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THE RELATION OF THE PHYSICAL STRUCTURE OF THE FRUIT BUD OF THE PEACH TO HARDINESS

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PURPOSE AND SCOPE OF THE WORK.

The purpose of this research is to determine the relationship of the structure of fruit buds of the peach to hardiness, and, to add to the general knowledge regarding death of plant tissue resulting from exposure to low temperatures. Peach buds were selected for this experiment on account of their large size, their abundance, and their relatively high lower-thermal-death-point.

The causes of death of plant tissue on exposure to low temperatures have interested many leading horticulturists and botanists, e. g. Sachs, Molisch, Wiegand and others. The botanists are interested for scientific reasons, and the horticulturists for economic reasons. Much work has been done showing the relationship of chemical and physico-chemical characteristics of plant tissue to death by freezing. Molischl from experiments with freezing potatoes and submerged aquatic plants, came to the conclusion that "death by freezing is essentially death by drying out." This view has been questioned, and, in a measure, modified by later investigators.

¹Molisch, Hans. Untersuchung ueber das Erfrieren der Pflanzen. pp.16-26 Gustav Fischer. Jena 1897.

Pfeffer and Chandler found that drying (wilting) of plants before exposure to cold increases their resistance to death by freezing.

The problem has been considered from a physical and physico-chemical point of view with special reference to the osmotic strength of cell sap, and its relation to the freezing (death) points of plant tissue. Fairly direct relationships have been established from these investigations. In this work it is the intention to deal only with the relation of the physical structure of the buds and bud-parts to death from cold. The results are made comparative as far as possible to eliminate the influence of extraneous factors.

Sources of Material.

The peach buds used in this investigation were obtained from the orchard of the Missouri Agricultural Experiment Station at Columbia, Missouri, and from that of the Experiment Station, at Experiment, Georgia. The intention was to obtain all material from the Missouri orchard, but the severely cold weather of January 1912, in Missouri, rendered this impossible by killing all of the fruit buds of the peach. The buds secured from the Georgia Experiment Station orchard were in an advanced stage of development, the pink tips of the petals showing in some varieties.

¹Pfeffer, Pflanzenphysiologie; Ed. 2, 1904, p. 315

²Chandler, W. H., Missouri Agricultural Experiment Station, unpublished data 1912.

From the Missouri Agricultural Experiment Station orchard, buds of the following varieties were obtained: Crawford's Late, Oldmixon Free, Heath Cling, Seedling Elberta, Ringold, Fitzgerald, Oldmixon Cling, Bell's October, Crawford's Early, Early Michigan, Champion, Golden Gate, Magnum Bonum, Hill's Chili, Gold Dust, Gold Drop, General Lee, Lewis, Elberta, and Ortiz.

From the Georgia Experiment Station the following varieties were obtained: Greensborough, Bell's October, Oldmixon Free, Oldmixon Cling, Carman, Belle of Georgia, and Hill's Chili.

Preparation of Material.

The conditions of the investigation required both microscopic and macroscopic study of the buds, and actual freezing of buds in the laboratory.

The buds were prepared for microscopic study as follows:

Large well developed buds were selected from peach trees of different varieties and from various portions of the trees in order to include any possible variation that might result from differences in their location on the tree. The buds were immediately taken to the laboratory, where the outer, heavier scales were removed in order to facilitate sectioning. The first lot were placed in Flemming's Solution of the following strength.

25 cc. 1% Chromic acid

10 cc. 1% Osmic acid

10 cc. 1% Acetic acid

55 cc. Water

¹ Duggar, "Fungous Diseases of Plants" p. 44. Ginn & Co. 1909

The buds treated with this solution became so brittle as to make sectioning difficult. For this reason Gilson's mixture of the following strength was substituted for Flemming's Solution:

15 cc. 80% Nitric acid

4 cc. Glacial Acetic acid

20 grs. Corrosive sublimate

100 cc. 65% Alcohol

880 cc. Water

It was found that in this solution the buds remained soft, and they did not become brittle in the subsequent processes. Further treatment consisted of dehydration in alcohol of increasingly higher grades, treatment in xylol, xylol-paraffine, and final imbedding in paraffine of 52° melting point. Sectioning was done with a rotary microtome, the sections being cut 3 to 8 microns in thickness. The sections of 5 microns in thickness were most satisfactory with the softer buds. Thicker sections were necessary with the harder, more brittle buds, and also those which retained some of their scales.

