EFFECT OF SEVERAL ENVIRONMENTAL FACTORS ON THE HARDENING OF PLANTS[1]

S. T. DEXTER[2]

(WITH FIVE FIGURES)

In several papers (**1–3**), DEXTER, TOTTINGHAM, and GRABER have outlined a method for determining the injury which plants suffer as a result of freezing. This method involves the determination of the exosmosis of mineral matter from the injured tissues, by measurement of the electrical conductivity of the water in which they have been immersed. Since the method is very sensitive to changes in the hardiness of plants, it appeared that the influence of various environmental conditions on hardening could be readily studied. This paper deals with the influence on hardening of light of different day-lengths, and of darkness. The influence of light with and without carbon dioxide (*i.e.*, with and without photosynthesis) was also studied. Several constant temperatures were used, and various combinations of alternating temperatures, both with and without carbon dioxide. Alfalfa, wheat, cabbage, and tomato plants were used for experimental material.

Experiment I

On August 25, pots of alfalfa plants of an especially uniform Turkestan strain were transferred from the greenhouse to a large cold room. These plants were grown from seed sown in May, and furnished by Professor L. F. GRABER, of the University of Wisconsin. The pots were one-quart ice cream cartons, paraffined thoroughly. There were generally five plants in each. The plants had never been defoliated. For this experiment, plants under four conditions during hardening were studied: (1) The tops were removed from the plants, and the pots were kept at 0° C. in darkness. (2) The tops were not removed, but the plants were kept in darkness at 0° C. (3) The tops were not removed, and the plants were illuminated for seven hours each day, in a gradually changing temperature which reached a maximum of about 10° C. after four hours of illumination; when the lights went off, the temperature gradually fell to 0° C. Two 1000-watt lamps were used, giving an intensity at the pots of from 900 to 1200 foot-candles, according to the Macbeth illuminometer. Thermograph records were kept. (4) The tops were not removed, but the plants were kept at 0° C. both during illumination and while in the dark. The illumination period was for seven hours, with a slightly greater intensity than in (3).

[1] Contribution from the Hull Botanical Laboratory.

[2] National Research Council fellow in the Biological Sciences.

The temperature at the pots always rose a degree or two when the lights went on, in spite of very thorough stirring of the air with an oscillating 12-inch electric fan.

Each sample tested consisted of the roots of the plants from two pots, trimmed to weigh 5 grams when clean and dried of surplus surface water. The plants were very uniform. The samples were placed in 1×8-inch pyrex test-tubes and frozen in an alcohol-ice slush bath at $-7°$ C. for four hours. They were thawed by immersion of the tubes in a water-bath at 2° C. for one hour. After thawing, 25 cc. of distilled water at 2° C. were added to each tube in turn. Exosmosis was permitted to continue for 20 hours at 2° C., when the liquid was drawn off and the electrical conductivity determined at that temperature. Table I shows the results of these determinations after various periods of hardening treatment.

TABLE I

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF ALFALFA ROOTS FROZEN FOR FOUR HOURS AT $-7°$ C. INTERVAL OF 20 HOURS ALLOWED FOR EXOSMOSIS AT 2° C.

TREATMENT AND CONDITION	DAYS OF HARDENING TREATMENT					
	0	4	7	14	28	42
1. Tops removed, dark, 0° C.	755	845	840	502	551	515
2. Tops not removed, dark, 0° C.	755	894	817	478	509	537
3. Tops not removed, 7 hours' light, alternating temperature	755	799	573	472	389	408
4. Tops not removed, 7 hours' light, constant temperature	755	662	577	468	346	341

Figure 1 shows these results in graphic form. It would appear that the four conditions divide roughly into two classes. The plants in the dark hardened much the same, whether with or without tops; those in the light hardened finally much the same, whether with constant or alternating temperature. At the end of 10 days, since the division into two groups was then becoming evident, two pots were transferred from condition no. 4 into a constant temperature (0° C.) in the dark, with tops removed. At the end of the experiment, these roots gave a conductivity reading of 551, as against 341 for those continuing in the light. This indicated clearly that hardening had not continued in the dark chamber, although it was well started in the light. Another pair of pots was transferred from condition no. 1 and from condition 2 into the dark but with the alternating tempera-

