

history. For comparison the following intra-racial (inter-individual) coefficients of variation for fecundity in other forms are tabled:

Constants of Variation in Fertility and Fecundity in Various Animals

Organism	Character	Coefficient of Variation	Authority
Poland-Chinaswine.	Size of litter	27.411	Surface ⁵
Duroc-Jersey swine.	Size of litter	25.997	Surface ⁵
Mouse	Size of litter	37.5	Weldon ⁶
Horse	Fecundity ⁷	24.771	Pearson ⁸
Man	Number of children	48.41	Powys ⁹
Domestic fowl.....	Annual egg production	34.21	Pearl and Surface ¹⁰

It is plain that the individual variability in "size of litter" shown by this ewe is of the same general order of magnitude as that found in other organisms for fecundity characters.

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THE INDUCTION OF NONASTRINGENCY IN PERSIMMONS AT SUPRANORMAL PRESSURES OF CARBON DIOXIDE

In a previous issue of this periodical,¹ I reported the results of some experiments to determine the relation of different pressures of carbon dioxide to the rate at which persimmons are rendered non-astringent by means of that gas. Two varieties were used in those experiments, *Taber 129* and *Hyakume*, as these are understood at the Alabama Experiment Station. I had already found that

⁵ *Biometrika*, Vol. VI., pp. 433-436, 1909.

⁶ *Biometrika*, Vol. V., pp. 442, 1907.

⁷ Fecundity in this case means the fraction which the actual number of offspring arising from a given number of coverings is of the possible number of offspring under the circumstances.

⁸ *Biometrika*, Vol. I., pp. 289-292, 1902. Actually only the moments of this fecundity curve are given at the place cited. From the moments we have calculated the coefficient of variation.

⁹ *Biometrika*, Vol. V., p. 251, 1905.

¹⁰ U. S. Dept. Agr., Bur. Anim. Ind., Bull. 110, Part I., pp. 1-80, 1909.

¹ Lloyd, F. E., "Carbon Dioxide at High Pressure and the Artificial Ripening of Persimmons," *SCIENCE*, N. S., 34: 924-928, December 29, 1911.

under normal pressure of pure and of approximately pure carbon dioxide² these varieties occupied from six to eight days in losing astringency, the *Hyakume* being the slower to respond to treatment. Under a pressure of 15 pounds of pure carbon dioxide, the period was found to be reduced to less than 46 hours. To be more explicit, 24 hours was found to be insufficient for either variety, while at the end of 46 hours all astringency had totally disappeared. The minimum period required at this pressure was not determined at the time for lack of material. It was, however, quite evident that the time necessary to render these varieties non-astringent at normal pressure of carbon dioxide can be reduced to less than one fourth at 15 pounds. It then remained until the season just closed to determine these relations more accurately and with reference also to still higher pressures. It is upon this work that I desire to submit at this time a preliminary report.

Meanwhile the results of experiments made by Dr. H. C. Gore³ on the effect of carbon dioxide at normal pressure have appeared. The varieties which he studied include *Taber 23* and *Hyakume*, so that his results are distinctly pertinent in the present connection. Gore used a metal receiver especially designed by him to meet practical requirements, and, as indicated by his controls, is doubtless as efficient for exact experimentation as a glass receiver. The experiments with *Hyakume* were done at Macclenny, Fla., so that the fruits of this variety were not subject to the exigencies of transportation. As to these conditions, therefore, Gore's experiments may be regarded as directly comparable to my own, which also were done on the ground in metal glass and wooden receivers. Gore's *Taber 23* fruits were processed in Washington. As to the numbers of fruits used in Gore's experiments, only three of *Hyakume* were available,

² The protocols of these and the remaining experiments will be published elsewhere in full.

³ Gore, H. C., "Large Scale Experiments on the Processing of Japanese Persimmons, with notes on The Preparation of Dried Persimmons," U. S. Dept. of Agri., Bur. Chem., Bull. 155, May 10, 1912.

while of *Taber 23*, 68 fruits were processed, 71 serving for control. The three *Hyakume* were treated *in vitro*; the *Taber 23* in the metal receiver.

