if transpiration is rapid, but the plants recover if the soil drains promptly. If the soil remains saturated more than 24 hours permanent injury occurs. Stem elongation usually ceases, epinasty often occurs, the leaves turn yellow and die, beginning at the base and progressing up the stem, and the roots and base of the stem begin to die and decay. Injury develops more rapidly in warm than in cool soil.

The initial wilting is caused by a sudden decrease in permeability of the roots to water. Prolonged flooding causes injury and death of the roots because of deficient aeration, but this is aggravated by the activity of microorganisms which destroy the roots and plug the xylem in the base of the stem. It also is possible that toxic substances produced by microorganisms or in the dying root cells contribute to injury of the shoots. The role of microorganisms in flooding injury deserves further investigation.

Wilting and injury to flooded plants can be prevented by forced aeration of the soil, and plants growing in soil at field capacity can be caused to wilt and show symptoms of injury by displacing the soil atmosphere with CO_2 or N_2. Use of CO_2 produces wilting sooner than N_2.

Although injury to plants in flooded soil is caused by deficient aeration it is more complex in nature than injury from O_2 deficiency alone because in most instances microorganisms appear to be involved.

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SUBCOOLING AND ICE NUCLEATION IN LEMONS [1]

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Subcooling of living tissue has been the subject of periodic study for several decades. The characteristic of plant tissue, in particular, to cool somewhat below its freezing point before ice begins forming in it may be of practical importance because, as is well known, subcooling in itself does not cause freezing damage (3, 4, 23, 24, 29). Several reviews concerning tissue subcooling have appeared, although most are only portions of publications dealing with low temperature damage in general (16, 18, 34, 39). Müller-Thurgau (23, 24) investigated subcooling by inserting a mercury thermometer into the plant part cooled and obtained the temperature curve now recognized as characteristic for subcooling with subsequent freezing. This work showed that the difference between the lowest temperature attained before freezing and the tissue freezing point, referred to as the amount or degree of subcooling in this paper, ranged up to 10°C. Later, Diehl and Wright (4) used thermocouples but otherwise similar methods and observed 4°C subcooling in apples. Single grape berries subcool 3°C on the average according to Carrick (2), and Schoonover et al (28) suggest Navel oranges are able to subcool 1.5°C in the orchard.

The theory of nucleation promises to provide a sound fundamental basis upon which to develop a study of subcooling in living tissue. This theory concerning the mechanism of initiation of the more stable phase within a metastable phase is reaching a stage in which it may be profitable to apply it to the study of subcooling in a complex system such as that of plant tissue. Several reviews dealing with nucleation theory have appeared (1, 12, 14, 38). The fundamental assertion of this theory is that the stability of a metastable phase lies in the existence of an activated complex or nucleus stage through which at least one group of molecules must pass before the more stable phase

can grow. The energy of activation for nucleation, W, is due to the relatively large contribution made by the interfacial energy to the average energy per molecule in the nucleus. Ice nuclei in subcooled water, for instance, may contain on the order of only 80 molecules (40).

Volmer and Weber (35) employed the fluctuation theory of Einstein (7) to obtain the general expression for the rate of nucleus formation, I, per unit volume of homogeneous or pure metastable phase:

$$I = Ke^{-(W/kT)} \qquad (1)$$

where K is a constant later evaluated by Farkas (8) for liquid nucleation in a supersaturated vapor and by Turnbull and Fisher (31) for crystal nucleation in a subcooled liquid, k is Boltzmann's constant, T is the absolute temperature, and W is calculable by a formula derived by Gibbs (9). This equation accurately predicts the experimentally measured rate of liquid nucleation in supersaturated vapors free of dust and ions (37). Turnbull's (32) determinations of the rate of crystal nucleation in those samples of sequestered mercury droplets which subcooled most (homogeneous nucleation apparently took place in these) are also in good agreement with equation (1).

