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Correlation of Chemical Composition With Hardiness in Brambles

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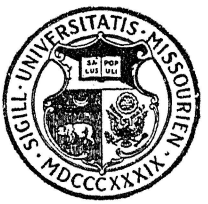

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Correlation of Chemical Composition With Hardiness in Brambles

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Abstact.—Determinations were made of percentages of moisture, bound water, pectin, total nitrogen, protein nitrogen, and pentosans in the bark of brambles. Observed hardiness was correlated with percentages of bound water, as determined with the dilatometer. A correlation coefficient of 0.67 ± 0.06 existed between percentages of bound water and pectin. A correlation coefficient of 0.60 ± 0.07 existed between percentages of protein nitrogen and bound water. A fall cover crop of oats with an application of sodium nitrate produced the greatest degree of hardiness found in blackberry canes. A fall cover crop of oats materially increased the hardiness of raspberry canes. The removal of the first two crops of young shoots from the Cuthbert raspberry caused a significant increase in hardiness.

The hardiness problem is one of vital interest to every fruit gower. The northward distribution of many of our most important horticultural crops is limited by low temperatures, yet damage by freezing is not limited to the colder fruit growing regions. It is a problem as important in temperate regions for less hardy fruits and in the sub-tropics in relation to tender species as it is in relation to the hardier species of the northern fruit-growing regions. The extremes of a single night in the more temperate regions may cause greater damage than a long period of comparable temperature in a naturally colder region. Likewise, injury from freezing is an important problem of the citrus grower of the subtropics, for a whole crop may be destroyed in a single night of exposure to a temperature which would be considered only moderately low in a region of more hardy fruits. It is quite evident then that hardiness is an important problem wherever fruits are grown, with the exception of the tropics. Therefore, it is not strange that for over one hundred years a great deal of attention has been devoted to the general problem of cold resistance in plants with special attention to the process by which plant tissues are killed on exposure to low temperatures, and to the factors determining the resistance of hardy species to cold. Although a vast amount of information on the various phases of the subject has been accumulated, the problem is as yet unsettled.

The present investigation was undertaken with the purpose of adding, if possible, another link in the chain of evidence showing why some plants are more hardy than others, what chemical constituent, if

*This publication was prepared by Richard V. Lott, a graduate student in the Department of Horticulture, year 1925-26, and presents the information given in his dissertation submitted in partial fulfillment of the requirements for the Degree of Master of Arts, University of Missouri.

any, is associated with hardiness, and to what extent hardiness can be increased in horticultural plants by cultural treatments.

HISTORY AND PLAN OF THE EXPERIMENT

Excellent reviews of the voluminous literature on the hardiness problem have been made by Abbe [1], Blackman [4], Chandler [6], Rosa [20], and Newton [16]. This paper will include only the history of the work done at this Station and a short review of important recent contributions.

The first important work at the Missouri Station was done by Chandler[6]. This work, begun during the season of 1904-05, concerned the effect of certain cultural methods on the hardiness of the fruit buds of the peach. In 1908 Chandler started a series of experiments to determine whether an increase in the sap density of plants lowered the temperature at which death results from cold exposure. This work was extended from year to year and the results published in 1913. Resistance to low temperatures was correlated with sap density of the tissues, but the freezing point depression from the increased sap density was in no case large enough to account for the extreme hardiness of the tissues of some plants.

Researches on the hardiness problem were continued by Rosa[19] and Hooker[12]. Rosa found that hardiness to cold may be developed by treatments that check vegetative growth. Other changes accompanying the checking process were an increase in percentage of dry matter, greater depression of the freezing point of the sap and an accumulation of sugar, starch and polysaccharides.

Hooker found a correlation between hardiness and pentosan content. He suggested that due to the colloidal state pentosans hold water in an absorbed or colloidal condition. Hardy plants having less free water but more colliodal water are, therefore, able to withstand the desiccating effect of extreme cold.

Rosa[20] in extensive investigations with vegetable plants found that cabbage hardened by various treatments contained a larger amount of "unfree" or not easily frozen water, as measured by the dilatometer. The increment corresponded to the extent by which growth was checked and both of these paralleled the degree of cold resistance. The actual amount of water remaining unfrozen at any given temperature was greater in hardened than in tender leaves, although the total moisture content was less. Hardened cabbage plants had a lower rate of transpiration and a slower rate of water-loss on drying, which together with the smaller amount of water lost during ice formation was taken as an indication that hardening developed an increased water-retaining capacity. The total pentosan content was greater in hardened than in ten-

der plants regardless of the kind of hardening treatment. In plants possessing potential hardiness, such as cabbage, kale, and lettuce, the fraction of the pentosan content soluble in hot water was larger than in tomato, eggplant and sweet potato, which do not possess potential hardiness. Rosa considered the water-soluble pentosan content to represent more nearly the amount of pentosan in the protoplasm and that this might function more specifically as water-retaining material. In the group of plants susceptible to considerable hardening the increase in total pentosan content upon hardening was largely an increase in the hot water soluble fraction while in the tomato the hot water soluble fraction did not increase on subjecting the plants to hardening treatments.

Newton[16] in a study of winter wheat varieties found that hardened tissue retained its water content against great force, since no appreciable amount of sap could be expressed at 400 atmospheres pressure, even after severe preliminary freezing, from tissue containing about 70 per cent of moisture. All varieties increased in amino-nitrogen and water-soluble nitrogen during the hardening process. The hardiest variety had the largest content of water-soluble nitrogen, but the relation was not uniform throughout the series.