Two staining solutions were tried - Delafield's haematoxylin and Bismarck brown. The haematoxylin stain was discarded on account of its tendency to stain the cell contents of the younger cells. Bismarck brown gave a beautiful light brown stain, which proved more satisfactory than the blue stain first employed. Back-ground staining occurred until the amount of egg-albumen fixative was reduced. The sections were mounted in Canada balsam.

In order to obtain information on the relative hardiness of bud parts, free-hand sections were made of the buds which

were killed while in the orchard. These sections required no staining, were mounted temporarily in water, and were studied with a low power lens.

Points of Structure.

From among those structural features which might probably affect the death point of peach buds, the following were chosen for study and comparison: (1) The relation of size of cells to hardiness; (2) the relation of thickness of cell wall to hardiness; (3) the relation of hardiness of various regions in the bud to hardiness of the bud as a whole; (4) the relation of bud scales as a protecting cover, to hardiness.

A study of the features mentioned seemed most likely to yield results for the following reasons: (1) It is known that a plant of dense structure will be more hardy than the same variety having a loose structure. For physical reasons one would expect smaller cells to be more resistant to cold.

Mousson¹ has shown that water in capillary tubes of less than 0.3 mm. or 0.4 mm. in diameter does not freeze until cooled to a temperature of -7° to -10° C. Also Molisch² says "The smallness of a cell is to a certain degree a protection against cold."

(2) We should expect thickness of cell walls to affect hardiness. Wiegand³ states that the degree of cold necessary

¹ Mousson, Die Physick auf Grundlage Erfahrung, 1 Ed., Part II,p.73

²Molisch, Hans. Untersuchung ueber das Erfrieren der Pflanzen. pp.16-26 Gustav Fischer. Jena 1897.

³Wiegand, "Buds and Twigs in Winter," Bot. Gaz. June 1906.

to cause separation of ice is proportional to the force which holds the water in the tissue. By increasing the thickness of cell wall a greater proportion of cell wall to water thereby obtains, with a consequent lowering of the freezing point.

- (3) From the known physical facts regarding insulation, the possible value of cutinized bud coverings in the form of scales surrounding the bud parts is apparent.
- (4) That the death of certain parts of the bud will affect others is evident. For example, the death of the pistil, or any essential part of it, inhibits its further development, and the death of the conducting tissue is certain to result in the death of the bud as a whole.

(1) Relation of Cell Size to Hardiness.

The majority of bud-sections were cut in order that comparisons might be made of the cellular structure of the buds of each variety and buds of different varieties, which had been selected from various locations on the trees. The intention was to secure a number of buds of each variety and in several stages of development, in order to determine whether or not differences in cellular structure might be correlated with differences in hardiness.

Measurements of many varieties were made and the follow-data taken as representative of the whole.

DATA showing comparative size of cells of peach buds, by regions, of different varieties,

	golow base of Pisti!	Conter of FISTI	10 of 1	Recordecte below 8n/her filon.ents	Base of	5 1de 04 87em	311169:013
VARIETY	Region I.	Region 2.	Region	Region 4	Region 5	Tregion G	Aver Size,
Crawford Late.	12,3	<i>6</i> 3	4,9	Э,	6,	10,	8,+
old Mizon free.	16,2	6,3	7.	/3,	8,8	10.	10.+
Old Mixon Cling.	12.	7.	6,	13,2	9,	10.	9.5.+
Elberta,	14:1	6,8	5./	10,5	7,5~	10,5	9,+
Fitzgerald.	15.	7	6.	10.	6,2	10,2	9,+
Ming gold.	13,2 ~	5,2	1 ,5	10,5	6,6	8.2	8.+
Bells October.	13.5 ~	4,5	6,	12.	6.	10.	8,57.