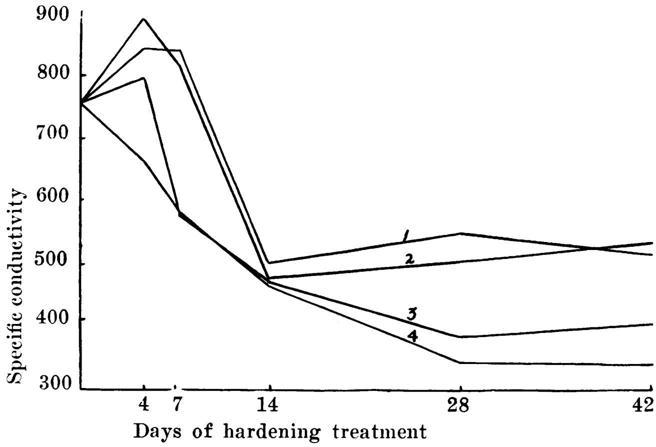

FIG. 1. Curves presenting data of table I in graphic form. The plants in treatment 4 (continuous cold, with light) hardened the most rapidly and the most completely; those in treatment 3 (alternating temperatures, with light) hardened almost as much. Plants in treatments 1 and 2 (in the dark) hardened much less than those in treatments 3 and 4.

ture of condition 3. They gave an average conductivity value of 517 at the end of 42 days. Thus hardening was not notably more efficient with the alternating temperatures in the dark than with the constant temperature in the dark.

On the last date, after 42 days of hardening treatment, the samples were so prepared that buds were left at the crown, in order that recovery in the greenhouse could be noted after freezing and exosmosis. Recovery of the plants showed that the treatments could be readily distinguished. Plants receiving light during hardening made much greater growth and fewer plants died.

Repetitions of experiment

Experiment 1 was twice repeated, with variations. In each case alfalfa plants in darkness hardened less completely than those in the light. Various day-lengths were used in the hardening chamber at 0° C. and at 10° C. The samples in these experiments were far from uniform, and definite conclusions could not be drawn. In general, hardening was favored by a long period of illumination, if the plants were kept continuously in the cold. In the case of alternating temperatures during hardening treatment, hardening was retarded by the long day at higher temperatures (PELTIER and TYSDAL 5), and elongation of stems was favored. Under this treatment, elongation of stems was especially evident if the plants had been given a short day (7 or 8 hours) previous to such hardening treatment. Figure 2 shows the change that occurred in previously short-day alfalfa plants during

FIG. 2. Condition of alfalfa plants, grown for several months in the greenhouse with a 7 or 8-hour day and then transferred to hardening rooms. The pot on the left received 14 hours of light each day at 0° C., and was kept in the cold continuously; the pot on the right received 14 hours of light each day at a room temperature of about 22° C. The first pot made no evident growth, while the second grew several inches in 21 days. The first treatment gives good hardening, while the second treatment stimulates top growth and almost prevents hardening.

a treatment in which they were given 14 hours of illumination at 22° C. for 21 days, with 10 hours of each day in the dark at 0° C. While such plants continuously in the cold room (0° C.), with 14 hours of illumination, hardened from a specific conductivity of 889 at the start to 559 at the end of 21 days, similar plants receiving equal light in the warm room hardened to only 751. The same general relation was found in the case of plants which received light for about 14 hours each day previous to hardening. These plants hardened from 797 to 497 when kept continuously in the cold room with 14 hours of light, while similar plants receiving 14 hours of light in a room at 22° C., and 10 hours in the dark at 0° C. each day, hardened to only 652. The short-day plants shown had received 7 or 8 hours of daylight in the greenhouse each day for several months previous to hardening treatment; the long-day plants had received 14 or 15 hours of illumination each day for the same period.

In no case did alfalfa plants harden as completely in the dark as in the light. Alternating temperatures were no more effective than continuous ones in any case studied. If the illumination tended to favor much extension of parts, hardening was retarded or prevented. Hardening was more thorough at 0° C. than at 10° C. Although these plants were in general very well stored with starch and other reserve foods, as shown by microinspection, hardening was always more complete in the light, when photosynthesis could furnish a continually replenished supply of carbohydrates for the processes of the plants.

Experiment 2

In this experiment, Minhardi winter wheat was grown in perforated soil-temperature cans (6×9-inch cylinders) in soil which was thoroughly sifted and mixed. The seed was sown on November 25, the plants were thinned to 15 plants per can, and, from the first, were exposed to two day-lengths in the greenhouse, at about 62° F. One set received ordinary daylight (reinforced with Mazda lamps on especially dark days) for 8 hours each day; the other set received the full day-length, with lights (four 200-watt, about 2 feet away) extending the illumination period until 10 P. M., which gave approximately a 14-hour day. At an age of six weeks, the short-day plants were much smaller and somewhat greener than the long-day plants. Fifteen short-day plants weighed, entire, 3.78 grams; 15 long-day plants, 7.0 grams. The short-day plants (leaves) had a percentage dry matter of 14.0; the long day, 13.0. The percentage ash (wet-weight basis) was slightly higher in the short-day plants than in the long-day plants, 2.22 as against 1.96. The electrical conductivity of the ash solution, after converting the basic oxides to carbonates and made to volume on a wet-weight basis, was about 2 per cent. higher in the short-day plants.

