should be noted that the stimulation of growth, particularly of lateral growth, on the addition of niacin to the thiamin-pyridoxin medium indicates that niacin may exert an effect on growth which is not due to any "sparing" action on vitamin B_6 .

It is evident that the data obtained so far on the replacement of pyridoxin by glycine, on the synergistic effect of glycine plus niacin in basal medium plus glycine and niacin, and the synergistic effect of glycine plus pyridoxin in basal medium plus thiamin, pyridoxin, niacin, and glycine, demonstrate a connection between vitamin B_6 and glycine or niacin in the clone of excised tomato roots studied here. The metabolic basis of this connection is being examined by analytical and further nutritional studies.

SUMMARY

The clone of excised tomato roots studied here requires thiamin, pyridoxin, and niacin for "optimal" growth but can be maintained in a medium supplemented only with thiamin and pyridoxin. Pyridoxin was replaceable by pyridoxal or pyridoxamine. The order of activity was pyridoxal > pyridoxin > pyridoxamine. Niacin was replaceable by niacinamide. Niacinamide was, in general, the more active.

Pyridoxin could be replaced in part by glycine. This replacement was greater in the presence than in the absence of niacin. In basal medium plus thiamin, pyridoxin, and niacin, the addition of glycine at certain concentrations of pyridoxin increased level of growth to that obtained with "optimal" concentration of pyridoxin.

Morphogenetic effects of glycine and pyridoxin were similar although glycine appeared to exert an independent effect upon the initiation of laterals. Root morphology was controlled by the balance of growth factors supplied in the medium.

LITERATURE CITED

- BOLL, W. G. Studies on the growth of excised roots. V. Growth of excised roots of two inbred lines of tomato and their reciprocal crosses in media supplemented with various growth factors. New Phytol. (In press.)
- 2. Boll, W. G. Effect of sucrose concentration, length of passage, minor element nutrition and pH on growth of excised tomato roots. Bot. Gaz. (In press.)
- BOLL, W. G. and STREET, H. E. Studies on the growth of excised roots. I. The stimulatory effect of molybdenum and copper on the growth of excised tomato roots. New Phytol. 50: 52-75. 1951.
- KREHL, W. A., BONNER, D., AND YANOFSKY, C. Utilization of niacin precursors and derivatives by the rat and *Neurospora*. Jour. Nutrition 41: 159–172. 1950.
- KUN, E. A study of the metabolism of glyoxal in vitro. Jour. Biol. Chem. 194: 603-611. 1952.
- MARDSCHEV, S. R. and SEMINA, L. A. Enzymatic synthesis of glyoxylic acid from glycine. Doklady Akad. Nauk. S. S. S. R. 74: 537. 1950.
- SNELL, E. E. A correlation of chemical, enzymatic and growth promoting properties of vitamin B₆. Symp. sur le Métabolisme Microbien. II^e Congrès International de Biochimie. Pp. 47. Paris. 1952.
- UMBREIT, W. W., WOODS, W. A., and GUNSALUS, I. C. The activity of pyridoxal phosphate in tryptophane formation by cell free enzyme preparations. Jour. Biol. Chem. 165: 731-732. 1946.
- WHITE, P. R. Further evidence on the significance of glycine, pyridoxine and nicotinic acid in the nutrition of excised tomato roots. Amer. Jour. Bot. 30: 33-36. 1943.
- WHITE, P. R. Handbook of plant tissue culture. Jaques Cattell Press, Lancaster, Pennsylvania. 1943.
- 11. WILLIAMS, R. J., EAKIN, R. E., BEERSTECHER, E., and SHIVE, W. The biochemistry of the B vitamins. Rheinhold Publishing Corp., New York. 1950.

