—table I).

Table I. *Growth of Cabbage Leaves at Different Levels of Insertion on the Plant*

First true leaf taken as leaf No. 3. Date of sowing seed taken as day 1. All values are for unhardened plants, except column 4.

Leaf No.	Age of plant when leaf measurable	Life span		Maximum growth rate	Leaf age at max. growth rate	Total growth period	Max. area
		Unhardened	Hardened from 43rd day				
	<i>days</i>		<i>days</i>	<i>cm²/day</i>		<i>days</i>	<i>cm²</i>
3	10	40	45	1.8	10	29	19
4	12	43	48	3.3	11	33	34
5	15	50	53	5.0	11	41	47
6	18	54	62	6.3	10	47	64
7	21	64	68	6.5	9	51	86
8	24	67	75	6.9	9	53	102
9	27	75	83	7.7	10	59	121
10	28	85	84	8.8	11	67	140
11	31	85	100	8.8	12	69	135
12	34	86	108	7.2	12	71	126
13	37	88	108	7.4	13	73	126
14	40	88	108	7.2	13	87	120
20	58	90	112	5.9	15	84	135
25	73	90		4.9	13	82	108
30	83	88		2.3	14	75	93

Table II. *Frost Hardiness and Growth of Cabbage Leaves at Different Levels of Insertion and Hardened From Forty-third day*

Leaf No.	Age of plant at max. hardiness	Age of leaf at max. hardiness	Maximum hardiness	Time hardened at maximum hardiness	Leaf growth during hardening
4	49	37	-4.5	6	0
5	49	34	-6.5	6	2
6	62	44	-10.0	19	5
7	71	50	-13.0	28	10
8	71	47	-15.0	28	14
9	76	49	-16.0	33	22
10	76	48	-17.0	33	31
11	98	67	-18.0	54	35
12	98	64	-20.0	54	40
13	128	91	-20.5 ¹	85	36
14	125	85	-21.5 ¹	82	13

¹ Due to their small size, leaves 13 and 14 were frozen while still attached to the stem, instead of removing them from the stem before freezing as in the case of the other leaves.

In general, frost resistance increased during the first week under each succeeding hardening regime, followed by a leveling off or slight decrease in the second week. At -3° , however, the reverse occurred. Due to inadequate temperature control ($\pm 1^{\circ}$), this treatment resulted in repeated freezing and thawing which must have affected the hardening process. The age of the leaf when it achieved its maximum hardening, changed very little with leaf number, at least up to the tenth leaf (table II). Yet the maximum hardening attained, increased with leaf number, at least until the twelfth to fourteenth leaf. This parallels the increase in number of days during which each leaf was exposed to the hardening temperature, before reaching its maximum hardness (Fig. 2). It also parallels the total growth period of the unhardened leaf (Fig. 2). A further relation between growth rate and hardness is indicated in Fig. 1. As the leaf number increases, the time of maximum growth rate approaches closer and closer to the date at which hardening was initiated, until leaf number 11 which was at its maximum growth rate when hardening began. Thus, the more rapid the growth rate on exposure to the hardening temperature, the more it can harden. But maximum growth rate decreases after the eleventh leaf (table I), though hardness continues to increase (table II). Similarly, the total growth produced during hardening, parallels the maximum hardening up to leaf 12 (Fig. 3), but not beyond it.

In those cases where the leaves were in the straight-line portion of the growth curve, the Q_{10} was 2.0 to 2.2 (table III). A similar result (Q_{10} 2.4-2.5) was obtained when growth rates during the first 24 hr at the hardening temperature were compared with those of the controls (table IV). The Q_{10} 's for growth stages 1 and 3 are meaningless, because the control and hardened plants do not remain at the same stage due to the more rapid rate of growth in the former.

Brief Hardening. One of the complications in experiments involving prolonged hardening, is a lowering of hardness due to senescence. Brief hardening periods avoid this problem, at least in the

Table IV. *Growth Rate of Control and Hardened Leaves During the First 24 Hours that the Latter were Exposed to the Hardening Temperature*

Leaf No.	Growth rate		Unhardened	
	unhardened	hardened	Hardened	Q_{10}
	<i>cm²/day</i>		<i>ratio</i>	
9	5.2	1.4	3.7	2.5
10	7.8	2.2	3.5	2.4
11	8.8	2.4	3.6	2.4

case of actively growing leaves. A 24 hr hardening period increased the frost resistance of the leaves by as much as 7.5° (table VI). Again, as in the case of the prolonged hardening, the more rapid the growth rate on exposure to the hardening temperature (table V), the greater the increase in hardness (table VI). This relation held true also for the growth rate during the 24 hr hardening period, though this was markedly decreased in all leaves (table VI and Fig. 4). Even the relative growth rate (the increase in area per unit of leaf area) showed this same relation to the increase in hardness up to leaf No. 11 (table VI). The very small leaves (13 and 14) were exceptional in having the largest relative growth rates and the smallest increase in hardness.