The catalyzing action of various particles and of spots on solid surfaces upon nucleation has received attention (12, 36). Such surfaces evidently reduce the energy of nucleation activation and in this way cause heterogeneous nucleation. Volmer (36) proposed adapting equation (1) to account for this effect by multiplying W by a factor containing the equilibrium contact angle between the stable phase and catalyst surface in the presence of the metastable phase. Using this suggestion Turnbull (32) found excellent correspondence with the rate of crystal nucleation in groups of mercury droplets which subcooled less than the maximum. Particles which catalyze ice nucleation in subcooled water are known as freezing nuclei by meteorologists.

As the rate of nucleation is an exponential function of the temperature, it is extremely temperature sensitive. This temperature sensitivity is exemplified in the experimental findings that homogeneous crystal nucleation in subcooled mercury occurs in a temperature range measured in tenths of a degree (32), and that heterogeneous ice nucleation due to a particular freezing nucleus repeatedly occurs within a few tenths of a degree of the same temperature (5, 40). It is clear that nucleation is a probability occurrence only in so far as the chance of there being present a foreign substance capable of causing heterogeneous nucleation under the ambient conditions is concerned.

Apparatus and Procedure

With the exception of some vesicles which were held in a walk-in freezer for microscopic examination, all tree parts were cooled in a closed wooden container, 30 cm on a side. Fruits were supported by their stems, which were clamped in alligator clips lined with Scotch electrical tape. In several runs the entire cut stems were maintained above 0°C by inserting

them into heating caps made of spiraled nichrome wire and covered with a 0.5 cm layer of asbestos (fig 1). The specimen container was cooled in a compartment of a household reach-in freezer while the temperature between it and the compartment walls was maintained uniform with a small fan. A temperature fluctuation of ± 1°C outside the container assumed negligible proportions inside it, as its walls contained a 1.5 cm thickness of glass wool. The thermostat was controlled by clockwork so that the rate of temperature decrease was a constant 1.1°C per hour. This rate compared favorably with that in the orchard on winter nights and also provided for a reasonably uniform temperature within the specimens during cooling.
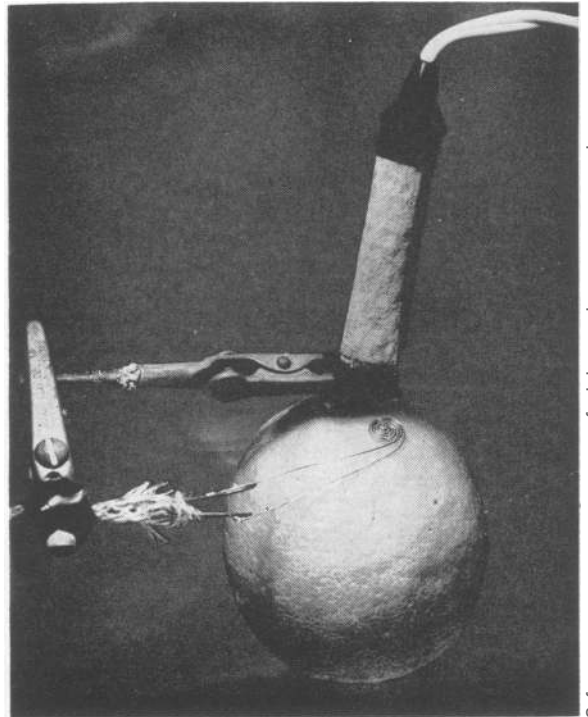

Fig. 1. Lemon in specimen container with thermocouple and stem heater in place.