STUDIES ON THE CHEMISTRY OF THE LIVING BARK OF THE BLACK LOCUST IN RELATION TO ITS FROST HARDINESS. VII. A POSSIBLE DIRECT EFFECT OF STARCH ON THE SUSCEPTIBILITY OF PLANTS TO FREEZING INJURY^{1,2}

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Under conditions of low autumnal temperatures not far removed from the freezing point, an increase in the frost hardiness of plants is often observed which is accompanied by a disappearance of the starch and an increase in the soluble sugar contents

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² Paper No. 2998, Scientific Journal Series, Minnesota Agricultural Experiment Station. This study was supported by a grant-in-aid from the Herman Frasch Foundation for Chemical Research.

³ Present address: Division of Chemistry, Department of Agriculture, Science Service, Ottawa, Canada. of the perennial tissues. This response is well exemplified in the seasonal changes in the living bark tissue of the black locust tree during late fall (8). It is not surprising that much of the extensive literature on frost hardiness in plants is devoted to a consideration of the extent to which this apparent conversion of starch to sugars may be involved in the actual frost-hardening mechanism. Of the many theories advanced in this regard, nearly all have ascribed the effect to the increase in concentration of the sugars which are produced. Theories of frost hardiness based wholly or in part on the effect of increased sugar concentration seem attractive from the physico-chemical point of view because of the relation of sugar concentration to a lowering of the freezing point of the cell contents or to a possible stabilizing or protective effect on cell colloids. These theories and the evidence in support of them have been discussed in recent reviews on frost hardiness (3, 4). As a first step in the appraisal of the possible role of soluble sugars in hardiness, it would appear that the degree of correlation between the soluble sugar concentration in the cell and the hardiness of the tissue would have been thoroughly examined. If sugar concentration were an important factor in frost hardiness, a positive correlation between these factors would be maintained and observed at all times and under all conditions. Yet when this relationship was examined in the cells of the bark of the black locust under seasonal as well as unseasonal conditions (5) such a positive correlation was not observed. A similar lack of correlation between the hardiness and the sugar content or the osmotic pressure of the tissues of other plants is reported generally in the literature (1, 2, 3).

Regardless of whether sugar concentration is a factor in the frost hardening mechanism, a possible direct effect of the elimination of starch from the cells on their frost hardiness has been either overlooked or discounted. Indeed Levitt (3), after examining the controversial evidence pertaining to the relation of soluble sugars to hardiness and the effect of starch to sugar transformations on hardiness, poses the question, "might not the presence of starch be harmful rather than the presence of sugars beneficial?" The possible injurious effects of starch contained in cells of tissues when they are frozen and, conversely, the beneficial effects which would be derived, if such an injurious effect exists, from the prior transformation of this starch to sugar, should have been suspected in view of the recent evidence of the mechanical nature of frost injury (4).

A deleterious effect of starch on cell hardiness became increasingly suspect in the course of studies on other aspects of the chemistry of the hardiness mechanism in the black locust when it was noted incidentally that a definite measure of increased hardiness consistently accompanied any considerable decrease in the starch content of the bark cells of this tree as determined by iodine staining of tissue slices. An investigation was therefore undertaken to examine more closely the relationship between the variations in the carbohydrates of the black locust and its frost hardiness, with special reference to the role of starch. A part of this study, describing analytically determined variations in the starch and soluble sugar contents of locust bark tissue under a variety of seasonal and unseasonal conditions in normal and ringed trees and in cut logs, has been presented earlier (8). The present paper is concerned with the relationship of these variations in the carbohydrates to the variations which were observed at the same time in the hardiness of this tissue.

METHODS AND RESULTS

The analytical procedures employed in the estimation of sugars, starch, and water soluble protein contents of the bark tissues are those described earlier (7, 8). Frost hardiness of the tissues was measured by the plasmolysis test (6) and is reported either in terms of the highest molarity of salt solutions (NaCl: CaCl₂::9:1) to which the tissue could be subjected before experiencing a 50 % killing of the cells, or in terms of the percentage survival of the cells when the tissues were subjected to salt solutions of varying molarity.