These plants were taken to the hardening chamber on January 6 (six weeks old), where several pots from each set (long- and short-day) were put to harden in the following ways: (1) in the dark; (2) with 7 hours of light each day; (3) with 15 hours of light each day. All were at 0° C. Two 1000-watt lamps furnished the light, as in the preceding experiment. Precaution was taken to avoid injury to the plants by lowering the temperature of the room gradually, taking about 6 or 7 hours. Very little wilting was noted. The samples were prepared by removing the roots and leaf blades, and washing and drying the remaining crowns. Duplicate samples weighed 0.9 gram each; they were frozen for 2 hours at −10° C. in an alcohol-slush bath and thawed for 30 minutes in the water-bath at 2° C. Fifteen cc. of distilled water (2° C.) was then added to each tube. Exosmosis went on for 16 hours at 2° C., when conductivity measurements were made. Table II shows the conductivity values obtained in this experiment after the durations of hardening treatment indicated.

Table II shows consistent results throughout. The plants, whether long- or short-day in the greenhouse, hardened little if at all in the dark; they hardened materially in 7 hours of light, and still more when given 15 hours

TABLE II

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF CROWNS OF WINTER WHEAT FROZEN FOR TWO HOURS AT −10° C. INTERVAL OF 16 HOURS ALLOWED FOR EXOSMOSIS AT 2° C.

DAY-LENGTH IN GREENHOUSE	HOURS OF LIGHT IN HARDENING ROOM	DAYS OF HARDENING TREATMENT			
		0	3	7	21
Short	0	382	393	384	386
	7	382	262	222	113
	14	382	218	174	89
Long	0	447	446	431	387
	7	447	315	317	171
	14	447	307	224	138

of light each day. In the dark, the short-day plants hardened not at all; the long-day plants hardened very slowly, but perceptibly.

Since it was clear at the end of the first week that but little hardening was going on in the plants in the dark, it seemed desirable to determine the result when plants were given light, but not carbon dioxide. Two pots were taken from the long-day (greenhouse) set which had been hardening in the dark for 10 days. One pot was put under a bell-jar which was sealed with sodium hydroxide solution on the bottom; free access to air was provided through a soda-lime tube at the top. Paper soaked in sodium hydroxide solution was stuck to a portion of the side wall of the bell-jar to take up any carbon dioxide set free in respiration. The other pot was similarly covered with a bell-jar which was sealed with water at the bottom. To this latter jar a carbon dioxide generator was connected each morning. Phosphoric acid was placed in the generator flask, and 100 cc. of M/50 sodium carbonate were allowed to flow slowly, through a capillary tube, into the acid. This slowly set free the carbon dioxide for a period of about three hours, it flowing through the delivery tube into the bell-jar. In this way, between 40 and 50 cc. of carbon dioxide gas were supplied to the pot each day. The bell-jars were placed side by side at 0° C., and were illuminated for 15 hours each day. This hardening period lasted for 12 days, when samples were prepared and treated as usual. Table III shows the results of determinations of electrical conductivity with duplicate samples from three pots, of which one was in the dark during this period, the second in the light without carbon dioxide, and the third in the light with carbon dioxide.

TABLE III

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF CROWNS OF WINTER WHEAT FROZEN FOR TWO HOURS AT −10° C. INTERVAL OF 16 HOURS ALLOWED FOR EXOSMOSIS AT 2° C.

CONDITION DURING HARDENING	SPECIFIC CONDUCTIVITY
No illumination, 0° C.	387
Illuminated 15 hours each day, 0° C., no CO_2	394
Illuminated 15 hours each day, 0° C., given CO_2	222

The figures in table III show that hardening proceeded almost identically in the cases of plants in the dark and those illuminated, but without carbon dioxide in the surrounding atmosphere. The plants which received carbon dioxide hardened in the usual way. This seems to indicate that hardening is definitely associated, in these rather succulent tissues, with photosynthesis, and that the influence of light at this temperature may be largely due to carbohydrate synthesis.

At the end of the hardening treatment, two pots of short-day (greenhouse) plants, one from the dark, the other from the long-period illumination treatment, were harvested for the determination of dry matter. The hardened plants (from the light) were much higher in dry matter (20.8 as compared with 13.0 per cent.) than those which had not hardened (in the dark). Curiously, however, the total amount of dry matter in the hardened plants had not increased in any such proportion. There seemed, therefore, to have been a dehydration process going on in the hardening plants, in the light, which increased the percentage of dry matter by loss of water as well as by gain in carbohydrates. Further study may determine whether or not this is actually the case.

