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Supercooling and Ice Formation in Mulberry Trees

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Abstract

Under both natural and artificial conditions, the initiation and the process of freezing in potted mulberry trees were investigated. Measurements of the nocturnal course of temperatures in mulberry trees were also made during cold seasons in the open.

The presence of ice on the leaf surface was found to prevent supercooling of leaves in autumn and spring, although detached leaves could readily be supercooled to -4°C . On the other hand, ice formation in winter shoots took place at -3.0 to -5.6°C without ice seeding in a cold room as well as in frosty nights. Freezing in a leafy shoot was found to develop from a nucleation point along the xylem vessels supercooled to a temperature below -1°C . The progress of freezing through the shoots was observed under controlled temperature conditions in a cold room. The velocity of freezing in shoots was found to be approximately proportional to the degree of supercooling of the shoots. The velocity was about 34 cm per minute per degree of centigrade. Even in the trees in field during the defoliation period, freezing proceeded along the vessels from a few nucleation points and reached all parts in the shoots at a relatively high velocity.

Introduction

It has been well known that frost injury in woody plants is due to either intracellular or extracellular ice formation, or both. Under natural conditions, ice crystal formation has been only observed on the outside of the cells, while the possibility of the natural intracellular freezing has been merely assumed by some investigators (Asahina, 1954, 1956; Levitt, 1956; White and Weiser, 1964). Since the nonhardy tree tissues are killed even by extracellular ice formation, the initiation of freezing in the tissues should be at the threshold of injury. Therefore, information on supercooling and freezing in the tender plant tissues such as young leaves, flowers, and fruits, may be of practical importance for the protection against destructive frosts.

In the principal sericulture regions of Japan the nonhardy tissues of mulberry trees usually suffer more or less damage by occasional frosts. In late autumn, their twigs sometimes suffer injury by freezing. This has been reported in detail from the view point of both natural and artificial frost hardening by Aoki (1955) and Sakai (1955 a, 1955 b, 1962). On the other hand, the new lateral shoots of mulberry trees are frequently injured by spring frosts, which has usually resulted in considerable economic loss to sericulturists. The cause of injury in the leaves by spring frosts was ascribed to intracellular ice formation (Ando, 1919), but it has been found to be the result of extracellular freezing by the present writer (1966). These observations seem to suggest that the young mulberry leaves may be damaged by freezing even at high subzero temperatures, say at the leaf

temperatures of -1 to -1.5°C , and therefore it became necessary to investigate the relationship between supercooling and freezing in mulberry tree tissues from a view point of practical frost protection.

The present paper describes some experiments on supercooling and ice formation in the leaves or the shoots of mulberry trees by means of cold rooms as well as in cold open air. Some descriptions of the temperature course of the tree tissues in mulberry fields during frosty nights are also made.

I. Materials and Methods

Materials. Plants used for the freezing experiments were uniform 2-year-old mulberry trees, *Morus alba* L. var. Ichinose, on rough mulberry rootstock. The plants were transplanted from a nursery bed into fertilized soil in 25 cm pots in the early summer of the previous year and then were pruned at a height of 10 cm above the soil surface. The shoots, sprouting from the stump in the pot, were trained to single or double and each shoot had a length of approximately 110 cm before the cessation of growth in autumn. The shoot apex was not injured by low temperatures during the winter. The plants potted were cultivated in early spring in a greenhouse. They were used at various developing stages of new lateral shoots. Detached leaves and detached new lateral shoots from the plants potted were also used. The individual detached leaf used was the broadest leaf of each lateral shoot at the 4th leaf-stage, and new lateral shoots used were detached from the trees at 5th leaf-stage with about 5 cm of the old shoot.

The trees used for the field examination were 24-year-old. These had been cultivated in a mulberry field, in Hino, Tokyo, at The Sericultural Experiment Station. They were "summer cut" mulberry trees* (Fig. 1) and had a height of about 2 m and a stump diameter of about 15 cm.

Measurement of temperature. Copper-constantan thermocouples (diameter, 0.2 mm) were used for the measurement of air temperatures and the temperature of leaves, shoots and stumps of mulberry trees. The thermocouples were attached to a Multiflex-galvanometer accompanied by a photo-electric tracing recorder calibrated to read to 0.02°C and also attached to two sets of 12-point recording potentiometers calibrated to read to 0.1°C . The measuring junctions of the thermocouples were closely set respectively at the central area of the leaf surface by threading a few centimeters of a leaf to them or they were attached to the bark surface of the shoots with tape. The thermocouples used for the measurement of the temperature in the field trees were separately inserted into several measuring-points of the shoots (a to e in Fig. 1-B) and into the stump (r and s) at various heights above the ground at the beginning of September. The wound

* "Summer cut" method is most commonly used in Japan. The method of cultivation was detailed by Tazaki (1959). The pruning method used is usually root training, namely the shoots developing from the stumps are cut in early summer, and then the stumps are at a height of several centimeters (about 25 cm in the present materials) from the soil surface. Many new shoots develop afterwards, and their leaves are harvested for silk worm rearing in August and September. Foliages remaining on the shoots are killed by severe frosts in late autumn and the trees are left bare of leaves till following spring. After the winter-dormancy period, new lateral shoots develop from the wintering shoots and are harvested in May. Thereafter, the shoots cut again.

