# STOCK-SCION RELATIONSHIPS IN TOMATOES

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# INTRODUCTION

The practice of grafting is employed in great measure because of the belief that stock and scion retain their individual characteristics. However, the reciprocal influences of stock and scion are well established in many fruit trees and considerable practical use has been made of the results and experiences gained through experimentation. Problems regarding the mechanism of this interaction have attracted the attention of horticulturists from time to time. Most of the workers consider these reciprocal influences as quantitative ones and suggest that the only way in which each part is influenced is through changed nutrition. It is generally assumed that apart from nutritional effects there is no interchange of qualities between scion and stock, each retaining its own identity intact. However, during recent years qualitative influences, such as the translocation of alkaloids across the graft unions, have been demonstrated in herbaceous plants, but still the observed phenomenon of stock-scion interaction in fruit trees is mostly explained as exercised indirectly through growth reactions.

The results concerning the effect of the stock on the nature of the scion, reported by Daniel (1897, 1904, 1911, 1920), are not in accord with those noted as having met the general acceptance. He has described many cases in which, as a result of grafting, a shoot was produced combining characters of scion and stock and he considers that such variations may be inherited. A similar concept of "vegetative hybridization" has been held by certain scientists in Russia where a strong controversy has been going on since 1930 between the "Mendelian" and "non-Mendelian" geneticists. The "non-Mendelian" school of genetics has been headed by Lysenko. The principal experimental basis for Lysenko's new genetical theories is formed by evidence on effects of grafting. Hudson and Richens (1946) in their review of the new theories, after carefully analyzing the data of graft hybridization in every detail, mention the difficulty of interpretation. They emphasize the urgent need for a repetition of some of Lysenko's experiments under strictly controlled conditions. In summarizing they state that "the evidence for genetic interaction between stock and scion is not compelling but suggestive."

It was with the aim of analyzing some of these highly controversial points in the field of genetics and plant physiology that this problem of stock-scion interaction was selected.

## REVIEW OF LITERATURE

Prior to 1946, several historical and theoretical papers have appeared in various journals on the genetics controversy in the U.S.S.R. (Sax 1944, Pincus 1940, Espinasse 1941, Haldane 1940, Mather 1942). The first comprehensive information in English on Lysenko's school of thought was made available in 1946 by Dobzhansky's translation of Lysenko's book "Heredity and its Variability", which was first published in 1943. Hudson and Richens (1946) provide additional information on "The New Genetics in the Soviet Union" and discuss the results obtained by Russian scientists.

The most important and radically different views held by Lysenko, as revealed by Dobzhansky's translation, center around the idea of inheritance of acquired characters. Heredity of organisms according to him is controlled by the environment. He considers "heredity as the property of a living body to require definite conditions for its life, its development and to react definitely to various conditions". However, according to Mendelian theory which has been generally accepted, heredity is controlled by the genes. This view of heredity regards that the characters of an organism are the result of an interaction between its hereditary genotype and the environment in which it develops. Altering the environment may change these characters but such a change is not inherited; this is what is called non-inheritance of acquired characters. A permanent hereditary change can be accomplished only by altering the genotype.

Davies (1947) considers that Lysenko's interest in genetics grew out of his classical work on vernalization. Probably his success in altering the phenotype through an environmental change led him to conclude that the distinction drawn by the Mendelian group between phenotype and genotype was not correct, and that inheritable changes could be produced by modifications of the environment in which the organism developed. Davies states that "the rejection of orthodox genetics and its replacement by the form of Lamarckism advocated by Lysenko, Prezent, Dolgusin and their followers is quite unjustified by the experimental evidence which they submit".

Fyfe (1947) while mentioning Lysenko's ignorance of the progress in genetics and disagreeing with his theories suggests the withholding of judgment on Lysenko's recent experiments until they have been adequately tested.

Stern (1946) and Goldschmidt (1946), reviewing Lysenko's conception of heredity and its variability, criticize the main points. Stern states that "Lysenko may or may not have discovered new facts. In any case his attempt to ignore nearly a half-century of scientific achievements seems to have resulted in replacing rational insights by hunches and vague generalities".

Darlington (1947), in his article "A Revolution in Soviet Science", quotes Lysenko's views with regard to heredity from Dobzhansky's translation and criticizes the line of argument followed by him. He interprets Lysenko's main idea as follows: "Heredity is development. The environment can change development. Therefore the environment can change heredity".

Ashby (1946) finds that the experimental technique followed by Lysenko and other workers of his school is poor, that the plant material is heterozygous, the physical controls employed are insufficient and the statistical analysis of data is lacking.

In view of the disagreements in the reported observations and conclusions of the Russians and the generally accepted views in genetics it seemed desirable to attempt the repetition of at least the most striking of the Russian experiments. As mentioned earlier, Lysenko has placed special emphasis on grafting experiments, of which he cited several results in support of his theories. It would not be out of place, therefore, to review the "graft-hybrid" concept and some of the earlier work in this connection.

Weiss (1930) and Jones (1934) have reviewed the various problems relating to graft hybridization. Jones states that "the nature of 'graft hybrids' has been conceived so differently by different workers, and often with such a lack of precision, that it is impossible to know exactly what is implied in any given case without further definition." The most clearly expressed version of a true graft hybrid is, that it results from the fusion of somatic nuclei of scion and stock in a manner analogous to that in which fusion between gametes or sex-cells give rise to a seed hybrid. However, some workers' conception of a graft hybrid, that it combines the qualities of scion and stock without the occurrence of nuclear fusion, is rather peculiar and hard to explain.