With conditions thus similar to those of my own experiments, Gore found that under normal pressure of carbon dioxide the *Taber 23* became nonastringent in two days and the *Hyakume* in 36 hours.³ Previously⁴ these had been found to yield to treatment in three days' exposure to vapor of alcohol of 5 per cent. and 25 per cent. strength in wooden tubs, the alcohol replacing, in this experiment, the *saké* of the empirical method of the Japanese. This result of Gore's I had overlooked at the time of my previous communication, or otherwise I should have been compelled to point out a discrepancy as between Gore's data and my own obtained with alcohol vapor for which I could not at that time have suggested an explanation. The discrepancy lies in the period required to render the fruit nonastringent with either carbon dioxide or alcohol vapor. Setting aside the *Taber 23* used by Gore as different from the *Taber 129* used by me, and therefore not comparable, the *Hyakume* in Gore's experiments yielded, under similar conditions, in less than one fourth the time required in mine; and, with reference to carbon dioxide alone, Gore succeeded in processing this variety under normal pressure in 36 hours, just the time, as it eventuated this year, required for apparently the same variety under 15 pounds. I do not argue from this that any doubt is thrown on Gore's work, nor would it be just to maintain the converse. It seems entirely probable rather that he and I have been working on different kinds. I have lately found that Japanese nurserymen on this continent recognize two subvarieties (using this term as a convenience) of *Hyakume*, the fruit of one of which becomes nonastringent while still firm and on the tree, while that of the other does so only after softening. It can scarcely be doubted that the former would ap-

pear to yield to the carbon dioxide treatment more readily than the latter. Furthermore, these physiological differences may not be confined to different races of the persimmon only, but may be highly individual, and different even in the same individual from year to year. In support of this I have to cite an important observation on the fruit of *Taber 129* growing at Auburn, Ala., on the station grounds. There are two trees, from which was obtained all the fruit for my experiments in 1911 and 1912. In 1911 the fruits from both the trees were, unless processed, uniformly highly astringent until softening was advanced. To render them nonastringent while still firm and crisp occupied 6 days in carbon dioxide alone at normal pressure. The fruit was tested each day, and there can be no doubt of the substantial correctness of the statement. In 1912, much to the surprise of both myself and my former colleague, Dr. F. A. Wolf, who aided me in carrying out the experiments to be presently mentioned, the *Taber* fruit apparently yielded to carbon dioxide at whatever pressure used, however low, and no matter how brief the treatment. But it was soon discovered that the real fact of the case was that *all the fruit was already non-astringent on the tree*, and this was true equally of fruits which were quite green and of those which were more or less of the definitive color, from yellow to deep orange. There were, moreover, in these fruits a very large number of reddened tannin masses, so that the inner portion of the mesocarp looked quite brown. That, however, this reddening of the tannin, due to oxidation, was not the cause of nonastringency is shown by the fact that the pulp was equally nonastringent where the oxidation had not occurred at all. I had previously ventured the opinion that the apparent absence of tannin from the nonastringent varieties might be due to the oxidized condition of the tannin, since I had been informed by Mr. Geo. C. Roeding, of Fresno, Cal., that the flesh of such kinds is freely interspersed with reddened cells. It now appears more probable that the reddening of the tannin masses is consequent on the nonastringency, and is not the cause of

³Gore, H. C., "Experiments on the Processing of Persimmons to render them Nonastringent," U. S. Dept. Agri., Bur. Chem., Bull. 141, September 29, 1911.

it; is indeed rather a result of the death of the tannin idioblasts,⁵ and can be seen to proceed *in vitro* equally well, either spontaneously, but rather slowly, or rapidly under the effect of certain acids. It seems true, therefore, that the cause of nonastringency in all the varieties, whether they are the so-called nonastringent kinds or not, is the same. I have advanced an explanation of the phenomenon elsewhere⁶ and the present issue does not call for a restatement.

In view of the possibilities in the case, therefore, I am bound to retain faith in the significance of the experiments upon which I based the conclusion that the higher the pressure of carbon dioxide, the shorter the period required to render the fruit nonastringent, especially as repetition and extension, in 1912, of the experiments of 1911 have discovered no discordant evidence.

The experiments were done during the first two weeks of September, 1912, at Auburn, Ala. The receivers used were four in number, all alike in construction. Each consisted of a piece of four-inch gas pipe two feet long, capped at both ends. A pressure gauge was inserted laterally, while each cap carried a gas cock, thus allowing the entrance and exit of the gas and air. This apparatus was cheap and efficient, but was not altogether easy to manage. It was necessary to work in a machine shop where pipe vises and large wrenches were at hand to screw home the large cap which had to be removed in order to examine the fruit. Graphite "dope" was used to make the joints tight. Though a pressure of 90 lbs. was in one case maintained for 24 hours, it usually fell a little, due to insufficient skill in getting every joint tight. The fall was, however, slight in any case, and, in view of the relations found, can be regarded as affecting the result but negligibly.