Discussion

The individual growth and hardness values obtained in this experiment are not to be taken as constants for this variety of cabbage. They will depend on the growing conditions, *e.g.* the pot size, mineral nutrition, spacing of pots, *etc.* Since all measurements were made on plants grown under identical conditions, they do permit an analysis of the relation of growth to hardness. The following is the most obvious explanation of the increase with leaf number in the maximum hardness attained. Due to the earlier age at which hardening commenced, and the parallel increase in life span with

Table III. *Growth of Control Unhardened Cabbage Leaves and of Corresponding Leaves Exposed to the Hardening Temperature From the Initial Hardening, Day 43 to Day 50 in Both Cases*

Q_{10} calculated for an average temperature of 20° for the unhardened plants (12 hr 25° , 12 hr 15°). Growth stage 2 is the straight line portion of the S-shaped curve.

Leaf No.	Growth stage	Growth from day 43 to day 50		Unhardened	
		unhardened	hardened	hardened	Q_{10}
		<i>cm²</i>		<i>ratio</i>	
7	3	10.5	5.7	1.8	1.5
8	2-3	17.2	7.3	2.4	1.8
9	2	30.0	9.7	3.1	2.1
10	2	44.0	15.4	2.9	2.0
11	2	52.4	17.2	3.0	2.1
12	2	51.2	15.5	3.3	2.2
13	1-2	35.3	8.0	4.4	2.7

Table V. *Growth of Unhardened Cabbage Leaves Just Before Exposure to Hardening Temperature*
Plant age was 43 days

Leaf No.	Leaf age	Growth rate	Leaf area	Growth rate per unit area	Frost killing temperature
	<i>days</i>	<i>cm²/day</i>	<i>cm²</i>	<i>per day</i>	<i>deg</i>
5	28	0.2	45	0.004	-3.0
6	25	0.7	59	0.012	-3.5
7	22	1.7	70	0.024	-4.0
8	19	3.3	74	0.043	-4.0
9	16	5.2	68	0.075	-3.0
10	15	7.8	58	0.130	-3.0
11	12	8.8	37	0.226	-2.5
12	9	5.6	15	0.373	-2.5
13	6	1.5	3.5	0.450	-2.5
14	3	0.4	0.5	0.800	-2.5

Table VI. *Relation of Growth and Frost Hardiness to Leaf No. After Hardening for 24 Hours at 5°*
Plant age was 44 days.

Leaf No.	Growth rate	Leaf area	Growth rate per unit area	Frost killing temperature	Increased hardness
	<i>cm²/day</i>	<i>cm²</i>	<i>per day</i>	<i>deg</i>	<i>deg</i>
5	0	44	0.000	-6.0	3.0
6	0.3	59	0.005	-7.0	3.5
7	0.45	72	0.006	-8.0	4.0
8	0.9	78	0.012	-8.0	4.0
9	1.4	71	0.020	-8.0	5.0
10	2.2	61	0.036	-9.0	6.0
11	2.4	41	0.059	-10.0	7.5
12	2.2	18	0.122	-6.0	3.5
13	0.8	4	0.200	-3.0	0.5
14	0.25	1	0.500	-2.5	0.0

leaf number, the period exposed to hardening conditions also increased with leaf number. Thus, the lowest leaves died a few days after hardening commenced and, therefore, had little time to harden. Whereas, the eleventh leaf remained alive for 57 days at the hardening temperature. But the growth rate just before exposure to the hardening temperature, and also the subsequent amount of growth during hardening, also increased with leaf number and, therefore, with the maximum hardness attained. This is the opposite of what would be expected if growth were antagonistic to hardening. On the contrary, growth appeared to be essential for hardening, since little hardening occurred in leaves exposed to the hardening temperature after growth was completed. This prevention of hardening may be due to senescence. But senescence cannot be involved in the actively growing leaves, and these showed an ability to harden which paralleled their growth rates at the time of exposure to the hardening temperature. Maximum hardening occurred in those leaves which were at or near their maximum growth rates when exposed to the hardening temperature.