REPETITIONS OF EXPERIMENT

Experiment 2 was repeated, with variations, with plants of three varieties of winter wheat grown out-of-doors until five weeks of age (September 23 to October 27). When these plants were given hardening treatment at 0° C. in the dark, with 7 hours and with 15 hours of illumination each day, for a period of 28 days, hardening was least complete in each case in darkness, more complete with 7 hours, and most complete with 15 hours of illumination. The average (for the three varieties) conductivity value at the beginning of hardening treatment was 884; in the dark this value was 398 at the end of the hardening period. With 7 hours of illumination each day the value was 292; and with 15 hours, 237. These plants, which were much more sturdy and higher in dry matter than the plants grown in the greenhouse, hardened considerably in the dark, whereas the succulent greenhouse plants did not. The alfalfa plants which were well stored with reserve foods (experiment 1) also hardened somewhat in the dark. In each case, however, hardening was more complete in the light.

The plants of the three varieties of wheat were also given 7 and 15 hours of illumination each day at 10° C., for 28 days. These plants, while hardening slightly at first, became very chlorotic and more tender as the experiment proceeded. As in the case of the alfalfa plants, those receiving the longer day at this higher temperature were more tender than those receiving the 7-hour day.

Experiment 3

In this experiment, the effect of the presence or absence of carbon dioxide in the air was studied at different temperatures and with four species of plants. Minhardi wheat, hardy alfalfa, cabbage, and tomato plants were used. The wheat, cabbage, and tomato plants were grown in sand culture with nutrient solutions, and were five weeks old at the beginning of the hardening treatment. The plants were very uniform in appearance. The alfalfa plants were nine weeks old when hardening treatment began. They were grown in soil and were somewhat irregular in appearance. The wheat received a short day (8 hours) in the greenhouse, while the other plants received a long day (about 14 hours). The photographs show the size of the plants. There were 30 wheat plants, approximately 30 alfalfa plants, 8 tomato plants, and 8 cabbage plants, respectively, in each can. Samples were prepared as follows. The wheat samples were prepared as in experiment 2, using duplicate 1-gram samples. The alfalfa root samples were prepared by removing the leaves at the crown, washing in distilled water, and drying the surface water. A single 0.5-gram sample (about 25 roots) was used. The tomato and cabbage samples were prepared by removing the roots, and washing the tops and drying between towels. Duplicate 3-gram samples of the tops of each were used. The wheat and alfalfa samples were placed in test-tubes and were frozen in an alcohol-ice slush at −8° C. for 2 hours; they were thawed in the water-bath at 2° C. for 30 minutes. To each tube in turn, 10 cc. of distilled water at 2° C. were added. Exosmosis continued for 16 hours at 2° C., when the electrical conductivity was measured at that temperature. The cabbage and tomato samples were placed in 1 × 8-inch test-tubes, frozen at −5° C. for 4 hours, thawed as indicated, and 25 cc. of distilled water at 2° C. added to each tube. Conductivity measurements were made after 14 hours of exosmosis. There is generally considerable super-cooling without freezing when plant tissues are subjected to a temperature no colder than −5° C. This experimental difficulty was met with regularly when this temperature was used. If, however, the tissues were moved about in the tube with a clean glass rod after they had been thoroughly chilled, or if the tube was sharply rapped against a solid body, freezing generally occurred rather promptly. In some cases, especially when the plants were partially hardened, it was necessary to start crystallization by placing the tubes for a moment or two in a colder bath (−7 or −8° C.), after which they were replaced in the −5° C. bath where freezing always continued without difficulty.

The plants in this experiment were hardened in the following way. Two glass-walled chambers, about 8 cubic feet each, were placed in the cold room at 0° C. Into each chamber a tube of air was led from a compressed air line. In the case of one chamber, the air was merely dehydrated (with sulphuric acid and glass wool); in the other chamber, the dried air was passed through a long tube filled with alternate layers of soda-lime, glass wool, and solid sodium hydroxide, to remove the carbon dioxide from the air. Since the chambers were by no means air-tight, and had to be opened to water the plants, etc., doubtless there was some carbon dioxide in the chamber in any case. To avoid this as much as possible, further sodium hydroxide solution was placed within the case. Into each case eight cans of plants were placed, that is, two cans of each of the four species. Both cases received light from a 1000-watt lamp for 14 hours each day. During illumination the temperature in the cases went up to about 2° or 3° C.

