soils, not given in the table, which become so dense during the centrifuging process that water will not pass through them. In some cases this condition has resulted when the centrifuge is brought up to speed too quickly, the soil becoming so compact water will not pass through it. This impervious condition has also been found when some of the soils were mechanically stirred in the centrifuge cups just before placing the samples in the centrifuge. In fact, in many cases standing water was found on the surface of the sample after centrifuging. We believe that under such conditions the moisture equivalent is meaningless. The results simply indicate that the soil was rendered impervious and the results obtained bear no relation to the textural properties of the sample.

The conclusion from our work with salt-treated samples is that more dispersion can be brought about by mechanical agitation than by salt treatment and leaching. These results indicate that the impervious conditions sometimes observed in the field, where the soil was pervious formerly, and attributed to the dispersion resulting from irrigation with salty water, may be brought about by mechanical working of the soil when too wet.

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## PIGMENTATION IN THE ROOT OF THE COTTON PLANT

IN connection with investigations of the effect of fertilizers on the incidence of cotton root rot (*Phymatotrichum omnivorum*) repeated observations have been made of consistent relationships between the pigmentation of cotton roots and stage of plant development, soil characteristics and fertilizer treatment. In field experiments on Wilson clay loam, a non-calcareous soil of the Blackland prairie section of Texas, fertilizer treatments in which phosphoric acid dominated tended to accelerate the appearance of root rot, as evidenced by above-ground symptoms, while fertilizers in which nitrogen dominated produced a converse effect. These same fertilizers had, respectively, the effect of hastening or retarding the physiological age of the cotton plant. Associated with these effects there was also observed a gradation in the pigmentation of the root bark; in mid-summer the roots of plants from unfertilized plats were a pale yellow, those from plats treated with high-nitrogen fertilizers were of a lighter tint, while high-phosphate plants displayed a distinctly reddish shade. Later in the season, the unfertilized and high-nitrogen plant roots acquired this reddish cast, while that of the phosphate-fed roots was intensified, consequently the gradient of color was maintained until all plants were matured.

The roots of cotton seedlings grown on the calcareous soils of the blackland section are also pale yellow; the red pigmentation appears about the time that the first squares are set, and becomes more intense as the season advances, so that at the end of the season the roots exhibit a deep red coloration, regardless of fertilizer treatment. The Wilson soils have a lower pH value than those of the Houston series. Limited observations of cotton plants of comparable age grown on the acid soils of east Texas indicate that this red pigment does not appear until later in the development of the plant, and at maturity the intensity of the color does not approximate that of plants produced on the less acid to alkaline soils of the blackland section. Thus it appears that the reaction of the soil, physiological age of the plant and fertilizer treatment are factors in the pigmentation of the roots of cotton plants grown in this region.

A systematic study of the pigmentation of the cotton root was begun in 1935 and continued during 1936, on samples taken periodically throughout the growing season. Roots, as prepared for carbohydrate studies, were extracted with boiling alcohol whose final strength after contact with the plant material was not less than 80 per cent. In 1936, the plants were divided into two parts, namely, bark and woody tissues. The alcoholic extracts of the woody part were yellow; these changed to orange toward the end of the season. Those of the bark were yellow until the stage of square formation at which time the red coloration appeared, and this latter color became more intense as the season advanced. The bark extracts, even though they were intensely red, were found to contain some of the yellow. The variations in the reddish color of the bark, which are discernible to the eye before heating with alcohol, are intensified in the alcoholic extract; the extracted bark-tissue is also much more highly colored than the unextracted.

Comparisons were made of extracts from roots of plants grown on Wilson clay loam with 0-15-0,[1] 3-9-3, 9-3-3 and 15-0-0 fertilizers; an unfertilized check plat was also used. The volumes of the extracts were adjusted so that the same ratio of liquid to plant material was maintained. Using the plant grown on the 0–15–0 fertilizer plat as a standard, the relative color intensity of the extracts from the other treatments were determined with facility by the use of the colorimeter. The intensities of the red pigment of the bark of plants produced with the 0-15-0 and 3-9-3 fertilizers were greater than that of the check, while those of the 9-3-3 and 15-0-0 samples were less; the extremes were produced by the 0-15-0 and 15-0-0 fertilizers. These differences were obliterated late in the season.

Wayne,[2] Drueding[3] and Power and Browning[4] have

[1] $N–P_2O_5–K_2O$.