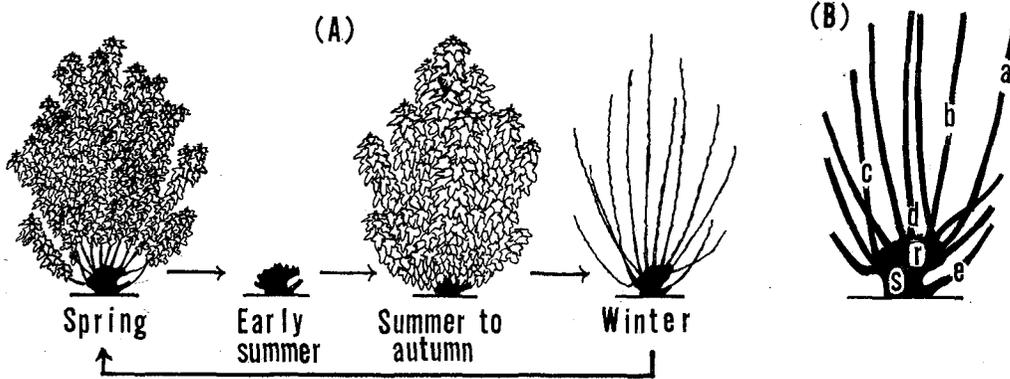


Fig. 1. Yearly cultivation cycle of "summer cut" mulberry trees (A) and measuring-points of a field tree (B) which thermocouples were inserted into the shoots (a to e) and the stump (r and s) (See also Table 4)

by the insertion of thermocouples completely healed after a few weeks.

Wet and dry bulb thermometers installed in a Stevenson screen, several glass thermometers, a whirling psychrometer and a recording whirling resistance psychrometer were used for the measurement of air temperatures and humidity in the mulberry field.

Method of cooling and freezing. The trees potted were cooled in cold rooms, $1 \times 1 \times 2$ m or $2 \times 2 \times 2$ m, in which cooling was achieved directly through the walls. Some detached leaves were cooled using a simple laboratory apparatus. The leaf was rolled up cylindrically and secured with a cotton thread. A thermocouple was put into the leaf-cylinder which was then placed in a fixed position in a test tube. The test tube was inserted into a cold bath. Other detached leaves and detached new lateral shoots were cooled in the cold open air. In some cases, the foliage of the potted trees and detached leaves were moistened by spraying with water prior to cooling. Some supercooled materials were inoculated with ice during the course of cooling.

Determination of freezing. Freezing of leaves, shoots and stumps was ascertained usually by a sudden rise in their temperature below freezing points on the recorded cooling curves. Ice formation in the leaves could be easily observed with the naked eye because some parts of the leaf appeared to become semitransparent simultaneously with the initiation of freezing. Freezing points of materials were determined by measuring the highest temperature of the materials attained during freezing.

Measurement of velocity of freezing through shoots. A special thermocouple, with two measuring junctions and a reference junction, was used for the measurement of the velocity of freezing through shoots. This thermocouple is shown schematically in Fig. 2 and was similar to that of Shinozaki (1954). The three junctions were inserted into a mulberry shoot at intervals of about 10 cm, and the wounds were closed with vaseline. The thermocouple was attached to a Multiflex-galvanometer. The potted tree with a single shoot was horizontally laid in the cold room and the part of the shoot to be measured was inserted into a glass container to minimize the fluctuation of temperature. Another three ordinary thermocouples were used for the measurement of air and shoot temperatures in the container. To initiate the freezing when the shoot was supercooled

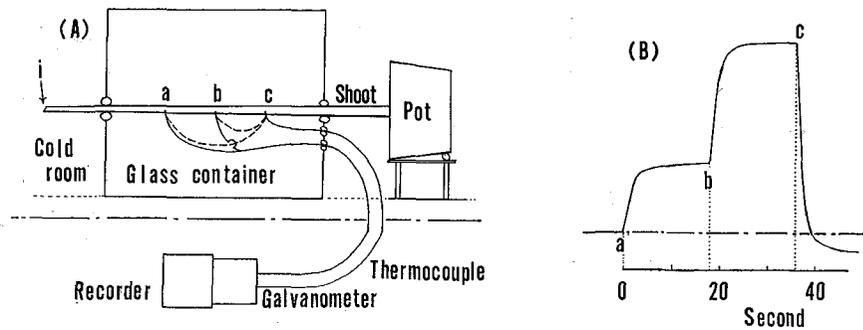


Fig. 2. The apparatus used for the measurement of the velocity of freezing through the shoot (A), and an example of records (B)