Temperature measurement within the container was by means of iron-constantan thermocouples of 30 gauge wire. Those employed inside tissue were coated with Glyptal. In several series of runs, temperatures were measured with thermocouples whose uncoated wires were wound in flat spirals around the junctions as shown in figure 1. Each junction was placed 1 mm above the surface of the fruit. The purpose of the spiral shape was to permit the lengths of thermocouple wire which could affect the temperature of the junction to be approximately the same distance from the fruit surface as the junction itself, and also to allow reproducible results. Temperatures were recorded by a twelve-point recording potentiometer (Leeds and Northrup, Micromax Model) with a range of − 18 to 93°C. The error of temperature measurement was ± 0.09°C, as determined by the standard

deviation from 0°C of a reference distilled water-ice mixture. This relatively small error was obtained by taking certain precautions: (a) thermocouple wire from the same spools of iron and constantan, respectively, was used throughout; (b) the potentiometer was calibrated with an ice bath after alternate runs; (c) dead space error was eliminated by causing the recorder to approach all temperatures of interest from the same direction (all odd-numbered points indicated the temperature of the relatively cold inside wall of the freezer compartment); (d) the error of reading the recorded temperatures was minimized by measuring the position of the points on the chart paper with a microscope equipped with an ocular scale. An additional uncertainty of ± 0.05°C was due to the circumstance that the temperature of each thermocouple was recorded at six-minute intervals.

Humidity was controlled by sulfuric acid solutions, and a lining of polyethylene film on the inside walls of the container served as a vapor barrier. Checks of actual humidities were made by the dew point method. The start of dew or frost formation on the surface of a copper bar projecting inside was detected visually while the temperature fall of the area observed was followed. The relative humidities read from appropriate psychrometric charts corresponded with those given for the various concentrations of acid used.

All specimens were obtained during the spring from a Eureka lemon tree grafted on grapefruit root. The tree was untreated with either an insecticide or a fungicide, and it received no special treatment during the course of the experiments. Lemons commercially graded as silver were used exclusively; their diameters varied from 41 to 54 mm.

The procedure for measuring the ice nucleation point, or the temperature at which freezing began, was unvaried throughout. Fresh specimens were placed in the container, which was then kept in a walk-in freezer held at 4 ± 1°C for approximately ten hours before being transferred to the reach-in freezer compartment. For the determination of the nucleation point of individual fruits, groups of nine were cooled three at a time. Preliminary runs established that, during cooling, the spiral thermocouples registered 0.42 ± 0.16°C less than thermocouples in the fruit centers, and 0.05 ± 0.11°C less than thermocouples imbedded 1.5 mm under the fruit surfaces and the same distance around the fruits from the stems as the junctions of the spiral thermocouples. The temperature of each spiral thermocouple clearly indicated the onset of freezing in the fruit over which it was held, as the readings of the thermocouple and point fruit temperatures, taken at one-minute intervals, were found to rise together.

RESULTS, ANALYSIS, AND DISCUSSION

SUBCOOLING IN VESICLES AND SPREAD OF FREEZING: Individual detached vesicles, 85 in number and resting on microscope slides, were placed in three closed Petri dishes in the wooden specimen container and the temperature lowered. The temperature was measured by means of a thermocouple inside an extra vesicle in one of the dishes. At –12°C no vesicle had begun freezing, but when the temperature had fallen below –18°C, 42 vesicles were frozen. A thermocouple whose temperature was about –12°C was thrust into several of the subcooled vesicles, one at a time. In each case, the vesicle froze very rapidly, and the temperature recorded by the thermocouple rose to approximately –2°C, showing that the low temperature attained without freezing was not due to a lowering of the freezing point in the detached vesicles.

Two sets of seven detached vesicles were placed so that the cut stalk ends were in separate pans of water while the bulbous portions remained in air. The pans and vesicles were placed in a –7°C atmosphere. In a few hours the water in one pan and all the surrounding vesicles were frozen. In the other pan neither the water nor any of the vesicles was frozen after 24 hours. At that time the water was inoculated with ice; it and all the vesicles around it quickly froze. This result indicates that freezing readily spreads through the stalks. As closer study of this process seemed desirable, several detached vesicles on microscope slides were placed in a walk-in freezer maintained at –8 ± 2°C and observed through a low-power stereoscopic microscope a few hours later. Within a few seconds after ice was pressed against the cut stalk ends very narrow, threadlike columns of ice penetrated to the point where xylem vessels terminate. The subsequent freezing in the bulbous portions of the vesicles was intermittent and quite clearly intracellular. The velocity with which the cellular freezing front progressed in the vesicles was several orders of magnitude less than the crystallization velocity in the stalks. These results suggest that rapid freezing spreads in stalks and throughout the vascular system of a fruit by way of the water conducting vessels.