In a continued investigation of the same problem Newton [17] found that the imbibition pressure of fresh leaves in the winter-hardened condition was in most cases directly related to hardiness and appeared to depend on the physical state of the cell colloids characteristic of living tissue, since this property was lost when the tissues were killed. The quantity of hydrophilic colloids contained in the press juice of hardened tissues was found to be directly proportional to hardiness. Newton states that "it appears probable that hardy varieties accumulate, during the hardening process, more protein in the form of cell colloids." The ratio of amino-nitrogen to total nitrogen increased in all varieties in late fall indicating an association of protein splitting with the later stages of the hardening process. He found some correlation between sugar content of hardened leaves and hardiness.

Briefly, the present status of the hardiness problem is somewhat as follows: Death from freezing is due to water loss from the individual cell beyond the point necessary to sustain life. Hardiness is in direct relation to the amount of "bound" or not easily freezable water present. The amount of bound water is correlated with the amount of hydrophilous colloids present in plant tissues capable of being hardened. What the specific colloid may be is still an unsettled question.

THE PROBLEM

The purpose of the present investigation was to determine whether there is any correlation between hardiness and one or more of the naturally occurring plant colliods. An effort was also made to determine whether hardiness can be increased in brambles by cultural methods and treatments.

Determinations were made of pectin, protein and pentosans since they are the commonly occurring hydrophilous colloids present in plant tissues. "Plant tissue which withstands freezing must be supposed to contain or be able to manufacture substances which will hold water in an adsorbed or colloidal condition. These substances must themselves be colloids, they must have a great water-holding and water-absorbing capacity, they must be known to occur in practically all plants capable of withstanding winter conditions, and they must be distributed generally through practically all plant tissues".[9] The three colloids mentioned above are the only one that seem to fit these conditions, and for that reason determinations were made to find whether any correlation existed between any one of them and hardiness.

The investigation was carried out mainly with blackberries, but the red and black raspberry, gooseberry and currant were used to a lesser extent.

For the experiment with blackberries four plots were selected, each containing one row of Early Harvest and one of Snyder blackberries with a buffer row of dewberries between. These blackberries were growing on a gently rolling hillside on one of the poorer types of loess soil on the University Fruit Farm located near Turner Station, Missouri. They received the ordinary summer cultivation and care. On August 25 the four plots were cultivated for the last time, with a five-shovel one-horse plow, and a cover crop of oats sown on two of the plots, at the rate of three bushels per acre. The other two plots were left bare. On September 19 an application of sodium nitrate was put on one of the cover crop plots and on one of the bare plots at the rate of 225 pounds per acre. The cover crop at that time was about five inches high with a well developed root system.

The object of this experiment was to determine whether hardiness can be increased by the use of cover crops and the effect of a fall application of a quickly available nitrogenous fertilizer both on the cover crop and on the hardiness of the plant studied.

The four plots were numbered as follows:

Plot 1. Cover crop without sodium nitrate.
Plot 2. Cover crop with sodium nitrate.
Plot 3. Ordinary cultivation with sodium nitrate.
Plot 4. Ordinary cultivation without sodium nitrate.

The first samples were taken on November 17. The weather conditions previous to that time had been unfavorable for the development of hardiness. Vigorous laterals, representative of the whole plot, were taken for samples.

The next samples were taken on December 19. At that time there had been more opportunity for hardiness to develop. There was no apparent winter injury at that time except occasional dead streaks in the bark. The samples were collected as before: only vigorous laterals comparable to those of the first samples were taken.

The last samples from the four plots were taken on February 25. They were left until this time in order to secure the maximum development of the hydrophilous colloids present, since they have been found to be increased by exposure to cold. There was very little winter killing apparent at this time, since there had been no extremely cold weather, the lowest temperature recorded being –3° F. on December 27. Occasional dead streaks were found through the bark, but very few laterals or canes were killed outright, and those that were killed were the weaker, stunted ones.

No samples were taken between December 19 and February 25 on account of the scarcity of comparable material. No samples were taken from the early Harvest rows after the first sampling due to a lack of enough material comparable to that of the Snyder rows.

At the University Fruit Farm at Turner Station, Missouri, an experiment was started with raspberries which were growing on a location similar to that on which the blackberries were growing. Two plots were selected, each containing one row each of Cuthbert and Cardinal raspberries. The two plots were cultivated the last time on August 25 and a cover crop of oats sown at the rate of 3 bushels per acre on one of the plots. The other plot was left bare.

No samples were taken from these plots until December 7 in order to allow them to develop as much hardiness as possible. At that time many of the laterals were killed, and only the vigorous laterals that had survived were used for sampling. No further samples were taken from these plots due to the fact that the cold weather of late December killed so many of the canes that there were not enough left for comparable samples.

Another experiment with raspberries was conducted at the University orchard at Columbia. One plot of one row each of Cumberland and Cuthbert raspberries had the young shoots removed on May 16 when they had reached the height of 8 to 10 inches. Shoots of the second growth were removed two weeks later when they had reached the same height. The third crop of shoots was let grow and was given the ordinary summer

cultivation and care. A similar plot containing one row each of Cumberland, Cuthbert, and Kansas raspberries received the standard care in regard to the young shoots and was cultivated similarly to the other plot. The object of this experiment was to determine whether the later crops of young raspberry shoots are naturally more or less hardy than the first crop.

Samples were taken from these two plots on December 10 and February 18. Vigorous laterals were used for sampling material. No more samples were taken on account of scarcity of suitable material. At the time of the first sampling the tips of a few laterals were killed and occasional dead patches were found in the bark. When the last samples were taken, a few laterals and weaker canes were found to have been killed.

It will be seen that throughout these investigations the material taken for all samples was the more vigorous, hardy material. For this reason the differences in hardiness and chemical composition found in the data may not be as great as might be expected.

METHODS OF ANALYSIS

In this work the following percentage determinations were made: freezable water, total moisture, dry matter, total nitrogen, pectin, and pentosans.

In all cases vigorous laterals were used for samples. They were brought immediately to the laboratory and the bark scraped off for analysis. The entire woody stem was not used since it was found that killing usually occurred first in the bark.