The Q_{10} of 2.0 to 2.5 for both the total growth of the first week of hardening, and the growth rate during the first day of hardening proves that the sole effect of the hardening temperature is on chem-

ical reaction rates, since this is the normal value for chemical reactions. There is, therefore, no evidence of an additional decrease due to a reversible denaturation of enzymes such as described by Brandts (1). This agrees with direct measurements of free and masked SH groups of proteins (Dear and Levitt, unpublished).

The fact that the leaves continue growing while hardening, proves, of course, that growth and hardening are not necessarily antagonistic. The decrease in growth rate which occurs at the low (hardening) temperature may, however, be necessary for hardening. Thus, the maximum area attained was less when part of the growth occurred at the hardening temperature than in the unhardened control leaf. On the other hand, the greater the fraction of the leaf's growth that had not been completed when hardening commenced, the more hardy the leaf became (Fig. 1). This indicates that maximum hardening is possible only if the leaf completes the major part of its growth at the hardening temperature.

The increases in hardness as a result of the brief (24 hr) hardening periods provide even more dramatic evidence of a direct relation between ability to harden and growth rate at the onset of hardening. In both experiments, the very youngest (and smallest) leaves were exceptions. This may mean that

only growth due to cell enlargement is directly related to hardening, and that growth due to cell division is inversely related to hardiness.

How can this direct relation between growth rate and hardening potential of cabbage leaves be reconciled with the fact that normal hardening of perennials in the fall occurs in the complete absence of growth? It seems obvious that the hardening process must require some factor common to both the growing cabbage leaves and the non-growing perennials which harden in the fall. A clue may be provided by the fact that both RNA and protein synthesis increase during the fall in the non-growing perennials, and that these increases parallel the increase in hardiness (4,5). In the case of the cabbage leaves, ability to harden paralleled growth rate during hardening and therefore metabolic rate in general and protein synthesis in particular. It is, therefore, proposed that those growing plants or organs which, unlike cabbage, cannot harden if exposed to the hardening temperature must cease growth at this temperature and, therefore, their protein synthesis is reduced to nothing more than turnover or perhaps less.

The concept on which this explanation is based is as follows. Proteins synthesized at normal, growing temperatures are aggregated irreversibly by freezing. Proteins synthesized at low (*i.e.* hardening) temperatures are less readily aggregated, and the lower the temperature of their synthesis, the more severe the freeze necessary to aggregate them. The ability of a plant to develop frost resistance at low (hardening) temperatures depends on its ability to resynthesize its proteins at these temperatures. On this basis, we cannot generalize that growth and

frost hardiness are always inversely related. Growing plants are unable to develop frost resistance only if their growth and active protein synthesis is brought to a stop by hardening temperatures. Non-growing plants are able to develop frost resistance only if they can develop an active protein synthesis which is uncoupled from growth at hardening temperatures. In short, a net protein synthesis at hardening temperatures is postulated to be the *sine qua non* of frost hardening; and if it occurs the plant will harden whether or not it is growing.

This concept does not necessarily apply to all the plant's proteins. It may conceivably apply only to a few key proteins, which due to their high content of hydrophobic groups are more likely to be denatured extensively at low temperatures (Brandts 1967).

Literature Cited

1. BRANDTS, J. F. 1967. In: Thermobiology. Anthony H. Rose, ed. Academic Press, New York. p 25.
2. KOHN, H. AND J. LEVITT. 1965. Frost hardiness studies on cabbage grown under controlled conditions. *Plant Physiol.* 40: 476-80.
3. LEVITT, J. 1956. *The Hardiness of Plants*. Academic Press, New York.
4. LI, P. H. AND C. J. WEISER. 1967. Evaluation of extraction and assay method for nucleic acids from red-osier dogwood and RNA, DNA, and protein changes during cold acclimation. *Proc. Am. Soc. Hort. Sci.* 91: 716-27.
5. SIMINOVITCH, D., B. RHÉAUME, AND R. SAHAR. 1967. In: *Molecular Mechanisms of Temperature Adaptation*. C. Ladd Prosser, ed. Washington: Am. Assoc. Advan. Sci. p 3.