Variations in the hardiness of locust bark tissue have been shown to be associated principally with changes in the water soluble protein content of the

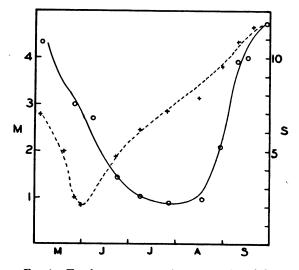


FIG. 1. Total sugar content (---+--) and degree of hardiness $(---\bigcirc --)$ of living bark tissues at various times during summer. Hardiness measured as molarity (M) of salt solutions required to effect 50 % killing of cells in plasmolysis. Sugar content (S) measured as % of dry weight of tissue. Abscissa indicates dates (May to September, inclusive) at which samples were taken.

cells (7). In order to detect any limiting relationship which might exist between hardiness and the starch or sugar content of the cells it is necessary, therefore, that concomitant variations in their water soluble protein content be taken into account.

SOLUBLE SUGAR CONTENT VERSUS HARDINESS OF BARK CELLS: Several lines of evidence have been obtained which indicate that no primary relationship exists between the soluble sugar content of a locust bark cell and its hardiness. For example, when the courses of the normal variations in the total soluble sugars and in the frost hardiness of the bark of intact trees are followed simultaneously throughout the summer season, results such as those illustrated in figure 1 are obtained. It is evident that the amount of soluble sugars existing in the bark tissues at any time bears little, if any, relationship to its frost hardiness at that time. In late spring the hardiness which existed previously in winter is still decreasing while the sugars have already passed a minimum and begun to accumulate. The sugars continue to increase steadily and progressively throughout June and July while hardiness passes through the minimum for the year. Fairly high levels of soluble sugars are reached in July, long before the hardiness for the following winter has begun to develop. It has already been shown (5, 7) that the trends exhibited here in hardiness follow closely the trends in the concentration of water soluble protein. In early spring, hardiness is slow to disappear in the bark. It does so in proportion to the extent to which the winter accumulation of water soluble protein disappears. Yet appreciable depletion of soluble sugars occurs very early in the spring by virtue of the apparent conversion of these sugars to starch (8) and by respiration. For these reasons the level of soluble sugars in the tissues in early May is much the same as in late July although the hardiness of the bark in May is much greater. A comparison of May and July bark tissues in these respects as well as in

TABLE I

THE RELATION OF SOLUBLE SUGAR, STARCH, AND WATER SOLUBLE PROTEIN CONCENTRATION TO FROST HARDINESS IN THE BARK OF NORMAL TREES IN JULY AND MAY

DATE SOLUBLE STARCH SOLUBLE RE SUGAR STARCH SOLUBLE FO PROTEIN N 10	Molarity of plasmolyzing	
	LUTION QUIRED R 50 % NJURY	
July 25 6.8 1.2 0.58	1	
May 15 6.3 5.8 1.00	4	

respect to the water soluble proteins and starch is shown in table I. But for the considerable accumulation of starch in the May tissues it is probable that their hardiness differences would be even greater.

If a tree is doubly ringed down to the wood in mid-summer so as to intercept, from above and below, a section of the bark on the trunk, translocation of carbohydrate to or from this section is arrested. It was shown (7) that if this ringing is performed in mid-summer and the variations in hardiness and soluble sugars are followed simultaneously in these sections from the time of ringing until early winter, the level of soluble sugar drops while that of the hardiness increases, although a degree of hardiness equal to that of normal trees is never reached. The hardiness tests and carbohydrate analyses made on such sections consistently indicate that the change in hardiness and the change in soluble sugars occur in opposite directions. Table II shows these variations in two such sections.