In a warmer room, at about 20° C., two similar cases were arranged, one with ordinary air and the other with carbon dioxide-free air. These plants

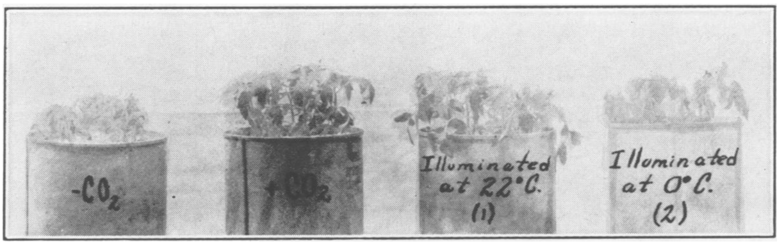

FIG. 3. Condition of tomato plants from experiments 3 and 4 after 2 days of treatment. On extreme left is the pot of plants which received no carbon dioxide; next to it, the one which received carbon dioxide. The plants receiving no carbon dioxide suffered very severe injury in the cold room, although they did not freeze; the others were uninjured. The two pots on the right are from experiment 4. The plants in the pot on the extreme right received illumination for four periods of 2 hours each at 0° C. with the other 16 hours each day at 22° C. They were badly injured by the cold. The other plants received an equal illumination, but at 22° C., with the remaining 16 hours at 0° C. They were uninjured by the cold. In this case, an opportunity for high photosynthesis and low respiration may well explain their ability to survive the cold for 16 hours, whereas the plants with an opportunity for high respiration and low photosynthesis could not survive.

received 14 hours of illumination each day at a temperature of approximately 24° C. Each day, at the end of the illumination period, the plants from both of the glass cases in the warm room were transferred to the cold room, where they remained for 10 hours in the dark at 0° C. They were then transferred back to the cases in the warm room, and the process was repeated. At the end of two days the photograph shown as figure 3 was taken. This shows that the tomato plants receiving light in the warm room, but no carbon dioxide, were very badly injured in the cold room, although they were not frozen. This injury was plainly evident after the first night in the cold room, following the first day without carbon dioxide. The plants receiving carbon dioxide were not injured perceptibly by the exposure in the cold room.

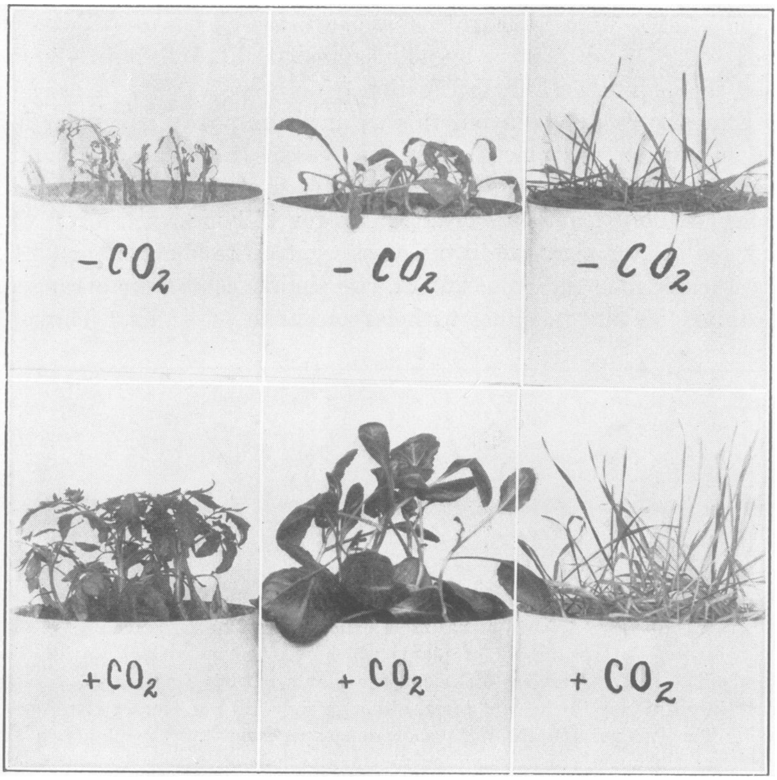

FIG. 4. Condition of the two sets of plants in the room at about 24° C. at the end of two weeks of treatment. Each set received 14 hours of illumination in the warm room, and 10 hours in the dark at 0° C. each day. The upper set received carbon dioxide-free air during the two weeks, and the plants were almost dead. The lower set received ordinary (dehydrated) air and were in good condition.

The other pots in figure 3 received an alternating temperature and light treatment as described in experiment 4. The uninjured plants received their light (four 2-hour periods of illumination) in a warm room, while the badly wilted plants next to them, on the extreme right, received equal illumination, but in the cold room. Again the injury was plainly evident after one day of treatment.

Photographs of the plants with and without carbon dioxide in the warm room, taken at the end of two weeks, are shown in figure 4. Here it is evident that the cabbage and tomato plants receiving light but no carbon dioxide were almost dead, and the wheat was wilted and yellow. The pots of alfalfa plants were omitted from the photograph, since the numbers of plants per pot were not identical. The alfalfa plants showed the starvation effect later than the other plants, but it was evident at the end of two weeks.