The fruits used were *Hyakume*, as stated, and *Tane-nashi*. For a supply of the latter I am indebted to Mr. C. L. Coleman, Fair-

hope, Ala., who was kind enough to send a large basket of choice material. For the name of this variety, I rely upon general acceptance in Alabama. The fruit answers also to the current horticultural descriptions. The *Taber 129*, which had been used in the experiments of 1911, were excluded for the reasons above stated.

The condition of the fruit as regards maturity was various, ranging from complete greenness to partly orange, the intermediate shades of yellow, yellow-green and yellow-orange being represented—this in *Hyakume*. The *Tane-nashi* ranged from pale yellow to orange. It was found that the rate of becoming nonastringent was *as rapid in the green as in the more mature fruits so long as they were under the influence of the carbon dioxide*. If, however, they were insufficiently processed, and then kept exposed to the air, the subsequent changes were more rapid in the more mature fruits. Maturity then did not modify the immediate effect, and the range of condition in regard of maturity rules out error due to difference of ripeness. The fact is one, moreover, of prime theoretical importance.

In charging the receivers, a corresponding number of sets of fruits was chosen in such manner that each set contained the same range of color. The charge consisted of 8 fruits in each instance. Each fruit was selected carefully for its soundness, and no bruised or otherwise injured fruit was used, except in the case of the *Tane-nashi*, which, on account of transportation, was in some instances more or less bruised. This was, however, found to have no immediate effect on the rate of processing, though the keeping of the fruit thereafter was affected, as was true also of the other kinds.

In the several experiments' pressures ranging from normal to 90 lbs., were employed, together with controls in air. By charging

⁵ Lloyd, F. E., "The Behavior of Tannin in Persimmons with some Notes on Ripening," *Plant World*, 14: 1-14, January 1, 1911.

⁶ Lloyd, l. c., note 5.

' While the results obtained with *Tane-nashi* are in general accord with those from *Hyakume*, they are not by any means as clear-cut and unequivocal. I shall therefore withhold them from the present discussion, but will embody them in a later account.

the receivers at different pressures and opening them at stated intervals, the period required for processing could be determined with all necessary accuracy, certainly within a very few hours. Both time and circumstance prevented a high amplitude of experimentation, so that pressures between 15, 25, 45, 75 and 90 lbs. were not tried. Concerning the highest of these (90 lbs.) it may be stated summarily that the fruit was killed, and on being taken out, at the expiration of 24 hours, was discolored (brown) and watery. It was, however, nonastringent. Whether the cause of death was due to rupture of the cells consequent on the too rapid increase or decrease of pressure, or on asphyxiation accompanied by change in permeability of the protoplast, I can not say. If the fruit was too soft, and the pressure released too rapidly, even when the initial pressure was no higher than 15 lbs., the fruit was burst, and microscopic examination showed that many individual cells were also in the same case.

The experiments were, moreover, designed to determine (1) the minimum period of time required to cause nonastringency at the pressures used; and (2) the after-effect of dosage at given pressures applied for periods insufficient in themselves to produce nonastringency. Aside from the theoretical interest attaching to the latter question, the possible economic application of short dosage, if followed by nonastringency within definite periods, was contemplated. The minimum period required to effect nonastringency was determined critically for normal, 15 and 45 lbs. pressure, the volumes of CO_2 being 1, 2 and 4, respectively. The after-effect of dosage at 45 and 75 lbs. was determined with sufficient accuracy to indicate definite quantitative relations.

It was found, in the first place, that at the end of 36 hours with 15 lbs. pressure (experiment 12 *a-d*, 1912) the fruits were nearly nonastringent. Further treatment for three hours produced complete astringency. It is probable that the period required is nearer 36 than 39 hours, but not less than the former nor more than the latter. At 45 lbs. 12 hours was found insufficient (experiment 11 *d*, 1912),

while at the close of 15 hours there was a scarcely perceptible astringency (experiment 9, 1912). It may be recalled that the variety in question (*Hyakume*, an "astringent variety") had been found in 1911 to require 6-7 days at normal pressure, and less than 46 hours at 15 lbs. Controls in air this year remained hard and astringent during the whole period of experimentation, nearly three weeks. It therefore emerges that, as I have previously maintained, there is a quantitative relation between the amount of CO_2 available and the rate at which the astringency disappears. Green fruits yielded as readily to both pressures as yellow or pale orange, which appears to indicate that, during the natural course of events, after the fruit has its full growth at any rate, the tannin cells are ready for the change leading to nonastringency, and that this change is induced by some condition set up in the tissue external to the tannin cells themselves. The graph determined by the data above indicated is that of a rectangular hyperbola, and this may be held tentatively as expressing the relation of time and pressure to the disappearance of astringency.