Conversely, if a tree is doubly ringed in winter and the tissues of the section of bark so intercepted are tested for hardiness and analyzed in June, it is found that while the soluble sugars in this section

TABLE II

CHANGES OCCURRING IN THE FALL IN THE SOLUBLE SUGARS, STARCH, WATER SOLUBLE PROTEIN, AND FROST HARDINESS IN THE BARK OF TRUNK SECTIONS OF TWO TREES INTERCEPTED BY DOUBLE RINGING IN MID-SUMMER

	DATE OF SAMPLING OF SECTION	%]	MOLARITY OF PLAS-		
Tree No.		Soluble sugar	STARCH	WATER SOLUBLE PRO- TEIN N	MOLYZING SOLUTION REQUIRED FOR 50 % INJURY
3-R	Initial section ringed and sampled July 25	n 8.3	1.1	0.66	2.5
3-R	Final Sampled Nov. 10	5.3	0.3	1.05	4.5
4-R	Initial section ringed and sampled Aug. 15	n 7.3	1.8	0.55	0.5
4-R	Final Sampled Nov. 15	4.6	0.3	0.65	2.7

have been depleted to almost the same extent as in normal trees at this time, the loss in hardiness is much less than in the bark of normal trees. This is illustrated in table III. The unusual retention of hardiness in June in such winter ringed sections occurs independently of the loss of soluble sugars and is associated with the retention of a level of water soluble protein more characteristic of winter tissues (5). Comparison of the level of sugars existing in the hardy ringed tissue of June 23 in table III with that in the normal unhardy tissues of July 25 in table I further emphasizes the lack of correlation of soluble sugar content with hardiness. These observations indicate that it is unlikely that soluble sugar concentration per se is a primary factor in frost re-

TABLE III

THE RELATION OF SOLUBLE SUGAR, STARCH, AND WATER SOLUBLE PROTEIN CONCENTRATION TO FROST HARDINESS ON JUNE 23 IN THE BARK OF A NORMAL TREE AND IN THE BARK OF A SECTION OF TRUNK ISOLATED BY RINGING IN WINTER

		%	MOLARITY OF PLAS-			
Date	Descrip- tion	Solu- ble sugars	Starch	WATER SOLUBLE PRO- TEIN N	MOLYZING SOLUTION REQUIRED FOR 50 % INJURY	
Feb. 6	Normal winter bark	12.8	0.1	1.29	> 5	
June 23	Section of trunk iso- lated by ringing in Feb.	6.4	0.5	1.25	5	
June 23	Normal tree	4.0	0.9	0.57	1.5	

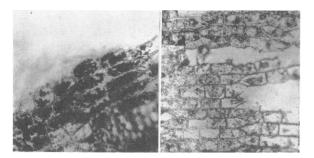


FIG. 2 a and b. Photomicrographs of iodine-stained normal locust bark tissue of (a) (left) high and (b) (right) low starch content.

sistance. Is, then, the association of a measure of increase in hardiness which is observed to accompany the replacement of starch by soluble sugars in the tissue purely fortuitous, with both processes occurring independently but at the same time seasonally? The following considerations and evidence would indicate that this is not entirely so.

THE EFFECTS OF STARCH ON HARDINESS: Starch is observed to occur in considerable quantities in the bark at certain times of the year (8). If, at these times, tissue sections are cut from the bark and stained with iodine, the characteristic and intense blue color is given by the large granules of starch present in the cells. This is illustrated in figure 2a. The intense staining reaction of such tissues is in sharp contrast to that given by those tissues which are completely devoid of starch (fig 2b). When starch is present in such high concentration the granules can occupy a considerable proportion of the protoplast. When such cells of high starch content are placed in strong salt solutions the cells plasmolyze with osmotic reduction in the solvent space of the protoplast but with relatively little reduction in the volume of the insoluble starch granules. The result is that, with contraction of the protoplast, the starch granules within it are packed tightly together and bulge out at its periphery as shown in figure 3a. This too is in contrast to the characteristic smooth indentation or concave plasmolysis of the protoplasts of cells devoid of starch when these are plasmolvzed in the same solutions (fig 3b). Moreover if the cells of both tissues were given time to round up in

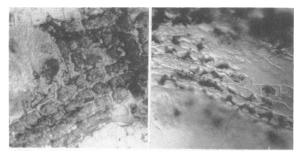


FIG. 3 a and b. Photomicrographs of plasmolyzed locust bark tissue (without staining) of (a) (left) high and (b) (right) low starch content.

smooth, convex plasmolysis the volume occupied by the protoplasts of cells with high starch would, because of the relative lack of osmotic reduction in the granule size, be greater than that occupied by the protoplasts of cells without starch. The dehydrated and contracted protoplasm and protoplasmic membrane in the starch-containing cells are not only distorted but stretched irregularly by extension over a greater area than in the starch free cell.