A comparison of the extremes of resistant qualities represented here by Crawford's Late and Bell's October, show that, though Bell's October has the more hardy buds, its cells average much larger in size than those of Grawford's Late buds. Comparing Elberta and Crawford's Late, the former known to be much the less hardy of the two, was found to be the smaller celled variety. Oldmixon Free and Ringold show the more hardy variety, (Ringold) to be the smaller celled. Elberta and Ringold compared as to cell size show that the Ringold, the more hardy variety, is the smaller celled.

Since the style, one of the most important parts of the bud, is killed first on exposure to cold (see page 10), it is very probable that this may be an important region for comparison. A comparison of cell sizes of Crawford's Late and Bell's October in this particular region (region 2 - see figure 1, Plate I) shows that Bell's October, the more hardy variety, is the smaller celled. A comparison of Oldmixon Free and Ringold show the former, the less hardy variety, to be the smaller celled.

From the present data no relationship between the average size of cells and hardiness is established, although such relationship is indicated, since Bell's October and Ringold with the smaller celled styles are the most hardy.

Size of Cells of More Advanced Buds from Georgia.

Examination of the pistils of buds from the Georgia Experiment Station, gathered March 8th, 1912, in which the pink color of the petals was becoming visible, showed a great development in length and a slight increase laterally. The growth had taken place almost wholly in the style near its center. The

sections showed that many cells were in process of division both laterally and transversely when killed. The development of the pistil and its cells, is in proportion to the development of the bud, irrespective of the variety.

The tissue of the style in the winter buds gathered in November in the Missouri Agricultural Experiment Station orchard was a loose shapeless mass of small cells averaging about 8 microns in thickness (see data on cell sizes, also Plat I). In the buds from Georgia the corresponding cells were much larger, averaging 16 to 25 microns in length by 10 to 14 microns in thickness, and were arranged in a more or less regular columnar order. The cells in the base of the pistil, the ovary, were still irregular in shape and arrangement, but were seemingly in a state of rapid division. They had not increased much in size.

of those buds frozen in the laboratory at -10° C., as given in the data on page 10, the styles were killed first, the stigmas second, and the ovaries third. This was in order of the size of their cells, but the size of the cell was probably not a great factor as it was also in order of activity of growth which is known to be an important factor in determining the killing point of a tissue. Death, in all cases in which the style only was killed, occurred in the most rapidly growing portions.

Surrounding cells of large size, petal-cells, which were more exposed to the surrounding cold air, and which were also in a state of rapid division, but which possessed thicker cell walls, did not die from the effects of the cold. It is probable that concentration of cell sap is a very important factor in determining the cell hardiness.

Data-showing retalive hardiness of different regions of the pistil in started buds.				
No. Buds. Style only, Itilled stigmoustyle killed Pistil completly dead.				
//	24	40,		

Total from 3 freezes temp to-10°C In4 hours at-10° I hour.

(2) Thickness of Cell Walls and Its Relation to Hardiness.

There is a marked relation existing between the thickness of cell walls in a tissue and the death point of that tissue. Wiegand publishes data which bears this out. Thick walled cells of seven species of woody plants did not form ice at -18° C.

Observations on six varieties of peach buds showed that the variation and limits of cell-wall thickness was remarkably constant in all of the varieties studied. The range in thickness of wall in each region was similar and nearly equal in the six varieties. The range of each important region is as follows:

Style .5 to 1 micron

Stigma 1 to 2 microns

Ovary 1 to 2 "

Petals 1 to 2.5 "

Scales 2 to 4 "

The relation of thickness of cell wall to hardiness is easily seen when this is compared with the data on page 10. The style is killed first, the stigma second, ovary third, petals (from previous years' observation) fourth, and the scales, the most exposed portion of the bud, withstood the severely cold weather of January 1912, at Columbia, Missouri, when all other parts of the bud were killed. As in Wiegand's work, mentioned above, the concentration of cell sap in these different regions must play an important part in determining relative hardiness, though it seems insufficient to account for the great differences observed.