[2] E. S. Wayne, *The Pharmaceutical Journal and Transactions*, 3: 64–65, 1872.

reported a red to purple material as a component of the root of the cotton plant; the color here described is apparently related to this material. Drueding also reported a yellow material obtained from the bark of the root. Although none of the workers established the identity of the red material, Wayne called it *Gossypic* acid due to its acidic nature. The red-purple pigment has been obtained in this laboratory by precipitation from the alcoholic extract of mature cotton plants with an excess of 10 per cent. sodium hydroxide, washing with slightly alkaline 80 per cent. alcohol, dissolving in water, and precipitating with hydrochloric acid; this gelatinous material was then washed with water. It dries to an amorphous powder of a red-purple color. When dissolved in 80 per cent. alcohol, it responds to the qualitative tests of the anthocyanins as given by Onslow.[5]

Although the yellow to orange material of the alcoholic extracts of the woody tissue of the root is less susceptible to isolation, qualitative tests applied to these extracts indicate the presence of a pigment which displays characteristics of the flavone and flavonol pigments. Early in the season the high-nitrogen fertilizers produced a higher concentration of the yellow than the high-phosphate, but as the season advanced the highest concentrations of the yellow of the woody tissue and the red of the bark were both produced by the same fertilizer treatment.

There has been observed a general correlation of the pigmentation of the cotton root with the physiological age of the plant, the reaction of the soil, the effect of fertilizers and the incidence of cotton root rot as observed in the field.

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## THE DORSOVENTRAL AXIS OF THE FORELIMB BUD IN AMBLYSTOMA MICROSTOMUM

THE following experiments were performed to determine the stage of development at which the dorsoventral axis of the forelimb bud of *Amblystoma microstomum* is established. It has been known since Harrison's studies[1] that the dorsoventral axis of a limb may not be determined until much later than the anteroposterior axis. Swett[2] showed that in *A. punctatum* the former axis in the forelimb bud is not irreversibly established until stage 35 (Harrison's stages), whereas in *A. tigrinum* Hollinshead,[3] using grafts only two somites in diameter, found that it is not until stage 38 that all grafts retain their prospective asymmetry.

The forelimb bud of *A. microstomum* is first visible externally as a rounded elevation beneath somites 3, 4 and 5 at stages 35 to 36. Round disks of tissue 3 to 3½ somites in diameter were removed from this region at stages varying from 27 to 34. In order to reverse only one axis, left limb buds were transplanted to the right flank in a location midway between the fore and hind limbs. This orientation resulted in an inversion of the dorsoventral axis, while retaining for the anteroposterior axis a normal relationship.

In all, 104 operations were performed, but in 30 cases the host died. In those surviving, 9 grafts were resorbed and 25 produced limbs which were too imperfect to be interpreted. The remaining 40 cases formed supernumerary limbs: 17 with the asymmetry reversed and 23 were inverted left limbs with asymmetry not reversed.

Of the eleven positive cases operated on at stages 27 to 31, ten had reorganized the dorsoventral axis and formed right limbs (Table 1). The eleventh case, performed at stage 29, formed a double limb, the primary member of which was an inverted left limb. It is believed that either this case was an error or that the transplant was larger than 3½ somites. No inverted limbs were obtained in the seven positive cases transplanted at stages 30 and 31.

TABLE 1
SHOWING THE DETERMINATION OF THE DORSOVENTRAL AXIS IN AMBLYSTOMA MICROSTOMUM

| Stage | Total | Harmonic right | Inverted left |
|---|---|---|---|
| 27–29 | 4 | 3 | 1 |
| 30 | 2 | 2 | 0 |
| 31 | 5 | 5 | 0 |
| 32 | 12 | 7 | 5 |
| 33 | 9 | 0 | 9 |
| 34 | 7 | 0 | 7 |
| Totals | **39** | **17** | **22** |

Stage 32 proved to be the transitional stage. In about half of these cases there developed right limbs and in the other half, inverted left limbs. This variation may be explained on the basis of slight differences in the size of the graft or in the age of the donor.

At stage 33 there were nine grafts which developed limbs and all nine had retained their prospective asymmetry. The dorsoventral axis had been established at the time of the operation and had continued its devel-

[1] R. G. Harrison, *Jour. Exp. Zool.*, 32: 1–136, 1921.

[2] F. H. Swett, *Jour. Exp. Zool.*, 47: 385–439, 1927.

[3] W. H. Hollinshead, *Jour. Exp. Zool.*, 73: 183–194, 1936.

[3] C. C. Drueding, *ibid.*, 8: 245–246, 1877.

[4] F. B. Power and H. Browning, *The Pharmaceutical Journal and Pharmacist*, 93: 420–423, 1914.

[5] "The Anthocyanin Pigments of Plants," 2nd edition, 1925, Chapter 4, by M. W. Onslow.