At the end of one week of hardening treatment, and at the end of two

TABLE IV

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF SAMPLES OF THE PLANTS IN EXPERIMENT 3. SAMPLES OF WHEAT AND ALFALFA WERE FROZEN FOR 2 HOURS AT $-8°$ C.; THOSE OF CABBAGE AND TOMATO FOR 4 HOURS AT $-5°$ C.; FOURTEEN HOURS OF ILLUMINATION DAILY

Species	Carbon dioxide	Days of hardening treatment			Remarks
		0	7	14	
Continuously in cold at 0° C.					
Alfalfa	Present	297	274	210	
Alfalfa	Absent	297	314	317	
Wheat	Present	650	367	226	
Wheat	Absent	650	499	345	
Cabbage	Present	804	658	486	
Cabbage	Absent	804	1027	1445	
Tomato	Present	1060	1006	1250	Both sets almost dead
Tomato	Absent	1060	1159	1385	
Illuminated at 24° C., and 10 hours in dark at 0° C.					
Alfalfa	Present	297	263	296	
Alfalfa	Absent	297	292	320	
Wheat	Present	650	567	621	Decided elongation in both cases
Wheat	Absent	650	635	700	
Cabbage	Present	804	976	707	Approximately doubled in size
Cabbage	Absent	804	1093	1709	Almost dead
Tomato	Present	1060	1052	946	Approximately doubled in size
Tomato	Absent	1060	1081	1385	Almost dead

weeks, samples from the four sets of plants were prepared for testing, as previously described. The conductivity determinations are given in table IV.

From the figures in table IV, it is evident that hardening was favored by the presence of carbon dioxide in the air. The different reactions of the four species are of considerable interest. Thus wheat and alfalfa, and to a less extent cabbage, hardened much more thoroughly when kept continuously in the cold. The tomato plants, however, were severely injured by the conditions of hardening before the period was over, with the exception of the plants receiving carbon dioxide and light at the higher temperature. The tomatoes would not survive the occasional exposures to a temperature of 0° C. unless given illumination at a higher temperature for a part of the day. Experiment 4, which follows, brings this point out further. It is also of interest to note that, of the plants in the cold room continuously, but largely without carbon dioxide, the winter wheat alone hardened. Wheat plants of the same set in the same room in the dark showed no hardening whatsoever. In experiment 2 it was shown that when carbon dioxide was completely excluded, wheat hardened almost precisely the same in the light as in the dark.

As the photographs in figure 4 show, the tomato and cabbage plants grown in the warm room, with carbon dioxide, grew extensively in the two weeks' period, and almost doubled their green weight. This was, assuredly, unfavorable for hardening. It may well be also that the long day was a further aggravating tendency. The work of HARVEY (**4**) would indicate that a temperature of from 5° to 10° C. lower might have caused the cabbage plants to harden much more completely under the conditions of alternating temperatures; in fact, fully as completely as those kept continuously in the cold room. His photographs indicate that there was no perceptible difference in the size of the plants hardened with constant low, and alternating high-low temperatures. This difference in the growth behavior of the plants would readily account for the difference in hardening response in the two experiments.

The winter wheat plants which received a long day at 24° C. elongated their leaf sheaths very notably, whether they received carbon dioxide or not. The plants receiving no carbon dioxide, however, were much more chlorotic.

Experiment 4

In this experiment, four species of plants were again used, and of the same kinds and ages. However, the alfalfa was of another variety, and the cabbage and tomatoes were grown in soil. Only the wheat samples were identical with those used in experiment 3. The samples were prepared as before, except that 4-gram duplicate samples of the tomatoes and cabbage were used. Freezing and other details were carried out as described for experiment 3.

In this experiment, an attempt was made to provide conditions during hardening which would combine the effects of alternating temperatures and "cold shocks" with those of increased photosynthesis or respiration. The plants were hardened in five ways: (1) Illuminated for 2 hours at 22° C., then moved into the dark in the cold room at 0° C. for 2 hours, again into the light in the warm room; repeating four times. Thus the plants received 8 hours of interrupted illumination in the warm room, with a total of 16 hours each day in the dark at 0° C. (2) Precisely as the previous case, except reversed; that is, illumination was given only in the cold room, darkness only in the warm room. There was, therefore, 8 hours of interrupted illumination at 0° C. and 16 hours in the dark at 22° C. (3) These plants received the same temperature treatment as (1) but no light; that is, 8 hours, interrupted, in the room at 22° C. and 16 hours at 0° C., all in the dark. (4) Continuous dark, at 0° C. (5) Continuous light at 0° C.

In no case were the plants which were moved kept in absolute darkness; they were covered with heavy cardboard boxes, but could receive a fraction of a foot-candle of illumination when presumably in darkness.