The dilatometer method as described by Bouyoucos[5] and Rosa[20] was used to determine the amount of freezable water present in the different tissues. This quantity subtracted from the total amount gives the quantity of unfreezable water in the sample at the temperature of the experiment. The quantity of unfreezable water is an index of the hardiness of the tissue in question and also amounts to an indirect measurement of the sum of the osmotic and imbibitional pressures of the plant cells. That the percentage of bound water present in the tissue is a reliable index of hardiness has been demonstrated beyond doubt by the recent investigators of the hardiness problem. This has been mentioned in the review of the problem.

The dilatometer method is undoubtedly a very satisfactory means of measuring hardiness since the tissue fluids are studied under as nearly natural conditions as possible. When the plant fluids are extracted for study, there is always the possibility that the specific substances which we desire to study may be left in the residual tissue or that at best only a part of them may be extracted. By the dilatometer method all the

tissue fluids are subjected to a low temperature when in a condition almost identical with that in which they occur in nature.

Freezable Water.—A 10-gram sample of the green bark freshly scraped off was placed in the bowl of a 75-c.c. dilatometer, the dilatometer filled with petroleum ether (boiling point 40-60° C.) all air bubbles excluded and the bowl tightly stoppered with a rubber stopper. A thermometer and a 25-c.c. burette with a glass stopcock were inserted through the stopper, the thermometer so placed that the bulb was in contact with the plant tissue and the burette used as a reservoir from which to add more petroleum ether as the amount in the side arm of the dilatometer fell, due to contraction on cooling. After being stoppered the dilatometer was placed in an ice and salt bath, mixed in such proportions that the temperature was slightly lower than that desired in the dilatometer. The temperature of the plant tissue in the dilatometer was usually one or two degrees higher than that in the bath outside. A temperature of −6° C. for the contents of the dilatometer was used throughout the investigation. As the contents of the dilatometer cooled the column of petroleum ether in the graduated side arm of the dilatometer fell rapidly and more was added from the burette reservoir in order to have the ether column at a point where it could be read easily. When the contents of the dilatometer had been at the desired temperature for several minutes and the column of petroleum ether in the side arm had become stationary its height was read and the time recorded. As the water in the sample froze the column of petroleum ether rose in the graduated side tube, due to the expansion incident to freezing. The height of the column was read again at the end of one hour and the difference from the initial reading taken as the expansion due to the freezing of the water in the sample The side tube on the dilatometers used was graduated to one-hundredth of a cubic centimeter. The expansion from freezing multiplied by 10 gives the number of cubic centimeters of water frozen in the sample, since one gram of water increases approximately one-tenth of its volume on freezing. The water content of a comparable sample was determined (see moisture) and the percentage of total water frozen calculated by dividing the number of c.c. of ice formed by the total water content of the sample.

Moisture.—A tared 150-c.c. wide-mouth bottle was filled with the scraped off bark and dried to constant weight in an electric oven at 65° C. and the percentages of moisture and dry matter calculated.

The dry sample was ground in a small corn mill until it would pass through a 60-mesh sieve and saved for the determinations of total nitrogen and pentosans.

Total Nitrogen.—One gram of the dry powder was used to determine nitrogen by the Kjeldahl-Gunning-Arnold method.

Protein: Protein was determined by a slight variation of the method described by Loomis[13]. A ten gram sample of the bark (phloem and cambium) was scraped off and ground as finely as possible in a food chopper. It was then placed in a beaker, covered with 75 c.c. of 95 per cent alcohol and let stand for 3 hours. It was then transferred to a filter paper and washed with 55 per cent alcohol until the volume of the filtrate and washings amounted to 250 c.c. The sample was then dried to constant weight in an electric oven at 65° C. and ground to pass through a 60-mesh sieve. A nitrogen determination was then made by the Kjeldahl-Gunning-Arnold method.

The protein is precipitated by standing in the 95 per cent alcohol and the alcohol soluble nitrogenous compounds as well as other alcohol soluble materials are then washed out by the 55 per cent alcohol.

Pectin: The method of Ahmann[3] was used to determine pectin. A 20-gram sample of the freshly scraped off bark was ground as finely as possible through a food chopper, placed in a one liter Erlenmeyer flask and about 500 c.c. of triply distilled CO_2-free water added. The pectin was extracted by the following method: The flasks containing the samples were placed in a water bath at 90° C. for 12 hours, the supernatant liquid decanted, more water added and the process repeated, making four extractions in all. After the last extraction the sample was placed on a Buchner filter, washed thoroughly and the residue discarded. The extract as soon as extracted was placed in a 2200-c.c. evaporating dish and evaporated to a fairly concentrated solution. Each extraction was added in succession as extracted, evaporation was continued and the whole eventually concentrated to about 800 c.c. The extract was then transferred to a one liter volumetric flask, previously steamed out to exclude CO_2, made up to volume and sealed. Each flask was fitted with a soda lime tube to exclude CO_2 and a siphon to withdraw the solution.

A 100-c.c. aliquot was siphoned off and pipetted into a 250-c.c. volumetric flask. Into this was pipetted 100 c.c. of an approximately 0.2 normal NaOH solution free from CO_2, and the whole made up to volume. Each flask was fitted with a rubber stopper and a soda lime tube to exclude carbon dioxide. The flasks were then placed in a water bath at 55° C. for 12 hours for saponification. A 50-c.c. aliquot was then pipetted into a beaker, a few drops of phenolphthalein added and titration made to the end point with HCl about one-fourth as concentrated as the NaOH solution. The amount of pectin present was calculated from the amount of NaOH used up (combined with the pectin). A blank determination on the NaOH solution was made with each series of four flasks.