A thermocouple (solid line: copper; broken line: constantan), having two measuring junctions (a and b) and a reference junction (c), was used. An ice piece (arrow with i) was touched to the cut surface of the shoot apex at -1.5 to -3.0°C

to a certain constant temperature, the shoot apex was touched with a piece of ice. The shoot apex was cut off previously to allow for a successful inoculation of ice. When the ice front reached each junction, sudden changes of temperature were recorded by the photo-electric tracing recorder attached to the galvanometer. The velocity of freezing was calculated by the period of time read from records in which the ice front proceeded a known distance along the shoot between the junctions.

II. Results and Discussion

Initiation of freezing in mulberry leaves. Using five detached leaves per measurement, the initiation of freezing in leaves was investigated under various conditions in the cold bath and in the open. Ice formation in detached leaves began at leaf temperatures as low as -5°C or lower, although some leaves remained supercooled to almost -7°C for 30 minutes or more (Table 1). There was no apparent correlation between the cooling rate per minute at 0°C and the initiatability of freezing in the leaf. A markedly rapid cooling was recorded by exposing detached leaves directly to the cold open air. Freezing in the leaves did not, however, occur at such a rapid cooling but took place at leaf temperatures below -5°C or in relatively slow cooling during the nocturnal temperature fluctuation (Fig. 3-B). Simultaneously with a sudden rise of the leaf temperature, the leaf always showed a watersoaked and semitransparent appearance. The semitransparent leaves usually withered and died after thawing.

To examine whether the supercooling above -5°C in detached leaves is stable or not, the leaves supercooled at -4.3°C in the open were jarred or struck hard with a stick. In spite of such mechanical shocks, freezing did not occur. On the other hand, they were easily frozen with a piece of ice held against their surface. When detached leaves were cooled together with ice particles in contact with their surface in the cold bath, supercooling of the leaves was inhibited (Fig. 3-C). For further determination, five detached leaves were moistened and cooled individually in the cold bath. On three of them, the surface moisture, film or drops of water, initially froze at the respective

Table 1. Supercooling and freezing in detached leaves* from mulberry trees

| Method | Cooling Temperature (°C) | Mean**cooling rate per minute at 0°C (°C) | Mean time before leaf froze (min) | Minimum leaf temp. before freezing | | | Mean freezing point (°C) |
|--------------|--------------------------|---|-----------------------------------|------------------------------------|-----------|-------------|--------------------------|
| | | | | Highest (°C) | Mean (°C) | Lowest (°C) | |
| Cold bath | -7 | 1.7 | N.F.*** | -4.1 | -5.3 | -6.9 | - |
| | -9 | 2.0 | 5 | -5.0 | -5.8 | -6.5 | -2.1 |
| | -12 | 7.3 | 2 | -5.7 | -6.3 | -7.2 | -2.2 |
| Open air**** | -1.5 to -2.2 | 10.0 | N.F. | -3.9 | -4.0 | -4.3 | - |
| | -2.4 to -3.5 | 13.5 | N.F. | -5.9 | -6.4 | -7.5 | - |
| | -2.4 to -3.5 | 18.0 | 4 | -5.2 | -6.6 | -7.6 | -2.4 |

* Leaf length, moisture content, and freezing point depression of sap of the leaves used were respectively from 6 to 8 cm, from 79 to 84%, and from 0.66 to 0.84°C

** Mean of 5 leaves

*** N.F.: Leaves remaining in unfrozen state for 15 to 45 minutes

**** The leaves used were transferred one after another under cold and dry outdoor conditions from the greenhouse at 20°C. The leaf was then supported horizontally by sewing to a thermocouple fixed at a height of 30 cm above the ground. The measurement at air temperatures of -2.4 to -3.5°C was carried out the same night, and half of the leaves used were frozen during a nocturnal temperature fluctuation (see Fig. 3-B) while the other half did not freeze