Figure 3 shows the sharp difference in behavior in the tomato plants of treatments 1 and 2, photographed at the end of the second day. From this reaction it would appear that the alternating temperature behavior might well be associated with increased photosynthesis and reduced respiration in treatment 1 as contrasted with slow photosynthesis and high respiration in treatment 2. Figure 5 shows the plants from treatments 1, 2, and 3 at the end of 13 days of hardening treatment. Again the pots of alfalfa plants are omitted. (During the second week, there were two periods of illumination of 4 hours each, rather than four periods of 2 hours each.) When the photographs were taken, the tomato and cabbage plants of treatment 1 had made considerable vegetative elongation; those of condition 3 (alternating temperature in darkness) were almost dead; and the tomatoes in treatment 2 (light given at 0° C.) were also almost dead. In the two remaining treatments, 4 and 5, none of the plants made apparent growth, while the tomato plants gradually died, although those receiving light survived longer than those in darkness. Of the five treatments, but one set of tomato plants survived the experiment, namely, treatment 1. This was precisely the result found in the experiment previously described, in which it was shown that the tomato plants appeared to require an opportunity for photosynthesis at a fairly high temperature if they were to survive occasional exposures to a temperature of 0° C.

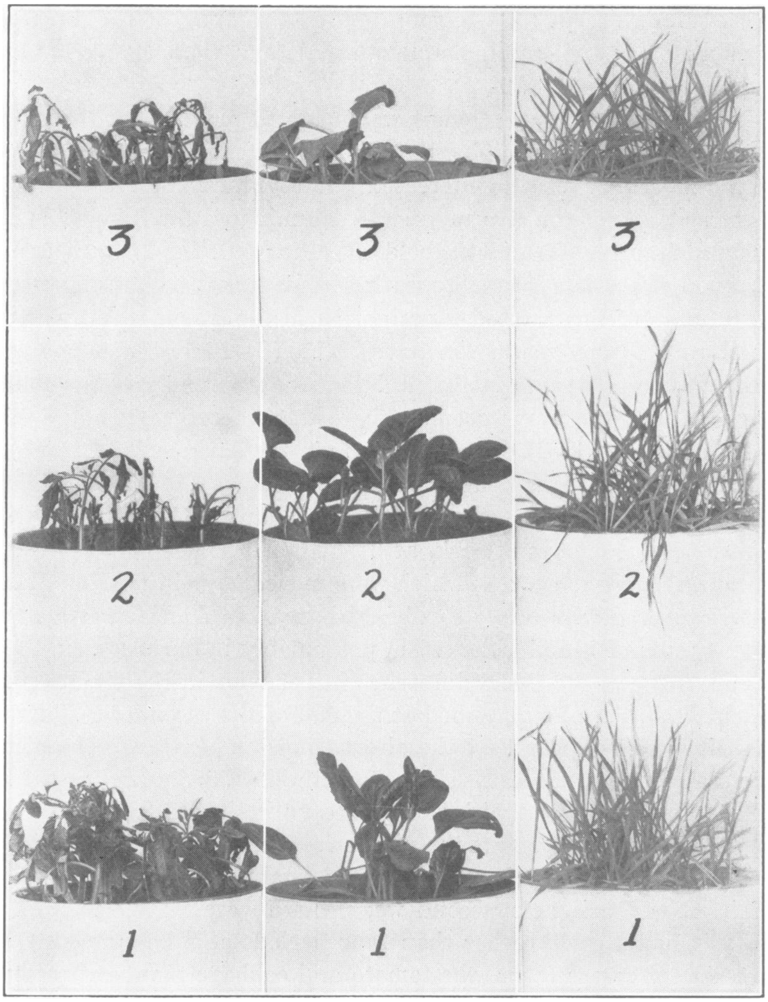

FIG. 5. Condition of the plants after 13 days of treatment. The top row of pots received alternating temperatures in the dark (treatment 3); the middle row received light in a cold room (0° C.) in four 2-hour periods, and the remaining 16 hours in the dark at about 22° C.; the lower row received light in a room at about 22° C. in four 2-hour periods, and the remaining 16 hours in the dark at 0° C. The tomatoes in the bottom row were almost uninjured, whereas the others were severely injured. The cabbage plants in the top row were almost dead. All sets of wheat were adversely affected by the warm temperature treatment, but those of the top row were in a very chlorotic condition and almost dead.

The leaves of the cabbage plants from treatment 3 were so badly wilted and shrunken that it was impossible to get strictly comparable samples, but the conductivity value is included in the table. Comparable samples of the tomato plants could not be obtained, since, in all except treatment 1, only stumps of the stems remained alive. Table V gives the conductivity values of the samples at the beginning of the trial, and after two weeks of hardening treatment.