Pentosans.—A modification of the method developed by Abbott[2] was used in the determination of pentosans. Five grams of the dry sample from the moisture determination were hydrolyzed for 3 hours in 100 c.c. of 1 per cent HCl under reflux condensers. The sample was then filtered, neutralized with NaOH and made up to 250 c.c. Two 25-c.c. aliquots were taken from this for total reducing power of the solution. Reducing power in all analyses was determined by the Shaffer and Hartmann method[21]. A 100-c.c. aliquot was pipetted off, one-ninth cake of Fleischman's yeast added, made up to a PH of 4.9 with acetic acid, and fermented for 8 hours at 35° C. A 50-c.c. aliquot was made up to a PH of 6.7, one-ninth cake of Fleischman's yeast added and allowed to ferment at 35° C. for four days. In each case the reducing power was then measured by the Shaffer and Hartmann method.

The 8 hour fermentation removes the hexose sugars. The four-day fermentation ferments off the pentose sugars as well. Then the total reducing power minus the reducing power left after fermenting the hexose sugars gives the reducing power of the hexose sugars. The total reducing power minus the reducing power left after the four-day fermentation gives the reducing power of the total sugars. The reducing power after the 8-hour fermentation minus the reducing power after the four-day fermentation gives the reducing power of the pentose sugars as determined.

The Shaffer and Hartmann method of sugar analysis used was as follows: A 25-c.c. aliquot was pipetted into a 300-c.c. Erlenmeyer flask and 25 c.c. each of Fehling's solution A and Fehling's solution B added. It was then brought to a boil in four minutes and boiled for two minutes, cooled to 40°C., exactly 25 c.c. of a KI-KIO_3 solution added, 20 c.c. of N/5 H_2SO_4 added at once and the flask rapidly rotated, 22 c.c. of a saturated solution of K_2CrO_4 added, and the whole titrated to the end point of sapphire blue with N/10 $Na_2S_2O_3$.

PRESENTATION AND DISCUSSION OF DATA

The results of the investigation are presented in Tables 1 and 2 and in Figures 1 to 3. Figure 4 shows the mean daily temperature from September 1, 1925 to March 1, 1926.

It was noticed throughout this investigation that there was a close correlation of the amount of actual killing observed in the field with the percentage of freezable water later determined with the dilatometer in the laboratory.

TABLE 1.—SAMPLES ANALYZED

No. of Sample	Date Collected	Series	Kind of Sample	Treatment
1	11/17/25	I a	Snyder	Plot 1. Cover crop without $NaNO_3$
2	11/17/25	I a	Snyder	Plot 2. Cover crop with $NaNO_3$
3	11/17/25	I a	Snyder	Plot 3. Ordinary cultivation with $NaNO_3$
4	11/17/25	I a	Snyder	Plot 4. Ordinary cultivation without $NaNO_3$
5	11/17/25	I a	Early Harvest	Plot 1. Cover crop without $NaNO_3$
6	11/17/25	I a	Early Harvest	Plot 2. Cover crop with $NaNO_3$
7	11/17/25	I a	Early Harvest	Plot 3. Ordinary cultivation with $NaNO_3$
8	11/17/25	I a	Early Harvest	Plot 4. Ordinary cultivation without $NaNO_3$
9	12/7/25	II	Cuthbert	No cover crop
10	12/7/25	II	Cardinal	No cover crop
11	12/7/25	II	Cuthbert	Cover crop
12	12/7/25	II	Cardinal	Cover crop
13	12/10/25	III a	Cuthbert	Young shoots removed
14	12/10/25	III a	Cumberland	Young shoots removed
15	12/10/25	III a	Cuthbert	Ordinary cultivation only
16	12/10/25	III a	Cumberland	Ordinary cultivation only
17	12/10/25	III a	Kansas	Ordinary cultivation only
18	12/19/25	I b	Snyder	Plot 1. Cover crop without $NaNO_3$
19	12/19/25	I b	Snyder	Plot 2. Cover crop with $NaNO_3$
20	12/19/25	I b	Snyder	Plot 3. Ordinary cultivation with $NaNO_3$
21	12/19/25	I b	Snyder	Plot 4. Ordinary cultivation without $NaNO_3$
22	2/18/26	III b	Cuthbert	Young shoots removed
23	2/18/26	III b	Cumberland	Young shoots removed
24	2/18/26	III b	Cuthbert	Ordinary cultivation only
25	2/18/26	III b	Cumberland	Ordinary cultivation only
26	2/18/26	III b	Kansas	Ordinary cultivation only
27	2/25/26	I c	Snyder	Plot 1. Cover crop without $NaNO_3$
28	2/25/26	I c	Snyder	Plot 2. Cover crop with $NaNO_3$
29	2/25/26	I c	Snyder	Plot 3. Ordinary cultivation with $NaNO_3$
30	2/25/26	I c	Snyder	Plot 4. Ordinary cultivation without $NaNO_3$
31	2/24/26	IV	One yr. currant	Ordinary cultivation only
32	2/24/26	IV	Two yr. currant	Ordinary cultivation only
33	2/24/26	IV	One yr. gooseberry	Ordinary cultivation only
34	2/24/26	IV	Two yr. gooseberry	Ordinary cultivation only

The data for the blackberry plots show that in all cases the percentage of bound water was highest in the samples from plot Number 2, which had a cover crop with an application of sodium nitrate. The canes in this plot showed less killing than those of the other plots. However, there was very little difference in degree of killing in the different plots, due no doubt to the relatively mild winter temperatures to which the canes were subjected. It is probable that had the plots been subjected to extremely low winter temperatures there would have

been a much greater difference in the degree of killing in the different plots.

The raspberry plots showed very clearly a correlation of winter killing with percentage of bound water.