It was found that vesicles subcooled to –7°C were not inoculated by ice held against their sides. To further investigate the mode of spread of freezing in the pulp, separate segments were cooled on glass slides. The segment membrane on each side was pulled back exposing the vesicles and two thermocouples were inserted into vesicles at opposite ends of the segments. When the indicated temperature reached –5°C, the vesicles punctured by one of the thermocouples were inoculated. In less than one minute the freezing spread to the vesicles around the thermocouple at the opposite end, as indicated by a temperature rise there similar to that in the inoculated portion. However, in segments from which the peel, including the inner wall of the outer portion of the segment and the vascular bundles connecting the vesicle stalks, had also been removed, the freezing did not penetrate to the vicinity of the other thermocouple in 10 minutes. It follows that freezing is disseminated throughout a segment by way of the vascular system and does not progress directly from one vesicle to another inside the segment.

A rough check on the rate of freezing spread in a subcooled system comprising a fruit and stem with attached leaves was made. The stem was held horizontally in the container, and the temperatures at four points were recorded: one thermocouple was inserted 0.5 cm into the cut stem end; another was imbedded in the midrib of a leaf; the third was inside the pulp of the fruit; and the last was 2.5 cm below the stem, midway between the fruit and the cut stem end. Several runs were made, and subcooling was followed each time by apparently simultaneous freezing throughout the system, as indicated by the fact that all the temperatures, taken at two-minute intervals, rose together. The recorded air temperature increased least so that air heated by the fruit, for instance, could not have caused the temperature in the leaf or stem to increase the recorded amount without freezing actually occurring there; the stem was placed in a horizontal position to reduce this possibility to a minimum. Freezing was found to spread from one fruit to another on the same branchlet in a similar manner. The crystallization velocity was found to be greater than 15 cm per minute at a subcooled temperature of $-5.2°C$. It is expected that a more precise measurement would show the crystallization velocity to be considerably above the lower limit established.

As xylem vessels are continuous tube-like structures containing a dilute aqueous solution, it is reasonable to expect the crystallization velocity in them to compare with that found in glass capillary tubes. Tammann and Büchner (30) give a value of 66.5 cm per minute in water subcooled $3.2°C$, which is well above the lower limit obtained for branchlets. That freezing spreads rapidly in subcooled plant tissue was suggested by Müller-Thurgau (24), who also obtained evidence that freezing spreads through water-conducting vessels in potatoes. Maurer and Murray (20) noted that ice first forms in and around these vessels. The finding by Seemann (29) that freezing spreads almost instantaneously in subcooled bean plants is similar to the result reported here. The fact that freezing spreads rapidly in subcooled tissue strongly suggests that ice nucleation ordinarily occurs at one point only in such a system, just as it does in small volumes of subcooled water.

A THERMODYNAMIC ANALYSIS: The results given above are used together with concepts of the theory of nucleation and the supporting experimental results to carry out a thermodynamic analysis of the phenomenon of ice initiation in a lemon. The system examined is composed of a fruit, its stem, and the surrounding atmosphere. It is postulated that ice nucleation occurs neither in the stem nor in the atmosphere. The tissue is idealized to consist entirely of healthy undamaged cells, and its interior is postulated to be in equilibrium with respect to water; that is, that the partial molal free energy with respect to water is uniform there. This does not mean, as Edlefsen and Anderson (6) have emphasized, that the freezing point is uniform. On the contrary, as proteins and other substances are concentrated at proto-

plasmic interfaces (15), a lower freezing point at those interfaces is to be expected. Such a decrease in freezing point is evidenced, for example, by the action of the outer protoplasmic membrane in inhibiting ice growth through it (3). For this reason, ice nucleation is not likely in the outer protoplasmic membrane, and the fruit is seen to be divided into two distinct parts in so far as ice nucleation is concerned: (1) the intercellular portion, and (2) the intracellular portion.