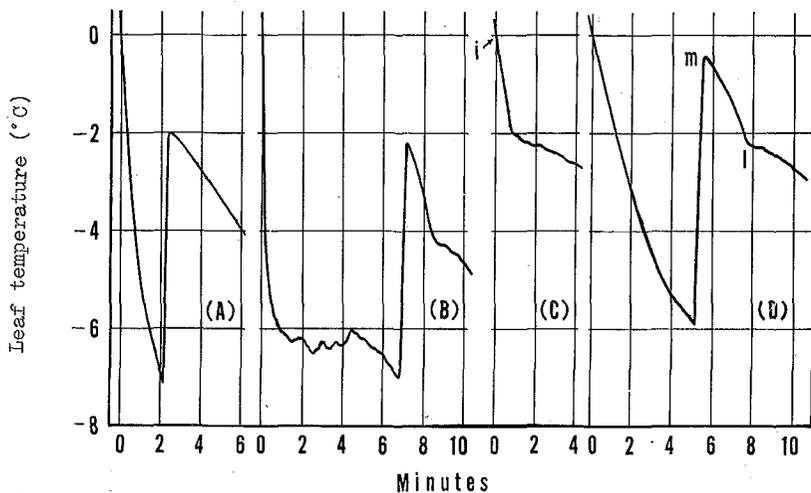


Fig. 3. Freezing curves for detached leaves from mulberry trees. The curves obtained respectively from the leaves with dry surface in the cold bath at -12°C (A), in the open at air temperature of about -3.5°C (B) and in the cold bath at -8°C where the leaf was cooled together with ice particles (arrow with i) touched to its surface (C), and from the leaf with a wet surface in the cold bath at -7°C (D), which ice formation (l) following immediately freezing of surface moisture (m)

temperatures of -0.9, -2.6 and -5.9°C. Ice formation in these leaves immediately followed the freezing of surface moisture (Fig. 3-D). In another two leaves, the freezing initiated in themselves at -4.8 and -6.0°C respectively, and external ice formation was not recorded. When these two leaves were taken out from the cold bath, the surface moisture remained unfrozen while the semitransparent symptoms were visible in the leaves.

From the aforementioned results, it may be said that without any ice seeding, the supercooling in detached mulberry leaves of approximately 3°C below their freezing points is stable, and that surface moisture can sometimes supercool to below the freezing temperature of leaves. The influence of surface moisture on the freezing of plant tissues has been reported by many investigators (Lucas, 1954; Asahina, 1954, 1956; Hendershott, 1961). In many cases, frozen water on the tissues was found to prevent supercooling, and the present data are also in agreement with these observations. In mulberry leaves, in particular, the relation between the duration of supercooling and freezing of surface moisture on the leaves exposed to subzero temperature is brought forward as a problem, because some dew-drops, prior to freezing, were observed upon the leaves on spring frost damage in mulberry plantations (Kitaura, 1961).

Effect of surface moisture on freezing of mulberry foliage. After it was ascertained that the trees potted at the 5th leaf-stage did not freeze at $-2.5 \pm 0.5^\circ\text{C}$ for 17 hours in the cold room, several leaves on such trees were wetted and the trees were cooled gradually from 0 to -5°C for about 1.5 hours. The measurement of temperatures was made on all of the wet leaves and on several leaves with dry surfaces of the same tree, or separate intact tree. The results obtained from 10 repetitions in each treatment are shown in Table 2. The minimum leaf temperature indicated in Table 2 was obtained from the respective first wet and dry leaf.

Table 2. The effect of surface moisture on freezing of mulberry foliage

| Minimum leaf temperature before freezing (°C) | Foliage freezing percentage | | | |
|---|-----------------------------|----------|---------------|----------|
| | Single tree | | Separate tree | |
| | Wet leaf* | Dry leaf | Wet leaf | Dry leaf |
| 0 ~ -1.0 | 30 | | | |
| -1.1 ~ -2.0 | | 30 | 40 | |
| -2.1 ~ -3.0 | 20 | 20 | | |
| -3.1 ~ -4.0 | 10 | 30 | 10 | |
| -4.1 ~ -5.0 | 30 | 10 | 20 | 10 |
| -5.1 ~ -6.0 | 10 | 10 | 10 | 20 |
| Unfrozen (below -5.1) | | | 20 | 70 |

* Surface moisture formed only on several leaves in which the temperatures were recorded.

The presence of surface moisture not only brought about freezing in the wet leaves at temperatures above -5°C but also greatly altered the degree of supercooling in the dry leaves on the same tree. In this treatment the wet leaf always froze earlier than the dry one. But the leaves on the intact trees began to freeze at temperatures below -4.8°C in a similar manner to the detached leaves represented in Table 1. These data suggest that freezing from a nucleation point may proceed in some way to the dry leaves within a tree, and that bedewed leaves may easily freeze by exposing them to a few degrees of subzero temperature.

To gain information on the actual course of freezing in mulberry leaves at the time of spring frosts, observations were carried out experimentally during two frosty nights

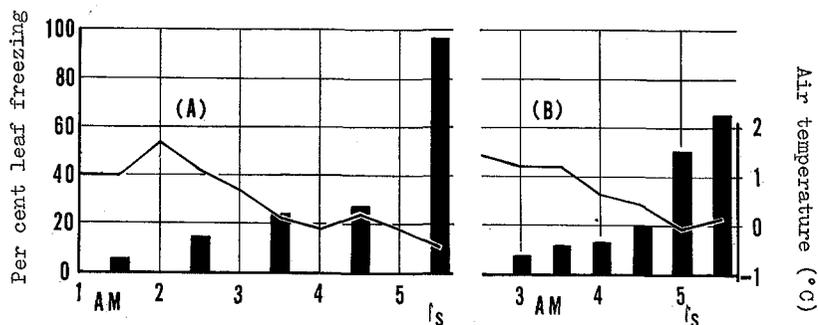


Fig. 4. Sequence of freezing in mulberry leaves during two frosty nights with copious dew