TABLE 2.—ANALYSES OF SAMPLES IN PERCENTAGES OF DRY WEIGHT

No. of Sample	Moisture	Bound Water	Pectin		Total Nitrogen	Protein Nitrogen	Reducing Substances	Total Sugars	Hexose Sugars	Pentose Sugars
			Fresh	Dry						
1	36.2	61.33	2.80	4.39	.94	.80	20.54	15.66	7.73	9.20
2	34.7	66.86	3.19	4.88	.75	.74	20.36	15.56	6.70	9.10
3	38.0	55.26	2.66	4.29	.75	.73	19.22	14.56	5.54	9.20
4	36.4	62.91	2.88	4.53	.89	.77	20.82	18.74	9.03	10.00
5	29.6	69.55	2.55	3.62	.97	.85	20.26	15.66	7.18	9.56
6	27.4	76.26	2.77	3.82	.98	.96	19.60	13.36	7.76	6.00
7	24.7	55.47	2.44	3.24	.95	.94	18.18	15.56	6.51	9.20
8	23.0	56.52	2.52	3.27	.88	.87	19.02	17.14	6.22	11.08
9	49.7	59.76	1.23	2.45	1.19	.99	21.68	18.18	6.73	12.36
10	34.6	55.16	1.54	2.35	.93	.86	21.50	17.62	5.13	13.00
11	51.2	67.77	1.29	2.64	1.19	1.02	22.36	18.18	5.66	13.00
12	38.0	68.42	1.79	2.89	1.20	1.00	22.16	17.70	6.92	11.54
13	46.0	59.78	1.60	2.96	1.30	1.20	21.78	18.36	5.73	13.08
14	51.1	70.65	1.57	3.21	1.16	.89	22.16	18.56	7.33	11.90
15	46.7	59.31	1.62	3.05	1.27	1.04	22.56	18.56	5.36	13.72
16	48.9	75.46	1.68	3.28	1.27	1.11	21.20	17.62	5.85	12.08
17	46.0	67.39	1.71	3.16	1.14	1.09	21.78	17.80	6.79	11.26
18	35.5	60.56	2.60	4.03	.75	.74	19.60	15.38	4.48	11.08
19	33.6	70.24	2.76	4.15	.74	.66	19.40	14.28	3.71	10.64
20	33.9	44.12	2.29	3.46	.61	.56	20.54	15.94	5.93	10.18
21	32.0	50.00	2.24	3.29	.71	.62	20.20	14.78	4.85	10.32
22	46.7	78.59	1.85	3.47	1.35	1.18	21.98	17.98	9.50	9.92
23	44.8	84.38	2.07	3.75	1.18	1.07	20.06	15.94	5.16	10.90
24	35.8	63.69	2.07	3.22	1.19	1.17	21.30	16.40	5.82	10.64
25	43.4	75.81	2.13	3.75	1.07	1.03	19.68	16.12	5.05	11.08
26	37.6	74.79	1.76	3.37	1.18	1.05	20.54	16.78	6.31	10.64
27	38.3	63.45	2.13	3.45	.68	.57	19.60	16.40	4.31	12.18
28	32.0	68.75	2.40	3.53	.61	.58	19.78	16.78	3.34	13.44
29	31.9	59.25	1.79	2.64	.62	.61	20.44	15.56	5.54	10.28
30	37.1	67.65	2.13	3.38	.68	.61	21.02	16.78	5.06	11.72
31	64.7	79.91	1.74	4.93	1.68	1.01	18.84	15.02	1.33	13.73
32	66.4	87.20	1.88	5.60	2.20	1.71	18.18	14.74	1.16	13.54
33	52.9	79.21	1.90	4.03	1.24	.86	20.26	15.94	6.08	10.72
34	50.2	72.11	1.85	3.71	1.10	1.01	20.16	15.94	5.45	10.72

TABLE 3.—PERCENTAGES OF BOUND WATER IN SAMPLE CANES

No. of Sample	Kind of Sample	Treatment	Percentage Bound Water
9	Cuthbert	Ordinary cultivation	59.76
10	Cardinal	Ordinary cultivation	55.16
11	Cuthbert	Cover crop	67.77
12	Cardinal	Cover crop	68.42
22	Cuthbert	Young shoots removed	78.59
23	Cumberland	Young shoots removed	84.38
24	Cuthbert	No shoots removed	63.69
25	Cumberland	No shoots removed	75.81

Table 3 shows a much higher percentage of bound water in the cover crop plots than in those which had no cover crop. At the time the samples were taken there were more canes winter-killed on the plot without a cover crop than on the cover crop plot. The data of samples 22-25 show that the percentage of bound water was highest in the samples from the plots which had the young shoots removed. At the time these samples were taken (February 18) there were more winter-killed canes on the plot from which no young shoots were removed. Winter killing was especially severe with the Cuthbert variety, over 90 per cent of the canes eventually dying where no young shoots were removed; while not over 10 per cent were killed in the rows with young shoots removed. It is of interest to note that the highest percentage of bound water of the whole investigation was found in the currant which is naturally the most hardy of the tissues studied.

An inspection of the tables and figures reveals the fact that there is evidence of correlation between hardiness, as measured by the percentage of bound or unfree water determined by the dilatometer, and the percentages of pectin and protein. The correlation for pectin is somewhat better as shown by a correlation coefficient of 0.67 ± 0.06, compared to a correlation coefficient of 0.60 ± 0.07 for protein. It is possible that more than one hydrophilous colloid may be intimately associated with the condition of hardiness. In fact, it seems possible that the condition of cold resistance may be a function of the development in the plant of one or more sets of related colloidal compounds which are hydrophilous in character. This would explain a possible correlation between hardiness and both pectin and protein as the data indicate.

The data do not make evident any correlation between hardiness and total sugar or total pentosan content and show only a moderate degree of correlation with percentages of dry weight. The percentages of total nitrogen parallel fairly closely those of protein nitrogen but are divergent enough to show that a total nitrogen determination is not a reliable index of the amount of protein nitrogen present in these plant tissues.