¹Wiegand, "Buds and Twigs in Winter", Bot. Gaz., June 1906.

We may conclude therefore that the death point, which is necessarily below the freezing point, (when cold is the immediate cause of death) tends to vary with the thickness of the cell walls, in plant tissue.

(3) Relation of Hardiness of Various Regions to Hardiness of the Bud as a Whole.

Some parts of the bud are so related to one another that the death of one of them may cause death of the entire bud. Sections of freshly killed buds in the orchard in January 1912 showed that the buds were entirely killed, with the exception of the bud scales, and the dead tissue extended down the vascular tissue of the stem which, in section, was in the form of a fish tail. (See Plate II). It is obvious that with death of the vascular tissue, complete death of the bud must follow. This was the case with the bud scales which survived the cold weather in the orchard. All of them died in the few weeks that followed.

exceptional conditions in the orchard while at the same time the other parts were unharmed and the fruit developed normally. How, ever, where the pistil alone was killed, which is often the case, the flowers may bear both petals and pollen, but the flower cannot produce fruit. The functional death point at least is that of the style, which is the portion of the pistil most easily killed. The death point of a bud is the death point of the most easily killed essential part. The functional death point of a bud is the death point of a bud is the death point of a bud is

(4) Relation of Bud Scales to Hardiness.

Upon casual thought it would seem that insulation of the essential flower parts from cold by the surrounding flower parts and bud scales is an important factor affecting the hardi-Some recent work by Chandler throws some importness of buds. The results given below, from experant light on this subject. iments conducted in the winter of 1909-10, however, are quite the reverse from what was expected. There was a difference in the hardiness of buds, when exposed in a cold chamber, dependent upon whether their scales were left intact or whether their scales had previously been removed. Those buds having the scales removed withstood the low temperature better than those buds remaining intact.

The following data is by Mr. Chandler.

Chandler, W. H., Missouri Agricultural Experiment Station, data unpublished.

		N	
DATA-WHichandler regarding	INSULATION	N By Bud S	icales,
Old Mixon Free.	No. Buds	No Killed	of Killed
Scales removed	100.	20.	20 %.
" not "	87.	65.	74.7
Scales removed.	80,	5.	13.8
" not " ,	137,	19.	13.8
Dec. 6 1909.			The same of the sa
	No Buds	No Killed	of Killed
All scales off.	50.	20.	40
Part " ".	310.	164.	49.6
All scales on.	200.	Q70.	93,/
		,	
Gen. Lee.	No Buds	No Willed	% Killed
Scales not removed	144.	75.	52.
"	149	56.	47.8
" removed	160.	106.	63,6
Bells October	No. Buds.	NoKilled	of Killed
Scales on	195.	79	40,5

These experiments were made in a moist atmosphere. From these data we must conclude that the presence of bud scales in a moist atmosphere is no protection to the life of the bud, and, moreover, that bud scales are negative in their insulating However, under conditions in which the presence or absence of moisture is a factor, we have a relationship indirectly to the life of the bud and bud tissues. In February 1912 all the fruit buds in the orchard were dead with the exception of the protected bud scales. The outermost scales were dead, and the inner ones were killed to just below the line of contact with the next outer scale. (See Plate II). The scales entirely protected were alive and remained alive for several weeks. Therefore since heat insulation is not a factor, protection is secured through the effect of the scales in retaining moisture. in experimenting with buds of Pinus laricio. Aesculus hippocastanum, and Syringa vulgaris in which he removed the scales from buds and exposed them along with entire buds, found that those buds with the scales removed, lost over 30% of their water content, against a loss of less than 3% by the entire buds. The foregoing would also strongly indicate the moisture retaining power of the bud scales.

EXTRANEOUS NOTES AND OBSERVATIONS.

(1) The entire bud, in case of Elberta was killed in January 1912. Scales of other varieties remained alive. (2) In some cases bud scales remained alive where wood below was dead and shrivelled. (3) Live buds were observed on some girdled branches after all others were killed.