A very important finding of nucleation studies is that homogeneous ice nucleation does not take place above $-39°C$ in subcooled water (27). This implies that the presence of freezing nuclei active at small degrees of subcooling is a prerequisite for ice nucleation at temperatures experienced in the orchard. The finding that vesicles subcool below $-12°C$, together with the fact that the location of initial ice formation has consistently been found to be in the intercellular spaces (3, 17, 23, 24), suggests that ice nucleation does not take place within the pulp but, rather, in the intercellular space of the peel. Small, freezing nuclei undoubtedly can diffuse into the intercellular space, as these nuclei need be only of a size comparable to ice nuclei (13). As a consequence, the reports that soil particles (27) and atmospheric dust (25, 27) catalyze ice nucleation in water subcooled a few degrees, lead, with one exception, to the following hypothesis. Ice nucleation in a lemon ordinarily occurs at or near its surface and is due to freezing nuclei of atmospheric origin. The exception is based upon the evidence of Dorsey (5) and Wylie (40) that solid surfaces rubbed together in water catalyze ice nucleation at a slight degree of subcooling. This suggests that lemons wet with dew at their point of contact and brushing against one another cannot subcool as much as other fruits. If the hypothesis is correct, varying conditions at the fruit surface should affect the degree of subcooling; it was on this basis that the experiments described below were made.

SUBCOOLING OF DETACHED LEMONS: The freezing points of a group of detached fruits were obtained by placing thermocouples in their pulps and cooling them in the specimen container. The highest temperature maintained for 15 or more minutes, once freezing had begun, was taken as the freezing point. The amount of previous subcooling had no apparent effect on the measured freezing points. The result is given in table I.

The ice nucleation point of fruits with (fig 1) and without stem heaters in place was determined under three conditions of humidity. Temperatures were measured with spiral thermocouples, as it was desired to have no foreign body whose surface could possibly aid ice nucleation projecting into the fruits. The surfaces of all the fruits in these five groups appeared dry to the naked eye throughout the tests. Frost formed on the inside surface of the plastic lining in the 90 % relative humidity runs but had no apparent effect on fruit subcooling. There was no significant difference in nucleation point among these five groups (table I).

TABLE I

EFFECT OF SURFACE CONDITIONS ON SUBCOOLING OF DETACHED LEMONS AND LEMON PARTS

GROUP	TREATMENT	ICE NUCLEATION POINT *
		° C
1	Freezing point of fresh fruits	– 1.4 ± .05
2	Detached vesicles	below – 12
3	Fruits in 90 % relative humidity	– 4.4 ± .20
4	Fruits in 90 % relative humidity and stems heated	– 4.2 ± .30
5	Fruits in 55 % relative humidity	– 4.0 ± .25
6	Fruits in 8 % relative humidity ...	– 3.9 ± .25
7	Fruits in 8 % relative humidity and stems heated	– 3.9 ± .29
8	Condensation on fruits	– 4.3 ± .15
9	Fruits in alcohol	– 6.1 ± .34
10	Half peeled fruits in alcohol	– 8.7 ± .40
11	Fully peeled fruits in alcohol	– 8.5 ± .36
12	Fruits rubbed on water droplets ..	– 2.6 ± .07
13	Fruits rubbed but not on droplets	– 4.1 ± .18

* The values are the mean of 9 fruits ± standard error.

The effect of surface moisture on the degree of subcooling was determined with two groups of fruits. Moisture was formed on the fruit surfaces of group 8 by condensation from relatively moist laboratory air which was forced into the specimen container at half-degree cooling intervals. Frost deposition on the inside surface of the plastic lining was prevented by a thin coating of glycerol. The fruits in group 13, whose treatment is given in more detail below, were cooled with distilled water droplets on their surfaces. Subcooling of fruits in these two groups was not significantly different from that of fruits with dry surfaces. This is somewhat similar to the finding of See-mann (29) that spraying bean plants with water immediately before cooling appears to slightly increase subcooling.