The data and figures show indications of a close correlation between percentage of pectin and percentage of bound or not easily freezable water, which has been found to be a reliable index of hardiness. The pectin curve follows that of bound water somewhat more closely for blackberries (Fig. 1) than for raspberries (Fig. 2). However, in each case the pectin curve is of the same general trend as is that for bound water.

In general, there existed a moderate degree of correlation between percentages of protein nitrogen and percentages of bound water. The

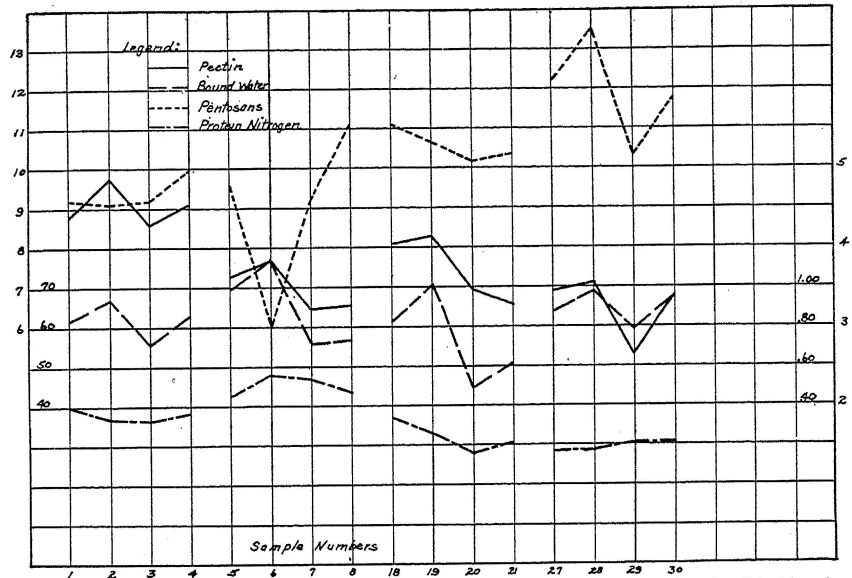

Fig. 1.—Percentages of Bound Water and Certain Hydrophilous Colloids in Blackberries.

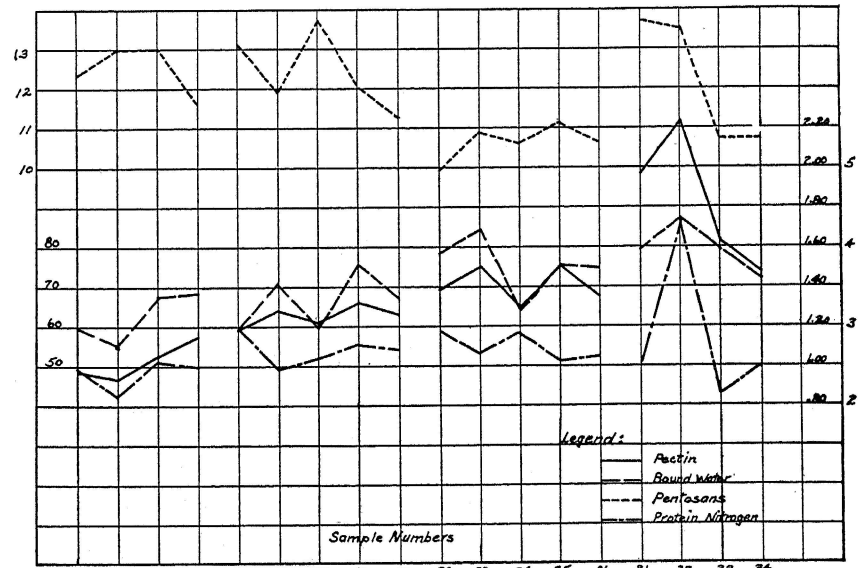

Fig. 2.—Percentages of Bound Water and Certain Hydrophilous Colloids in Raspberries.

degree of correlation was not as striking as that with pectin, but was much better than that of any of the other constituents determined.

In each group of blackberry samples the highest percentages of bound water and pectin were found in Plot 2, which had a cover crop with the addition of nitrate. In only one case, however, did the samples from this plot contain the highest percentage of either total nitrogen or protein nitrogen. The samples from this plot were higher in percentages of bound water and pectin than those from the plot which had a cover crop without nitrate. The third plot (ordinary cultivation with nitrate) was in all cases the least hardy as measured by the percentage of bound water It was, however, in some cases, very close to Plot 4 in hardiness.

From these data we may assume that a rank growing cover crop, such as oats, sown in late summer is effective in developing hardiness in blackberries, that a cover crop with the addition of sodium nitrate is somewhat more effective, and that the application of sodium nitrate without a cover crop has no advantage over ordinary cultivation.

The chief effect on hardiness of a fall application of sodium nitrate to plants of the blackberry type is apparently a stimulation of the growth of the cover crop. This more vigorous growth of the cover crop apparently takes up some of the soil moisture which would otherwise be taken up by the blackberry plants. This causes a greater drying out of the plants with a consequent increase in the development of hydrophilous colloids, which are very evidently directly associated with hardiness. What the subsequent effects of the nitrate application may be on yield and growth is uncertain, but from the hardiness standpoint alone it is doubtful if the increase is great enough to justify its application to blackberries, since a cover crop alone very probably causes the development of enough hardiness to withstand the average Missouri winter. However, more extended investigations may show its application with a cover crop to be very practicable for the more tender horticultural plants such as raspberries. The data give no evidence of the fate of the sodium nitrate. Certainly the percentages of protein nitrogen and total nitrogen were not consistently higher in the samples from the plo receiving nitrates. A general decrease from the first to the last sampling was noticed in total nitrogen.