The histograms indicate for each time the percentage of frozen leaves on the detached new lateral shoots which were set on the old shoots of the field trees at 1 m above the soil surface. Air temperatures (solid line) were measured in the Stevenson screen which stood approximately 35 m away from the field. Minimum air temperature was -0.9°C on the first night (A) and -0.4°C (B). Average relative humidity was 96% during both nights. Arrows with S represent the time of sunrise

in early spring by the use of ninety detached new lateral shoots. These new lateral shoots were set individually on the old shoots of field trees at 1 m above the soil surface at midnight. Numerous dew drops were condensed upon all leaves at 1 a.m. and also a film of water was observed on some leaves, particularly on the slanted ones. About 90 leaves on 20 detached new lateral shoots selected at random were examined each time. Counting of the number of frozen leaves were made by observing the watersoaked symptoms. In these observations the leaf temperature was not determined and the air temperature was measured in a Stevenson screen which stood at about 35 m distance from the field. Ice formation in moisture on the leaf surface was followed subsequently by freezing inside the leaf. Several frozen leaves appeared even at air temperature near $+1^{\circ}\text{C}$ in both nights and the frozen leaves progressively increased in number as the temperature fell (Fig. 4). Since the difference between leaf and air temperature (in a Stevenson screen) has been reported to be from 1.5 to 2.0°C at the time of spring frosts (Sugazawa, 1961), the leaf temperature can be inferred to be -0.5°C or lower at air temperatures of $+1^{\circ}\text{C}$. There were some water film on the surface of the leaves frozen before 4 a.m. On the other hand, the leaves, remaining supercooled until sunrise, were situated horizontally and their surface moisture remained unfrozen. The minimum air temperature in the first observation (A) was -0.9°C and that in the second was -0.4°C (B). Therefore, some leaves and their surface moisture should have been supercooled to at least -2.4°C for 2 hours.

From Table 2 and Fig. 4, it can be seen that the surface moisture on mulberry leaves was supercooled to almost -6°C or to a minimum temperature of -0.9°C throughout a frosty night. These results agree with the report of Hendershott (1962) who reported that some fruits of orange trees were supercooled to 25°F for 8 hours. If this always the case, some leaves, shoots, or trees in mulberry plantations should survive a spring frost due to supercooling. In fact, some surviving shoots with undamaged foliage were observed occasionally in mulberry fields after spring hoar frosts. The present writer has also actually observed a very rare case in which only one shoot with green foliage stood

out in a mulberry field where all the new lateral shoots were withered and blackened by frost damage extending over several hectares. On these damaged shoots, however, no new lateral shoots survived. A possible explanation on such a remarkable phenomenon should be given exclusively, on the one hand, by the durability of supercooling in the foliage or its surface moisture and, on the other hand, by the causal relation of freezing among the leaves or new lateral shoots within a shoot. To ascertain this, some further studies on freezing in the shoots were necessitated.

Ice formation in mulberry shoots. Supercooling and freezing in mulberry shoots were determined in the first experiment. The trees potted were used in two stages, *i.e.* winter-bud and 6th leaf-stage. These were cooled under dry conditions in a cold room with a cooling rate of 9°C per hour.

In the shoots with winter buds, ice formation took place at temperatures between -3.0 and -5.6°C without ice seeding and the average freezing temperature was -4.5°C from the measurement of 25 trees. The freezing point of these shoots were from -2.0 to -3.0°C . None of trees was injured by such slight freezing because their buds and shoots could withstand freezing at -10°C for 24 hours. After these determinations, some shoots were detached from the stumps and coated entirely with paraffin, and then they were cooled again. Ice formation in such coated detached shoots took place at nearly the same temperature as the previously determined one. After thawing, these detached shoots were cut into three or six equal parts (in lengths of about 30 or 15 cm) and frozen in the same manner. Supercooling points of these shoot parts became lower than the prior temperatures and were from -5.4 to -6.5°C in both one-third and one-sixth parts. Some of them were, moreover, divided into small pieces with various lengths of 1.5 to 6 cm. The degrees of supercooling in these pieces ranged from -5.1 to -7.3°C . In an additional experiment, the shoot pieces, having a length of 6 cm, were frozen repeatedly three times for 30 minutes at intervals of 21 hours. The variation in three measurements of supercooling points of the individual piece was only within 0.6°C .