Injurious effects produced on bark tissues by the processes of freezing, desiccation, plasmolysis are essentially similar in that the injury in all cases derives from the mechanical stresses exerted on the protoplasm or the protoplasmic membrane as these are rendered highly and even irreversibly deformable by virtue of the dehydrations (6). The effect of these stresses on a stiffened or coagulated protoplasm or protoplasmic membrane can be sufficient to cause rupture, with killing of the cell. As a protective mechanism against these effects of dehydration, the physical properties of the protoplasm of the cells of plants which are developing hardiness are so altered as to render the protoplasm less subject to injury owing either to excessive stresses or to irreversible changes in deformability when dehydrated. The extent to which these favorable properties are acquired in the development of hardiness in the cells of a plant becomes, in effect, a measure of what can be called the intrinsic or real frost hardiness of the plant, and provides the basis for the tests of frost resistance which have been described (6). At any stage of intrinsic frost hardiness of the protoplasm, it is conceivable that the presence of a considerable amount of insoluble starch in frozen, desiccated, or plasmolyzed cells could exaggerate the mechanical stresses incurred and enhance the probability of injury. The following evidence tends to support this hypothesis.

The maximum degree of hardiness which can be attained in those tissues which are completely devoid of starch is never observed in tissues where a considerable accumulation of starch is present, even though the soluble sugar or protein concentrations may be the same in both samples of tissue. Incontroversial evidence for this is, however, difficult to obtain because of the conflicting effects of variations which usually occur simultaneously in the water soluble proteins and in the starch. A striking illustration of this effect should have been obtained from the multiply ringed tree experiments described in a previous paper (7), where, because of arrested translocation, high accumulation of starch was observed to occur in late summer in the bark of those sections of the trunk situated above the uppermost rings. Levels of starch as high as 15 to 20 % of the dry weight of the tissue were encountered in September in these sections, in contrast to the 5 to 7 % level observed in normal trees at this time. A comparison of the hardiness of the bark of these sections with that of the bark of normal trees should have revealed considerable differences in hardiness if starch was effective in promoting injury. The hardiness of these

high starch tissues was indeed found to be lower than that of tissues of normal trees, as is indicated in table IV. Unfortunately the effect of ringing was also to retard the rate of synthesis or accumulation of protein in these ringed sections, as compared with normal trees. Consequently it was not possible definitely to separate, through comparison with normal trees, the effect of high starch from that due to lack of a normal synthesis of protein in these sections.

Alternatively, since the starch levels of late summer become greatly reduced with the incidence of lower temperatures in late fall, it would have been advantageous to follow, in these sections of abnormally high starch content, the corresponding effect on hardiness. However, here again (table IV) the synthesis of protein which occurred in these sections in late fall accompanying the lowering of the starch

TABLE IV

LEVELS, ON SEPTEMBER 21, OF SOLUBLE SUGAR, STARCH, WATER SOLUBLE PROTEIN, AND FROST HARDINESS IN THE BARK OF A NORMAL TREE, IN THE BARK OF A SECTION OF THE TRUNK ABOVE RINGS IN A MULTIPLY RINGED TREE, AND IN THIS LATTER SECTION IN NOVEMBER AFTER FUR-THER ISOLATION BY RINGING FROM ABOVE ON SEPTEMBER 21

		%	MOLARITY OF PLAS-		
DATE OF SAM- PLING	Descrip- tion	Solu- BLE SUGARS	STARCH	WATER SOLUBLE PRO- TEIN N	MOLYZING SOLUTION REQUIRED FOR 50 % INJURY
Sept. 21	Normal tree	9.1	6.8	0.97	4.5
Sept. 21	Section of a ringed tree above rings	7.0	15.6	0.74	2.5
Nov. 10	Same section as above, after having been isolated by ringing from above on Sept. 21		3.4	1.07	>5

content obscured the effects due to starch which might otherwise have been revealed.