The blackberry samples showed no seasonal development of pectin, in fact there was a falling off in the percentage present in most cases. The percentage of bound water showed no direct seasonal increase in the blackberry samples (Fig. 3). On the other hand, in the raspberry plots where a study of seasonal distribution could be made there was in all cases an increase in percentages of bound water and pectin. This may indicate that blackberries reach their maximum hardiness at an earlier date in the dormant season than raspberries. If such is the case the

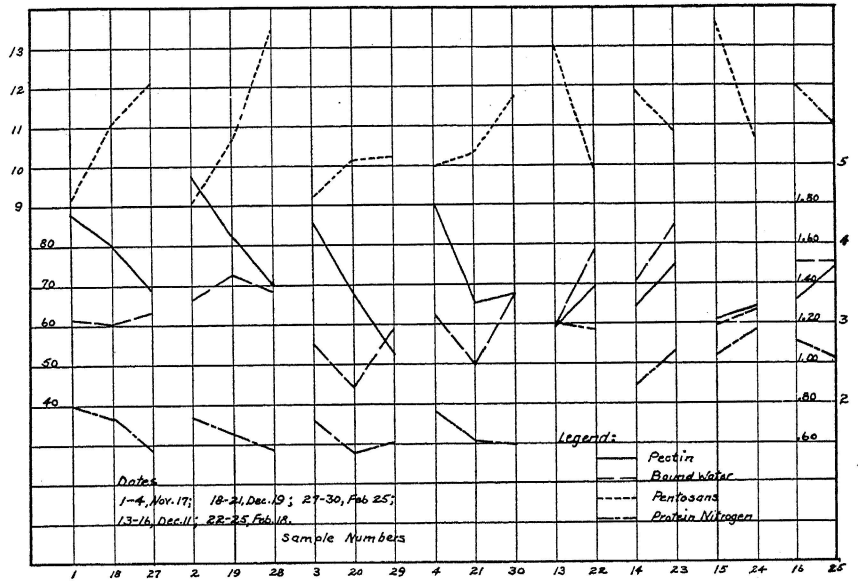

Fig. 3.—Seasonal Distribution of Bound Water and Certain Hydrophilous Colloids.

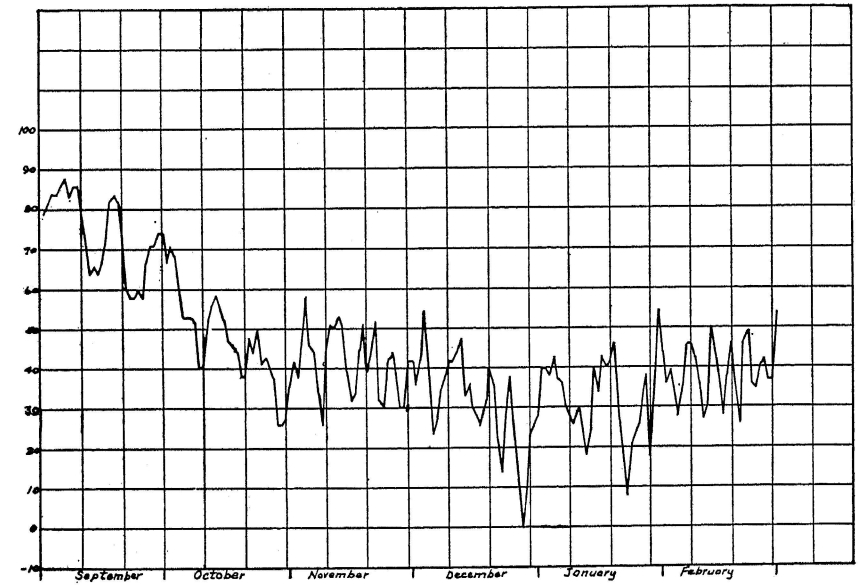

Fig. 4.—Mean Daily Temperature Sept. 1, 1925 to March 1, 1926 (Fahrenheit)

rather extensive killing of raspberry canes by low temperature in late fall and early winter is explained. On this hypothesis it seems quite possible that if the raspberry plants could, by some cultural treatment, be hardened earlier in the fall much of the winter killing could be avoided. That such hardiness can be developed is indicated by the data of Series II in which the tissues of the cover crop plot were materially hardier than those of the bare plot on December 7 (Fig. 2). Observation showed that there was less actual killing of canes on the cover crop plot than on the bare plot, and in addition it must be remembered that in all cases the tissues analyzed were those which had survived up to that time. Unfortunately there were not enough vigorous plants left in these plots for a seasonal study of hardiness. It is interesting to note that the percentage of protein nitrogen was higher in the two hardier samples (Nos. 11 and 12) of this series.

The percentages of pectin found in the last raspberry samples taken (Feb. 18) were in some cases as high as were several of those of the blackberry samples. This is not interpreted as showing that raspberries acquire as great a degree of hardiness as blackberries, but rather that some of the raspberry canes that live through the winter attain a considerable degree of hardiness. The data for the currant, which from observation is naturally the hardiest of the tissues studied, show the highest percentages of bound water and pectin found, and those samples were taken at a time when the buds had already begun to swell (Feb. 24). At that time it is quite possible that the amount of pectin had begun to decrease since the amount in the blackberry tissues had decreased appreciably. The data show that the highest percentage of protein nitrogen found in any sample was in the currant sample taken from two-year old wood, as were also the highest percentages of bound water and pectin. This further emphasizes the correlation of both pectin and protein with hardiness.

Of special interest was the large seasonal increase in percentages of bound water and pectin in the samples from the raspberry plots which had the first two crops of young shoots removed. The increase was very significant, especially with the Cuthbert, which is naturally the most tender variety in this section. There was practically no seasonal increase in the samples from the plots which had no young shoots removed. These results suggest a possible means of avoiding the widespread winter injury to this variety usually occurring in this latitude. It was observed that many of the Cuthbert plants, in the plot from which no young shoots were removed, died after the last samples were taken (Feb. 18). Death at this late date could not have been caused by exposure to low temperature, but must have been due to desiccation of the plant as a whole.