In the leafy shoots, the leaves usually froze earlier than the shoots under the conditions described above, for leaf temperatures were lowered to -5°C or less before the temperature of the shoot fell to -3°C . As soon as ice formation in the leaf occurred spontaneously, freezing in the shoot followed. Touching an ice particle to a supercooled leaf at about -4°C , caused not only an immediate freezing in the leaf but also a rise in temperature of the shoot as a result of ice formation within it. In such a case, the minimum temperature of the shoot before freezing was -2.5°C and its freezing point was about -1°C .

These results suggest that a temperature below -3°C can introduce freezing in intact shoots without ice seeding from the outside and that ice formation in a shoot plays an important role in freezing of the foliage within the shoot.

The effect of degree of the supercooling of shoots on the velocity of freezing (hereinafter are designated as V.C.) through shoots are illustrated in Fig. 5 where the values obtained from the three stages, winter-bud, budding and early leafy stage (1st to 3rd leaf-stage), of the tree were presented. In this experiment the air temperatures in the glass container (see Fig. 2), placed in a cold room, were always fixed to a given temperature during the measurement.

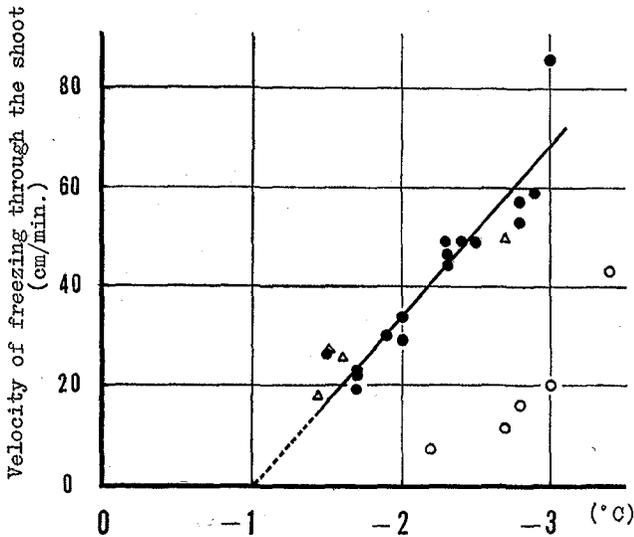


Fig. 5. The effect of shoot temperature on the velocity of the freezing through mulberry shoots

- : Winter-bud stage. △ : Budding stage
 ● : Unfolding stage (1st to 3rd leaf-stage)
 (See also Fig. 2)

The V.C. through shoots increased with the lowering in temperature. In a certain shoot temperature, the V.C. in the winter-bud stage was slower than in both the budding and the leafy stage. From the measurements in the early leafy stage, the relation between the shoot temperature (X) and the V.C. (Y) could be expressed by the formula,

$$Y = -34.7X - 34.9$$

The freezing points of the shoots were $-1.0 \pm 0.18^\circ\text{C}$ in the early leafy stage. The V.C. at the freezing point of the shoot may be considered as zero even though the freezing took place. This can be said because ice crystals co-exist with fluid at the freezing point. Such a consideration is additionally supported by the fact that the freezing point of the shoots was in accord with the temperature in $Y=0$ from the formula. Consequently, the V.C. was found to be approximately proportional to the degree of supercooling in the shoots. There is no apparent correlation between the V.C. and moisture content of the shoots, although it ranged from 62 to 73%.

It seems that freezing in shoots proceeds along the xylem vessels. The V.C. through the same shoot was determined two times before and after ringing, which was done by peeling a bark strip of about 3 cm in width midway between the a and b position shown in Fig. 2 and sealed with vaseline. No significant difference between these two V.C. was seen.

In the next experiment, to clarify the reciprocal relation of freezing among the shoots within a tree, the potted trees with double shoots were treated under a cooling rate of 5°C per hour in a cold room. When the leaf temperature fell to -1 to -6°C , the leaf on one of the shoots was inoculated with ice. The subsequent freezing in the shoot

Table 3. The progress of freezing* between two shoots sprouting from a stump of potted mulberry tree

| Symbol** | Description of potted tree | | Average temperature at the time of ice seeding | | | Average velocity of freezing*** (cm/min) |
|----------|----------------------------|-------------------|--|------------|------------|--|
| | Stage | Water content (%) | Leaf (°C) | Shoot (°C) | Stump (°C) | |
| D 8 | 5th-leaf | | -0.3 | +1.3 | — | 1.5 |
| D 26 | 6th-leaf | | -0.8 | +0.1 | +0.5 | 3.0 |
| D 27 | 6th-leaf | | -3.3 | -2.4 | -0.5 | 8.3 |
| S 28 | 7th-leaf | | -3.5 | -2.9 | -2.2 | 20.0 |
| S 17 | 5th-leaf | 60 | -3.9 | -3.4 | -2.3 | 36.0 |
| S 23 | 6th-leaf | | -4.2 | -3.1 | -2.3 | 40.0 |
| D 16 | 5th-leaf | | -5.8 | -5.2 | -2.0 | 17.3 |
| D 15 | 5th-leaf | 62 | -5.8 | -4.1 | -2.4 | 7.5 |
| D 13 | 8th-leaf | | -5.7 | -4.8 | -3.9 | 30.0 |
| S 14 | 5th-leaf | | -6.1 | -5.3 | -4.7 | 87.2 |
| D 21-a | Winter-bud | 48 | (-4.7) | -2.9 | -1.8 | 9.3 |
| D 21-b | Winter-bud | 48 | (-4.0) | -3.0 | -2.4 | 13.2 |
| S 19 | Winter-bud | 52 | (-3.9) | -3.3 | -2.4 | 17.0 |
| D 20 | Winter-bud | 45 | (-4.7) | -4.1 | -3.1 | 20.0 |