In the second place, the fact was established that nonastringency ensues the more rapidly, the longer the exposure to CO_2 , though this in itself is not sufficient to bring about the result. Pressures of 45 and 75 lbs. were used, the treatments being for 3, 6, 9 and 12 hours. After a pressure of 45 lbs. applied for 3 hours, nonastringency ensued in from 71 hours (for orange-colored fruits) to 192 hours (for yellow and green fruits). After 6 and 9 hours exposure, 45 hours were required, the evidence being that the period was somewhat less for the longer exposure. After 12 hours, the fruit became nonastringent in something over 18 hours. After 75 lbs. pressure, nonastringency was effected in a somewhat shorter period, but in view of the possible significance of the quantitative relations indicated above, but little difference should be expected between the results caused by 45 and 75 lbs. After 3 hours exposure, nonastringency followed in 62 to 185 hours; after 6 hours in something over 34 hours, after 9 hours less

than 34 hours, and after 12 hours in about 20 hours. In the receiver charged for 12 hours, however, the pressure fell from 75 to 50 lbs., so that no difference worthy of note may be seen as between this and the corresponding experiment for 45 lbs. The difference in effect of the 6 and 9 hour exposures was very small with both 45 and 75 lbs. pressure, a fact which I am unable to elucidate. Aside from this, the period required for becoming nonastringent was, roughly speaking, in inverse ratio to the time of exposure to the gas. It will be readily understood by those who have worked with such objects as ripening fruits that it is often difficult to fix upon a suitable indicator of the final limit of any of the physiological processes involved. Thus, in these experiments just described, the difference in rate of change in green and orange-colored fruits makes it difficult to decide on what is to be regarded as the final point at which nonastringency ensues. Furthermore, there is the more variation between different fruits the longer the period of ripening, and the end point is correspondingly difficult to fix.

I have, therefore, endeavored to apply a test different from that of tasting, at least for purposes of control. The mucous membranes are of course extremely sensitive; nevertheless, it becomes difficult, as the end point is approached, to judge clearly. It is furthermore of the highest importance to examine the physical characters of the tannin-mass, which has been shown to be a colloidal complex,⁵ in order to determine whether the condition reached by it when nonastringency has been accomplished quickly is identical with that reached after a slow process. A former study of the reaction of alkaloids⁶ with the tannin-mass showed me that as nonastringency is approached, the coarsely granular precipitate gives way to an increasingly finer one, so that an ultramicroscopic and eventually an amicroscopic suspensoidal condition is reached. The only change obvious on applying the reagent then becomes one of color,

⁵Lloyd, F. E., "The Tannin-colloid Complexes in the Fruit of the Persimmon," *Biochemical Bulletin*, 1: 7, September, 1911.

the tannin-mass becoming brown, lighter brown, darker and paler yellow as the definitive state is approached. By reflected light the changes are evident as a decreasing milkiness.⁹ By correlating these progressive changes with the disappearance of astringency, it has become evident that it is quite possible to decide whether a fruit is astringent or not without tasting it. This is because the physical condition of the tannin-mass is the same on the arrival of nonastringency, whether this has been accomplished in 15 hours or as many days.

There remains one further point of which to speak. When one uses such an expression as "the period required to become nonastringent," an incorrect notion may be implied. It is not to infer that in one case the process itself is slow and in another rapid (though this may be the case), but that the time required to start the process may differ. It remains to determine the actual fact, and this is necessary to an understanding of the whole matter.

Note.—The work, of which the above is a partial account, was done at the Alabama Agricultural Experiment Station as an Adams Fund Project. I have to thank Dr. F. A. Wolf for hearty and arduous cooperation in carrying on the experiments.

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THE society held its twenty-fifth annual meeting in Cleveland, Ohio, December 29, 1912, to January 1, 1913. Sixty-nine members were in attendance. Two executive sessions and six scientific sessions were held, two of the latter being joint sessions, one each with the American Society of Biological Chemists and Section K of the American Association for the Advancement of Science. The joint session with the American Society of Biological Chemists was opened with exercises in memory of the late Waldemar Koch. After the

⁹These changes are analogous to those seen first by Loew and others, and recently described in detail by Czapek. Czapek, F., "Ueber Fällungsreactionen in lebenden Pflanzenzellen und einige Anwendung derselben," *Ber. deut. bot. Ges.*, 38: 147-159, 1910.