TABLE V

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2°C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS FROM FROZEN PLANTS IN EXPERIMENT 4; FREEZING TREATMENT AS IN EXPERIMENT 3

PLANT	TREATMENT	DAYS OF HARDENING TREATMENT	
		0	14
Alfalfa	1. Warm light-cold dark	388	265
	2. Cold light-warm dark	388	263
	3. Continuous dark, warm-cold	388	321
	4. Continuous dark and cold	388	289
	5. Continuous light and cold	388	265
Wheat	1. Warm light-cold dark	650	500
	2. Cold light-warm dark	650	633
	3. Continuous dark, warm-cold	650	616
	4. Continuous dark and cold	650	659
	5. Continuous light and cold	650	234
Cabbage	1. Warm light-cold dark	1085	702
	2. Cold light-warm dark	1085	751
	3. Continuous dark, warm-cold	1085	1750
	4. Continuous dark and cold	1085	1047
	5. Continuous light and cold	1085	463
Tomato	All very severely injured in hardening process except (1), warm light-cold dark, which survived very well. No comparable samples obtainable		

Table V shows that hardening was more complete in treatment 1 than in treatment 2. In the case of the alfalfa, the roots were buried in the soil, of course, and did not actually receive the sharply alternating temperatures that the other parts tested did. The wheat and cabbage gave evidence of more complete hardening in 1 and in 2 by their conductivity values; the tomatoes by their survival at the temperatures used (figs. 3, 5). There seems to be no evidence that alternating temperatures as such (treatment 3) were beneficial in hardening these plants. In fact, on the average this would seem to have been the poorest treatment of the five used in this experiment. This seems not unreasonable, since it gave a considerable opportunity for respiration and no opportunity for photosynthesis, except during the intervals when the plants were being moved, or in the slight illumination under the covers. By all means the most efficient hardening treatment (with the exception of the tomatoes) was the continuous light with continuous cold. Tomatoes, however, did not seem to be able to endure this temperature without severe injury, unless receiving illumination at a higher temperature for part of the day. It seems probable that photosynthesis, with the tomato, is almost negligible at the temperature near 0° C.

Discussion and conclusions

The results of these experiments all point, in the opinion of the writer, to similar conclusions. The general proposition may be stated that hardening of plants is favored by conditions which tend toward the accumulation or conservation of carbohydrates and other reserve foods; that is, which further photosynthesis and lessen respiration and extension of vegetative parts. Hardening proceeded markedly in the dark in alfalfa or winter wheat plants that had an abundant storage of organic food, if the temperature was near 0° C. The more succulent wheat plants, grown in the greenhouse, gave no indication of hardening in the dark at 0° C., but hardened rather completely when illuminated. Alternating temperatures were not shown to be particularly favorable to hardening in either light or dark. A short period, with light, at a higher temperature was not especially deterrent in the hardening process, except in the case of winter wheat. This cannot be said of a longer day at the higher temperature, for in this case, with both alfalfa and wheat, and perhaps with cabbage and tomato, elongation of foliar parts was especially evident. The species are by no means identical, however, in their reactions. Winter wheat was much more adversely affected in its hardening behavior than alfalfa, cabbage, or tomato by a period of short duration at a higher temperature. In fact, tomato plants could not survive occasional exposures to a temperature of 0° C. unless they had opportunity for photosynthesis at higher temperatures.

Exposure to a long day in the hardening room at 0° C. gave no indication of the usual response in wheat and alfalfa of decided elongation of parts. On the contrary, the plants hardened more fully with such long-day illumination treatment than with a short day. Winter wheat plants which had received a short day in the greenhouse (60° F.) hardened more rapidly and more fully in the cold room than similar long-day (greenhouse) plants under either a long- or a short-day hardening treatment. The short-day plants were smaller, greener, and somewhat higher in dry matter.

Further evidence of the relation of organic foods to hardening is given by experiments 3 and 4. Winter wheat, alfalfa, tomato, and cabbage plants were hardened in several ways which would favor photosynthesis and decrease respiration, or the reverse. Removal of carbon dioxide from the air given the plants in all cases prevented or greatly depressed the degree of hardening. Plants receiving their light in a warm room and their dark period in a cold room were in all cases hardier than those receiving the opposite treatment. The first would seem to favor photosynthesis and depress respiration, while the second would tend to use the reserve foods of the plant without replenishment. Alternating temperatures, as such, without illumination did not appear to be helpful in the hardening of the plants. When marked top growth occurred, even under conditions favoring photosynthesis and depression of respiration, hardening was lessened.

Although the various species used differed considerably in their behavior, the general suggestion seems to hold that the development and maintenance of a high available carbohydrate supply, with much retarded vegetative growth, is essential before the cold-temperature reaction of hardening of plants will occur in an efficient manner.

University of Minnesota
St. Paul, Minn.

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