* Freezing was induced by ice seeding in a leaf or a shoot apex. Potted trees were cooled gradually with a cooling rate of 5°C per hour in the cold room

** The trees with double shoots, sprouting from adjacent positions on a stump, are indicated by S while the trees with double shoots with separate positions are indicated by D

*** The V.C. through two shoots and a stump was calculated from the time required for freezing to proceed a known distance between the ice-seeded point of one shoot and the farthest measuring point of another

reached the stump and then extended to the other shoot and its foliage. The lower the temperature at the time of inoculation of ice, the higher the average V.C. through the shoots and stump. There were some exceptions (Table 3).

The stump temperatures were higher than other temperatures owing to the heat supply from the soil in pots and had a considerable effect on the V.C. On the other hand, the positions on the stump, from which each shoot had sprouted, varied with the trees and also had some effect on the V.C. Propagation of freezing from a shoot through a stump was found to proceed more easily in trees with double shoots sprouting from adjacent positions than those with shoots from separate positions. It is an interesting fact that there were some shoots, in which the propagation of freezing the stump was relatively difficult such as shown in D 15 or D 16 in Table 3. Even at the winter-bud stage, freezing progressed from the ice-seeded point where the tip of the shoot was cut previously and extended to the entire tree at a similar rate of the average V.C. in the leafy stage. Such a freezing was reversible between the shoots, viz. D 21-b in Table 3 was frozen once more by inoculating the opposite apex with ice after D 21-a had been treated formerly.

Supercooling in mulberry shoots was found to be only a few degrees even in the winter-bud stage, although winter twigs of some deciduous trees may remain supercooled through the cold nights of -18 to -26.5°C (Wiegand, 1906). It has been reported that

the temperature which causes ice formation in intact plants varies depending upon many factors such as mechanical shock, cooling rate, surface conditions, moisture content and volume of fluid (Cooper *et al.*, 1954; Lucas, 1954; Chandler, 1954; Asahina, 1954, 1956). In the present data, mechanical shock and surface moisture were not contributing factors because the experiments were carried out under calm and dry conditions. Though non-turgid plant tissues may be supercooled at a higher rate than turgid tissues (Hendershott, 1961), the moisture content of mulberry shoots, especially in the winter-bud stage, was even less than in the leaves (Table 1 or 3). The moisture content may, therefore, be disregarded. In addition, the cooling rate seems to have no effect upon supercooling in mulberry shoots, for the twig pieces of mulberry trees have been found to freeze at -4 to -8°C without ice seeding under a cooling rate of about 1°C per minute from the freezing curves shown by Aoki (1955), while the cooling rate in the present experiments was about 0.15°C per minute. It thus appears that the factors in supercooling in intact mulberry shoots may also be involved in some states in the shoot tissues.

From the present results, the existence of moisture along the vessels of the shoots can be assumed as one of the factors affecting supercooling of mulberry shoots. The data obtained from detached shoots show that the cutting of a shoot into shorter parts can generally lower the previously determined supercooling points a few degrees. The division of the shoots means cutting the vessels in which moisture is contained. On the other hand, the detached shoot parts have cut surfaces where spontaneous freezing occurs easily. If the cut surface is effective in minimizing supercooling in the shoots, it may follow that their supercooling points were higher than the intact shoots. However, the effect of a cut surface on the supercooling of the cut shoots did not appear in the tests of dividing detached shoots but were noted only in the repeated freezing tests using small shoot pieces. These considerations led the present writer to the view that the state of moisture existing in the vessels plays an important part in the initiation of freezing in mulberry shoots which readily occurs under a very slightly supercooled condition. This view may be supported by the fact that the detached leaves or shoots showed a higher degree of supercooling than the whole tree in citrus plants (Cooper *et al.*, 1954; Hendershott, 1961, 1962).

Ice formation, proceeding through the xylem vessels, was observed in lemon fruits by Lucas (1954) as well as in potato leaves by Asahina (1954). Accordingly, freezing in mulberry trees may also progress by way of the vessels. The V.C. through the shoots, illustrated in Fig. 5, is estimated at temperatures below -2°C . The V.C. through the shoots is as rapid as or more than the freezing velocity of pure water in a U-tube, which was determined by Shinozaki (1955). It is expected that further experiments will show the freezing process in the vessels and may eventually clarify the reason for such a rapid V.C.