¹Wiegand, Bot. Gaz. June 1906, p. 41.

SUMMARY.

- 1. The purpose of this work is to determine the relationship between the physical structure of peach buds and their hardiness.
- 2. Most of the work done on the killing of plant tissues by exposure to cold has been from a chemical or physico-chemical point of view and this chiefly in respect to osmotic strength of sap.
- 3. Ordinary methods of killing, fixing and staining were followed. The methods followed were the ones used by Drinkard but with the substitution of Bismarck brown stain for Delafield's haematoxylin.
- 4. Points of structure studied were: (1) Relation of cell size to hardiness; (2) Thickness of cell walls; (3) Insulating effect of scales; (4) Relation of death of a part of a bud to the death of the bud as a whole.
- 5. In unopened buds in advanced stages of growth the pistil was the least hardy, the style being the least hardy portion of the pistil.

conclusions.

- 1. A relationship between cell size and hardiness is indicated though not proven.
- 2. Thickness of cell walls showed a definite relation to killing points of tissues. The thin walled cells killed first and the thickest last.

Drinkard, A. W., "Fruit Bud Formation and Development." Annual Report Virginia Polytechnic Institute 1909-10.

- 3. Bud scales have no beneficial effect on preventing loss of heat, but they do exercise the important function of conserving moisture.
- 4. Death of special parts may bring about death of the entire bud. Death of any part of the pistil is the functional death of the bud.

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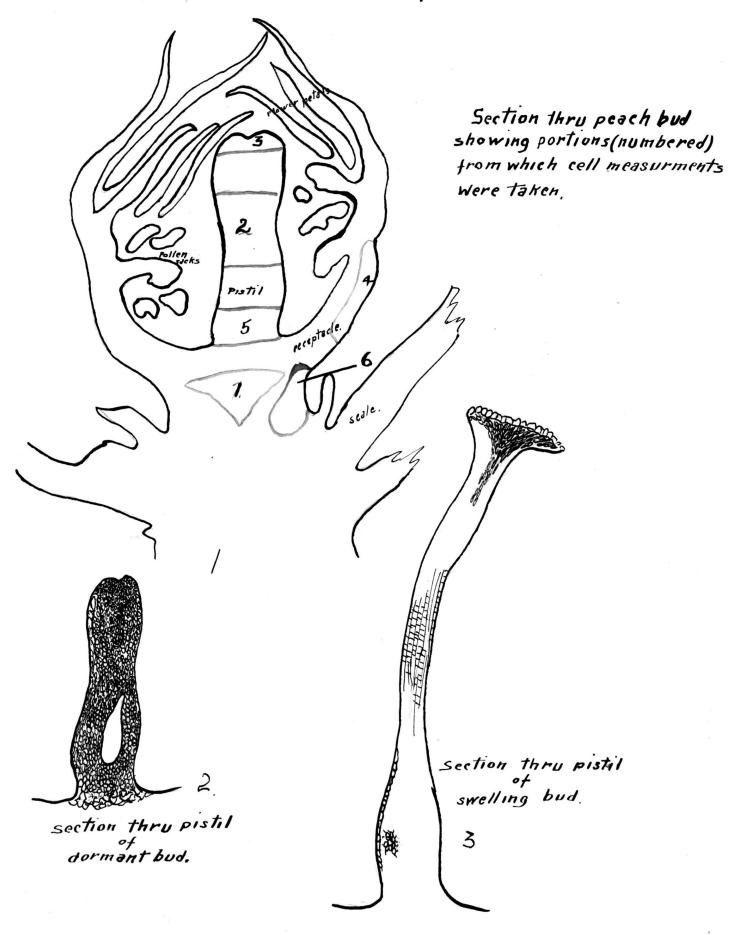
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1 Ed., Part II, p. 73

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Botanical Gazette, June 1906.



Section thru peach bud Feb. 28 12

showing portion of buds killed by

severecold of Jan + Feb 1912

shaded portions dead.



semi-diagramatic



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