This drought effect was evident at the time of last sampling although relatively few canes were dead. On May 1 it was noticed that at least 90 per cent of these canes had died, while not over 10 per cent of the canes from the other plot (young shoots removed) were dead. These dead canes apparently suffered from conditions similar to those reported by Emerson[8], who found that canes coated with paraffin or covered with snow were uninjured, whereas exposed canes died apparently from drought or loss of water from the plant as a whole. No such drought effects were evident in the plants from which the first two crops of young shoots were removed. Very little seasonal development of protein in this series was shown in the analyses.

Chandler[7] mentions several observations on winter injury which might be interpreted to indicate that either nitrogen or carbohydrates favored the development of hardiness. He states that, during the severe winter of 1917-18, in New York State, in every instance observed where, during the summer of 1917, leaves were removed by insects, sprayburn, or other causes, killing of the wood during the following winter resulted. This may be taken to indicate that the lack of foliage with consequent decreased manufacture of carbohydrates, led to a low resistance to cold in the trees in question. On the other hand, it might be interpreted as showing that lack of leaves caused poor growth, with an accompanying weakened condition. Chandler further states that young peach trees, which always have a large leaf surface in proportion to the size of the trees, suffered much less injury than old trees. This evidence might be interpreted as favoring carbohydrates or nitrogen or both. He says that apparently the greater the amount of foliage in proportion to the amounts of wood to be hardened, the more rapidly the hardening process takes place.

Chandler cites instances in which trees that bore a heavy crop in 1917 were badly injured the following winter while adjacent trees of the same variety that bore little or no crop that year were uninjured. Chandler concludes that some material is elaborated in the leaves during late summer and autumn that renders the wood more resistant to low temperatures, but that when the tree is ripening a heavy crop so much of this material may go into the fruit that, if the season is not a favorable one, the wood may not reach maximum hardiness. Additional weight is given to this idea by the statement of Macoun[14] that in Canada, in general those varieties that mature their fruit earliest in the season are the hardiest. Chandler suggests that from this evidence, the material or materials in question may quite probably be carbohydrate derivatives, since large quantities of carbohydrates would be used by the fruit. But Murneek[15] has recently shown that in the tomato, at least, the

development of a large crop of fruit led to a marked decrease of vegetativeness of the plant with the development of conditions typical of nitrogen starvation. The percentage composition of nitrogen in the tomato fruit was always higher than in any other part of the same plant. In view of this information it is possible that the condition described by Chandler may have been caused by nitrogen starvation, with accompanying decreased hardiness. However, it is possible that pectin may be the constituent concerned in the instances mentioned by Chandler. This would be suggested by the data of the present investigation and from the fact that the hot-water soluble pentosans which Rosa[20] found to be associated with hardiness in vegetables would comprise compounds similar to the pectin determined in the present study since in both cases the hot-water soluble extract was determined. Heinicke[11] found no reduction in hardiness from a late summer application of nitrogen to the soil even though considerable quantities were taken up by the trees.

Regardless of what the specific chemical constituent or constituents may be that cause the development of hardiness, it is without doubt an hydrophilous colloid, or a combination of two or more hydrophilous colloids, which make it possible for the protoplasm to withstand water loss from freezing.

That the material in question may be pectin or protein or the two acting at the same time is suggested from the data of this investigation.

The effect of other factors, such as acidity, hydrogen-ion concentration, various salt mixtures, etc. on the water holding capacity of the colloids must also be considered. The water-holding capacity of plant tissues must not be considered the only factor in hardiness, even though it is undoubtedly very important and explains many of the observed facts relating to winter injury. The degree of acidity may be influenced by the condition of the pectin present in the tissue. Ahmann[3] found that apparently pectin has twelve carboxyl groups, with probably seven of them covered by methyl radicals. It is possible that the number of methyl groups changes during the season with a corresponding change in the acidity of the tissue. If the degree of methylation does change a different neutral equivalent should be determined for each group of samples. In the present investigation the same neutral equivalent was used throughout. A change in methylation may then in part account for the lack of seasonal development of pectin observed in some of the samples. Further investigations of this problem should include determinations of this phase of the subject.

SUMMARY

From the data obtained in this investigation the following conclusions were drawn:

1. There was a direct correlation between observed hardiness and the percentage of bound water as measured by the dilatometer method. This correlation held not only for different degrees of hardiness naturally exhibited by the several species but also for the various degrees of hardiness observed within each species.

2. In blackberry and raspberry plants a close degree of correlation existed between observed hardiness, the percentage of bound water in the tissue, and the percentage of pectin present. A less pronounced but moderate degree of correlation existed between hardiness as measured by the percentage of bound water and the percentage of protein nitrogen found in the tissues studied. Little or no correlation existed between hardiness and percentage of total sugar or of total pentosans and only a moderate degree of correlation with percentage of dry weight.

3. A late summer cover crop of oats with an early fall application of nitrate of soda resulted in the highest degree of hardiness produced by treatment. An early fall application of nitrate of soda without a cover crop did not increase hardiness above that of the cultivated plot without sodium nitrate. A late summer cover crop of oats alone induced a degree of hardiness less than that of the plot with cover crop and nitrate. From this we may conclude that the only advantage of a fall application of sodium nitrate in relation to hardiness is secured through the greater growth of the cover crop, which increases hardiness through a greater drying out of the fruit plant. A late summer cover crop of oats was very effective in increasing hardiness in raspberries.

4. There was a more marked seasonal increase in hardiness in those raspberry plots from which the first two crops of young shoots were removed than in those plots from which no young shoots were removed. This is corroborated by direct observation of Cuthbert, for at least 90 per cent of the canes eventually died in the latter plots and not over 10 per cent in the former. This suggests a possible means by which to avoid the widespread winter killing usually associated with this variety in Missouri.

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