Temperature distribution with height and ice formation in mulberry trees during frosty nights. The temperatures of mulberry shoots and stumps were recorded in the field during the winter of 1961-62. Two trees were selected for the measurement. In both trees, the thermocouples were inserted into the shoots at the respective heights of 100 (a), 75 (b), 50 (c), 25 (d) and 10 cm (e) above the ground and into the stump at two depths of 2 cm (r, at 20 cm above the ground) and 5 cm (s, at 10 cm) (Fig. 1-B). The remainder of the thermocouples was used for the measurement of leaf and air temperatures. From

Table 4. Temperature distribution with height and minimum temperature, prior to freezing, in various portions of field mulberry trees during frosty nights

| | Measuring-point* | | Temperature difference** | | Minimum temperature before freezing**** (°C) |
|-------|------------------|---------------|--------------------------|--------------------|---|
| | Height (cm) | Depth (cm) | Autumn*** (°C) | Winter**** (°C) | |
| Shoot | a | 100 | 0 | 0 | -1.8 |
| | b | 75 | 0 | 0 | -1.5 |
| | c | 50 | -0.1 | -0.5 | -2.0 |
| | d | 25 | -0.6 | -1.6 | -2.9 |
| | e | 10 | -0.7 | -2.2 | -3.5 |
| Stump | r | 20 | 2 | -1.2 | -1.8 |
| | s | 10 | 5 | +1.7 | +0.5 |
| Leaf | | 100 | | -1.2 | |
| Air | | 100 | | 0 | |
| | | 50 | | -0.3 | +0.2 |

* See Fig. 1

** Difference of each point against point a in Fig. 1-B

*** From two frosty nights with copious dew on November 14 and 15, 1961

**** From dry frosty nights on January 13 to March 14, 1962. Mean of 16 nights

available records, the temperature distribution with height and minimum temperature before each portion was frozen were investigated.

At the first severe frost in late autumn with a minimum air temperature of -2.4°C (in a Stevenson screen), the leaf temperatures were recorded throughout the night; incidentally the foliage became entirely frozen and was shed within several days. On frosty nights during winter, the lowest temperature was seen at the point e of the shoot, and at lesser heights above the ground, shoot temperatures were lowered (Table 4). The stump temperature at a depth of 2 cm usually fell to a lower point than the shoot temperatures at heights above 50 cm throughout the winter nights.

In autumnal frosts with copious dew, ice formation in the leaves followed the freezing of dew-drops at leaf temperatures of -1 to -2°C . Freezing in shoots or stumps began about 2 hours later without supercooling as a result of the propagation of freezing from the formerly frozen leaves. In winter shoots, freezing was initially recorded at the point e or d in both trees at -3.0 to -4.4°C in the measurements during 16 winter nights. In this case, ice formation at other measuring-points started with a slight supercooling owing to the spread of freezing from the point e or d. The time required for such a freezing was about 5 to 105 minutes for all shoots while it was about 60 to 200 minutes for the whole tree. From the records, it can be seen that the more severe the natural cooling, the faster the progress of freezing was.

The order of the initiation of freezing at each measuring-point varied with the frosty nights. That order on a tree was classified in the 16 nights and represented in Table 5. As the order (d-r-b) was expressed almost constantly, it can be said that there is an intimate correlation of freezing between these three points. However, freezing at the point a happened later most frequently in the order. If such an order

Table 5. The order of the initiation of freezing in each measuring-point of a mulberry tree in the field and the frequency of its appearance on 16 nights during winter of 1962*

| Order of the initiation of freezing 1—2—3—4—5—6—7 | Frequency in 16 nights |
|--|------------------------|
| e-(d-r-b)-c-a-s | 5 |
| e-(d-r-c-b)-a-s | 1 |
| e-(d-c-r-b)-a-s | 4 |
| e-c-(b-r-b)-a-s | 1 |
| e-(r-d-a-b)-c-s | 1 |
| (d-r-b)-e-c-a-s | 1 |
| (d-r-b)-c-a-e-s | 1 |
| (d-e-r-c-b)-a-s | 1 |
| (d-e-c-r-b)-a-s | 1 |

* See Fig. 1 and Table 4

could be kept in a tree on any night, the shoot at such a point as a would be the last to freeze or may even survive.

These data show an agreement with the aforementioned results obtained from the freezing experiments in the cold rooms, with the exception of the lower temperature of stumps. Consequently, mulberry trees freeze on winter nights at air temperatures below -2°C in almost all cases. This may prevent the possibility of intracellular ice formation which usually occurs in plant tissues under much more supercooled conditions.

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* In Japanese with English summary.