In one group of normal trees, conditions existed in autumn which made possible the direct observation of the effect of starch disappearance at lower fall temperatures upon hardiness. In this group the protein accumulation reached its maximum early in September, before temperatures became low enough to stimulate starch disappearance in the tissues. Thus, while the effect of starch upon hardiness could not be shown to best advantage, because the starch variations are not as considerable in normal as in ringed trees, the effect was rendered more discernible in the absence of any significant change in protein. The results of a comparison in tissues from one such tree are shown in table V. Neither the differences in the soluble sugar level nor in the protein level will serve adequately to explain the hardiness changes indicated between these samples. These differences in

TABLE V

CHANGES IN SOLUBLE SUGARS, STARCH, WATER SOLUBLE PROTEIN, AND HARDINESS OCCURRING FROM SEPTEMBER TO MARCH IN THE BARK OF THE SAME TREE

DATE OF SAM- PLING	%	$\%~{ m Dry}$ weight			% SURVIVAL AT VARIOUS MOLARITIES OF PLASMOLYZING SOLUTION	
	Solu- ble sugars	Starch	WATER SOLUBLE PRO- TEIN N	3 M	4 M	5 M
Sept. 29 March 3	11.5 10.0	6.0 0.1	1.06 1.11	100 100	60 100	10 100

hardiness seem accountable only in terms of the variation shown in the starch level and are probably greater than is actually indicated, because 100%survival at the highest concentration of plasmolyzing solution used (5 M) does not always include the full potential of resistance of a tissue to freezing (6).

Other evidences of an effect of starch on hardiness are adduced from observations made in the spring of the year. An increase in starch content occurs in the bark in spring at the expense of soluble sugars and in response to increases in the average daily temperature (8). During this time (March to June), fluctuations in hardiness were observed to coincide with fluctuations in starch content even though there was little variation in soluble protein the hardiness decreasing with increasing starch concentration. With persisting higher temperatures at the end of May, there occurred a pronounced increase in the amount of starch, a corresponding drop in the sugar level, and a definite decrease in the hardiness (table VI). This decrease in hardiness could be only attributed either to the increase in starch or to the decrease in the sugar level because of the small variation in protein. This normal process of apparent conversion of sugars to starch, at this time of year, and the associated process of de-

TABLE VI

Changes in Soluble Sugars, Starch, Water Soluble Protein, and Hardiness in the Bark of Normal Trees in Spring and in the Bark of Log Severed from a Tree in May and Stored for One Month at $+3^{\circ}$ C

		%]	MOLARITY OF PLAS-		
DATE OF SAM- PLING	M- DESCRIP- SOLU		Starch	WATER SOLUBLE PRO- TEIN N	MOLYZING SOLUTION REQUIRED FOR 50 % INJURY
March 3	Normal	10.1	0.1	1.13	> 5
March 30	Normal	11.5	0.3	1.20	> 5
May 22	Normal	3.6	7.0	1.12	3.5
June 21	Log severed on May 22 and stored at +3° C	l 9.8	0.2	1.09	5.0

creasing hardiness could be reversed by holding the tissues at a temperature a few degrees above the freezing point. Thus, when, at the point of maximal accumulation of starch (May 22), a length of trunk with bark intact was severed from the tree and transferred to a refrigerator at +3° C, the starch almost quantitatively reverted to soluble sugars (largely sucrose) and most of the hardiness existing originally in the tree in late winter was restored. On the basis of the evidence which has been presented above, the variations in soluble sugars could not account for the changes noted in the hardiness of the tissues under these conditions. Since variations in protein are not involved, it would appear that the important factor concerned in the early normal drop in hardiness in spring-and in the restoration of this hardiness in the severed log through application of low temperature-is the variation in the starch content of the cells.