Another group not included in table I was treated just as was group 8 except that frost was allowed to form on the plastic lining. The mean of the ice nucleation points was – 2.4°C. The higher temperature was apparently due to ice inoculation of the fruits by frost particles from the lining. This result compares with those reported previously by several workers (21, 22, 28, 33, 39), and it suggests that either a similar phenomenon or rubbing, discussed below, may have affected their results.

During the course of preliminary condensation experiments, it was noted that the appearance of ice on a fruit surface was followed, a short time later, by freezing of the fruit. This finding compares with that of Harvey and Wright (11), who found that frozen water on tomatoes prevents their subcooling; of Wartenberg (39), who obtained similar results with seeds; and of Young (41), who states that citrus fruits covered with ice appear to subcool less. The heavy covering on some herbaceous plants which can prevent ice inoculation from the surface (10) is evidently not present on lemons.

Fruits completely submerged in C.P. methyl alco-

hol were cooled. The temperature recorded was that of a thermocouple resting on the top surface of each fruit. These fruits subcooled more than fruits cooled under both dry and wet surface conditions (table I), and the differences are significant below the 1 % level as determined by the "t" test. The freezing points of sample fruits obtained with thermocouples in the pulps during a second freezing exhibited no deviation from the freezing point of fruits not previously in alcohol. This appears to indicate that the lower nucleation point of fruits in alcohol cannot be attributed to internal dehydration. Two aditional groups of fruits were cooled in alcohol. In the first, the outer half of the peel was removed before the fruits were placed in alcohol; in the other, the entire peel was removed leaving only the outer segment walls and imbedded vascular tissue. The nucleation points of these two groups, given in table I, do not significantly differ. However, the difference between their subcooling and that of unpeeled fruits in alcohol is statistically significant below the 1 % level even though the lowering of freezing point in the peeled fruits is taken into account: the freezing point of half peeled fruits was about 0.5°C lower than that of other fruits, and that of fully peeled fruits was about 0.75°C lower. These results resemble those recently reported by Luyet and Gehenio (19), who found greater subcooling of tissues predipped in glycerol.

Immersion in alcohol undoubtedly greatly reduces the freezing point, and also the chance of ice nucleation, at and adjacent to the surface of the fruits. For this reason, the results indicate that ice nucleation occurred near the surface in the fruits cooled in air.

The prediction that rubbing together of wet fruit surfaces hinders subcooling was checked with group 12. When the temperature of a spiral thermocouple first dropped to – 1.5°C, and at each half degree below that, fruits wet at the point of contact were brought together and rubbed against one another. These fruits subcooled less than any other fruits tested, and the difference in each case is statistically significant below the 1 % level. As a check, fruits in group 13 were cooled with distilled water droplets on their surfaces and were rubbed together, but not at the location of a droplet. These fruits subcooled to an extent comparable to those cooled with no rubbing and no water droplets on them. The effectiveness of rubbing of wet fruit surfaces in reducing subcooling may explain reports that wind lessens subcooling in plants (22, 28).

The hypothesis offers explanations for many previous findings concerning subcooling of plant parts. Müller-Thurgau's (23) discovery that factors which affect degree of subcooling are not the same as those which determine freezing point in living tissue is in agreement with the concept that the kind of freezing nuclei present determines the nucleation point. That there is no relation between rate of cooling, cell size, or osmotic pressure of cell sap and amount of subcooling (34) also agrees with nucleation theory, which asserts that minimum temperature at a point determines

whether ice nucleation will occur there, all other conditions being the same. In addition, the hypothesis indicates that neither the temperature gradient nor the length of time at a given temperature determines whether or not ice nucleation will occur. It may be possible to explain the finding that subcooling ability increases as the cross-section of intercellular spaces decreases (34), if the assumption is allowed that freezing nuclei from the atmosphere penetrate more quickly and more deeply into tissue with larger intercellular spaces. The hypothesis may also lead the way toward an understanding of reports that plants subcool more as turgor decreases (22, 26, 29). Tissue under water stress may absorb sufficient moisture to lower the dew point of air near it; dew, which aids nucleation when rubbing occurs, would then form on the plant surface under higher atmospheric humidity or lower surface temperature than otherwise.