DISCUSSION

The capacity of a plant cell to withstand rupture or disruption of internal organization from changes in its protoplasmic volume, due to dehydration and rehydration, is a measure of its winter hardiness against extracellular freezing (6). The hypothesis is proposed that the presence of starch in a cell increases its susceptibility to such injury, the incompressibility of the granules causing the protoplasm to be subjected to greater shearing forces as it is stretched around the granules during dehydration. According to this hypothesis, with other conditions being equal, the presence of starch in a cell will produce an increment of decrease in the winter hardiness. Similarly, a disappearance of the starch will produce an increment of increase in hardiness. It is suggested therefore that the observed increase in hardiness which accompanies the disappearance of starch from the bark cells of the locust tree in autumn, when temperatures approach the freezing point, can be ascribed in part to this circumstance rather than to any effect due to an increase in soluble sugars which usually accompanies this disappearance of starch. The soluble sugar content of the cells has been demonstrated, under a variety of conditions, to manifest no detectable effect upon the winter hardiness of the cells.

While the hardiness increment associated with the presence or absence of starch in the cell would be of a lower order of magnitude than the increment of hardiness associated with the water soluble protein content of the cell (5, 7) it could, under proper conditions of winter temperature variations, mean the difference between death and survival of the tissues. Periods of warm weather in spring are quickly reflected in a resynthesis of starch in the cells (8). This may occur also during warm periods earlier in winter. Should such warm spells be followed by quick and radical temperature drops, cells that would normally be hardy to such temperatures could be killed because of the starch accumulated in the cell during the preceding warm spell. A temperature

induced variation in hardiness of trees and shrubs is often observed in the course of hardiness testing in horticultural practice. This may well be related to the reversible temperature-sensitive conversions of starch and soluble sugars and to the postulated direct effects of starch upon the susceptibility of the cells to frost injury. The ease, rate, and degree of reversibility of the conversion process will, of course, vary with the particular species or variety of plant and with the time of year. It is suggested further that differences in the susceptibility of different parts of the same plant may be attributable to localized variations in starch content.

It is obvious that the hypothesis presented in this paper has a bearing on the problem of hardiness only in those plants, like the black locust, where starch constitutes a major reserve carbohydrate.

SUMMARY

With the onset of low autumnal temperatures there is observed a simultaneous decrease in starch content and an increase in soluble sugar content of the living bark cells of the black locust. An increase in soluble protein content precedes the decrease in starch content or continues at the same time. An accompanying increase in frost hardiness of the cells is largely associated with the protein change but under circumstances where the protein change is minimal or zero during the time interval of the carbohydrate conversion some increase in hardiness is observable during the starch-sugar content change.

Evidence is presented that variations in soluble sugars in the bark tissue at other times bear no relationship to the frost hardiness of the cells. It is proposed therefore that the small but discernible hardiness change which accompanies the disappearance of starch is due to a detrimental effect which is exerted by the starch itself, at higher concentrations, on the dehydrated cells. Such a detrimental effect of starch is shown to be possible in view of the mechanism by which frost injury is produced in the cells; the starch granules serve to enhance stress and distortion effects in the protoplasm as engendered, through dehydration, by the freezing process.

It is suggested that readily reversible changes in frost hardiness which have been reported to occur in tissues of some woody plants in response to temperature fluctuations could be due in part to the temperature-sensitive variations in the level of the starch content of these tissues.

LITERATURE CITED

- ASAI, G. N. A study of frost injury and frost resistance in garden roses. Cornell University Ph.D. Thesis. 59 pp. 1943.
- LEVITT, J. and SCARTH, G. W. Frost hardening studies with living cells. I. Osmotic and bound water changes in relation to frost resistance and the seasonal cycle. Canadian Jour. Res. C 14: 267– 284. 1936.
- LEVITT, J. Frost killing and hardiness of plants. A critical review. 211 pp. Burgess Publishing Co., Minneapolis. 1941.

- 4. SCARTH, G. W. Cell physiological studies of frost resistance. New Phytol. 43: 1-12. 1944.
- SIMINOVITCH, D. and BRIGGS, D. R. The chemistry of the living bark of the black locust tree in relation to frost hardiness. I. Seasonal variations in protein content. Archiv. Biochem. 23: 8-17. 1949.
- SIMINOVITCH, D. and BRICGS, D. R. Studies on the chemistry of the living bark of the black locust in relation to frost hardiness. III. The validity of plasmolysis and desiccation tests for determining the frost hardiness of bark tissue. Plant Physiol. 28: 15-34. 1953.
- SIMINOVITCH, D. and BRIGGS, D. R. Studies on the chemistry of the living bark of the black locust in relation to frost hardiness. IV. Effects of ringing on translocation, protein synthesis, and the development of hardiness. Plant Physiol. 28: 177-200. 1953.
- SIMINOVITCH, D., WILSON, C. M., and BRIGGS, D. R. Studies on the chemistry of the living bark of the black locust in relation to its frost hardiness. V. Seasonal transformations and variations in the carbohydrates: starch-sucrose interconversions. Plant Physiol. 28: 383-400. 1953.

THE AVAILABILITY OF MAGNESIUM FROM ORGANIC CARRIERS IN ARTIFICIAL SUBSTRATES^{1,2}

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A mineral deficiency of apple trees known as "leaf scorch" and by various other names occurs very widely in apple growing areas. While the symptoms vary under different environmental conditions intervenal chlorosis of the leaves is most common. These effects develop to scorch, often attaining brilliant tints and may lead to severe defoliation. Mulder (7) illustrates the marginal chlorosis and scorching of foliage of apples grown in Holland. In many New Hampshire orchards, instead of chlorosis, bronzing or brown spotting occurs with no preceding chlorosis. The yield is lowered markedly in some cases, and preharvest dropping is increased.

The literature on the cause and control of leaf scorch has been reviewed recently quite thoroughly by Boynton (3). Most investigators are agreed that it is due to either a deficiency of magnesium in the soil or unavailability of the element. The effects of this deficiency may be aggravated by high amounts of potassium. The various phases of the interdependence of these factors are not clear. Mulder (7) found that the potassium content of leaves from magnesium deficient trees may approach twice the amount present in leaves from a healthy orchard. For control, numerous inorganic carriers of magnesium, such as magnesium sulfate, kieserite, and dolomitic limestone, have been tried in applications to the soil. Recovery of the trees is slow, often with little effect even after three years and with heavy applications. Apparently, under some circumstances,

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² Published with the approval of the Director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 149.

³ Part one of this paper is based upon a thesis prepared in partial fulfillment of the requirements for the M.S. degree at the University of New Hampshire. the roots of the apple are unable to absorb much magnesium in these forms from the soil. Spraying of apple foliage or injection of solutions of inorganic magnesium into tree trunks may give some temporary alleviation of the trouble.

That this deficiency is not due to fixation of magnesium in the upper layers of the soil and resulting unavailability to the roots, at least for some soil types, was shown by Lott (6) in his work with magnesium deficiency of grapes in North Carolina. He reported that delayed responses in the plants evidently cannot be attributed to slow movement of magnesium into the root zone and that a portion of the magnesium applied to the soil remains in the surface foot for more than a year. A similar deficiency in grapes was reported by Scott and Scott (8) in Maryland. They also found good penetration of magnesium into the soil from applications of magnesium sulfate, but slight response in correcting the trouble. Spray applications to the foliage gave prompt alleviation. A later paper (9) reported fairly good results from the soil applications but slower recovery than from sprays. Davis and Mc-Call (4) describe a magnesium deficiency in celery, which also does not respond to soil treatment, but is alleviated by spray applications.

It is apparent from this discussion that the usual inorganic sources of magnesium are frequently inadequate to correct this deficiency. Even though spray applications on foliage may help, such relief is temporary and the extra trouble and expense of application make this an unsatisfactory method. The use of organic compounds of magnesium, particularly those in which the metal does not readily ionize from the rest of the molecule, seemed to offer some possibilities in aiding utilization of this element by plants, and ultimately the alleviation of such deficiencies as