SUMMARY

Some low temperature phenomena in lemon tissue were investigated by means of cooling apparatus constructed for the purpose.

Detached lemon vesicles were found to subcool readily to $-12°C$.

Experiments and observations made suggest that freezing in lemon segments progresses by way of xylem vessels.

The spread of freezing in lemon tree parts, all portions of which are subcooled, was shown to be very rapid. The crystallization velocity in branchlets has been found to be in excess of 15 cm per minute. The rapid spread of freezing suggests that ice nucleation occurs at a single point in a subcooled tree part.

A hypothesis that ice nucleation in a subcooled lemon takes place upon freezing nuclei of atmospheric origin near the fruit surface has been developed. The following results are consistent with the hypothesis.

(a) Humidity of the surrounding air or moisture on the fruit surfaces had no effect on the ice nucleation point of detached lemons.

(b) Fruits submerged in alcohol subcooled more than fruits in air.

(c) Fruits peeled before submersion in alcohol subcooled even more than unpeeled fruits in alcohol.

The additional prediction that rubbing of wet fruit surfaces may greatly hinder subcooling has been experimentally verified.

The hypothesis has been found to offer explanations of some plant subcooling phenomena observed by others.

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THE RELATIVE SENSITIVITY OF XANTHIUM LEAVES OF DIFFERENT AGES TO PHOTOPERIODIC INDUCTION [1]

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Lang (5), in his recent review, has discussed the literature dealing with the relative sensitivity to photoperiodic treatment of leaves of different ages. The work of Moskov (8, 9) with chrysanthemum, Borthwick and Parker (1) with Biloxi soybean, and Naylor (10) with Xanthium has indicated that the first expanded leaf showed the greatest sensitivity of those tested. Hamner and Bonner (3) in 1938, and others since, found that Xanthium plants defoliated except for the youngest leaves of less than 1 cm² area did not appear sensitive to short day treatment.

The tacit assumption has been that sensitivity to photoperiod increased as leaves expanded until the leaf reached full size and then gradually decreased as the leaf became older.

In most short day plants several photoinductive cycles are required to induce flowering. Xanthium is known to differ from these other short day plants since with this plant one short day is sufficient to bring about flower initiation. It is possible, therefore, to measure precisely the relative sensitivity of young expanding leaves of this plant. The results of certain experiments in this laboratory made it appear desirable to reexamine the photoperiodic sensitivity of Xanthium leaves of all ages.

MATERIAL AND METHODS

Burs from the species of cocklebur, Xanthium pennsylvanicum,[3] were collected from an area around Chicago, Illinois. These burs were soaked overnight and then planted in flats. After the seedlings were about two weeks old they were transplanted into four-inch clay pots or 10-oz. plastic cups as indicated in each experiment. From the time of planting until the time of experimentation the plants were kept in the greenhouse under long days of at least 18 hours of light, supplementing the natural daylight from sunset until 2:00 A.M. The plants were not used for experimentation until they had at least five fully expanded leaves.

After the plants received experimental treatment they were put back on long day for 3 weeks. They were then dissected to determine the presence of flower initiation and the size of the inflorescence. For the degree of magnitude of flowering in Xanthium an

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[3] Xanthium pen(n)sylvanicum Wallr., synonym X. saccharatum Wallr., as defined in the Eighth Edition of Gray's Manual (1b). Specimens of the plant used in these studies have been filed at the U.C.L.A. Herbarium.