Jones (1934) has stated the views of Daniel who suggested that there is no justification for regarding seed hybrids, graft hybrids and chimaeras as fundamentally different types. Daniel considers that all are the result of the union of two plant strains by nuclear fusion of either sex-cells or vegetative cells. Commenting on the hypothesis, Jones states that "the occurrence of nuclear fusion between the genetically dissimilar vegetative cells leading to the production of graft hybrid is not theoretically impossible; such fusion is certainly exceptional and still awaits demonstration". Weiss (1950) is also inclined to the opinion that hereditary interaction between the stock and scion of grafts has not yet been demonstrated.

Hudson and Richens (1946), while tracing the basis of Lysenko's graft hybridization theory, mention Michurin's theory of vegetative rapprochement and Timirjazev's Darwinian notion of graft hybridization. According to Michurin, the genetical natures of the two elements of the graft should approach each other. He assumed that the mere action of grafting is sufficient to bring about hereditary changes. His assumption appears to have been supported by a number of Russian papers cited by Hudson and Richens.

Most of the recent experiments which form the important basis of Lysenko's genetical theories, involved grafting of tomato varieties differing in such characters as fruit size or color, number of carpels, leaf shape, and habits of vegetative growth. These experiments have shown that all these characters may be transmitted from stock to scion under prescribed conditions using mature plants for stocks and very young plants as scions. The most important paper which supports Lysenko's views is that of Avakian and Jastreb (1941). It has been thoroughly reviewed by Hudson and Richens. Especially interesting are the results of grafting the tomato variety Albino (scion) onto the red variety, Mexican 353 (stock). It has been claimed that the white scion shoot bore fruits of various colors ranging from red to pale yellow. Further records of $F_1$ and $F_2$ generations showed a gradation of colors. The reciprocal effect of scion on stock was also shown by Avakian and Jastreb by using Albino variety as stock and Mexican 353 as scion. In this case the characteristics of the scion were imparted to the stock.

Results of several other investigations in the U.S.S.R., involving grafting of herbaceous plants and depicting similar changes as discussed above, are presented by Hudson and Richens. In addition, some experiments on grafting of cereals like wheat, to combine some of the morphological characters of both stock and scion, are given.

Outside the U.S.S.R. very little evidence is found in support of the graft hybridization concept. As mentioned previously, Daniel's (1897, 1904, 1911, 1920) experiments, principally on fruit trees or Helianthus spp., did show that heritable modifications can be induced by mere grafting; but his findings have not been confirmed.

Wettstein and Pirschle (1938) found that a chlorophyll defective petunia could transmit leaf and shoot characters to normal plants by grafting. Heinicke (1927, 1936) noticed that some apple varieties, when top worked with other varieties produced fruits below the graft unions displaying characters of the scions. Removing stock leaves and ringing the branches below the fruits helped intensify the scion effect. Swarbrick (1930), and Tukey and Brase (1933) have dealt at length with the effects of various apple scions on their rootstocks. They have pointed out that the scion had, in some instances, an appreciable influence upon the growth and character of the rootstock.

Problems regarding the chemical relationship between the stock and scion have been investigated by many workers. Warne and Wallace (1935) have made an investigation of the possible relationships between rootstock effect and chemical factors. These workers conclude that many of the outstanding rootstock effects could not be explained on the basis of the chemical characters examined. Hofmann (1927) did not find the transfer of anthocyanin pigment through graft unions of reddish purple Refugee bean and white Navy bean. Similarly Biffen (1902) did not observe the movement of red pigment in reciprocal grafts of red beet and sugar beet. Daniel (1926) found no transfer of inulin from artichoke scions to sunflower stocks.

The transfer of certain alkaloids across the graft union has been demonstrated from time to time. Daniel (1894) showed that atropine passes readily from <u>Atropa Belladonna</u> scions into potato stocks. Kraevoi and Nechaev (1941) and Kerkis and Pigulevskaya (1941) found that atropine was translocated from Datura stramonium rootstocks to Lycopersicum esculentum scions. Dawson (1942) has shown that nicotine produced in tobacco roots is transported across the graft unions into tomato scions. Kuzmenko and Tikhvinskaya (1940), while working on the inheritance of nicotine and anabasine content of Nicotiana tabacum x Nicotiana glauca hybrids and interaction of stock and scion when these species are grafted, observed that when tabacum (which lacks anabasine) is used as the scion in a graft with glauca, a considerable amount of anabasine appears in the leaves of tabacum, and a precipitable amount in tabacum when it is used as the stock.

Lysenko has based his theories very firmly on results reported from grafting experiments. His claims are somewhat surprising, since the experimental evidence, as far as it is accessible, appears to be meager. It was deemed desirable to repeat some of his and his followers' tomato grafting experiments, following the techniques outlined in the Russian publications as closely as possible. In order to substantiate the studies, some physiological characters of the fruits of grafted and ungrafted plants -- total soluble solids, total acidity and ascorbic acid content -- were determined. In addition, a study was made on reciprocal grafts of normal plants and plants modified through the action of 2, 4-dichlorophenoxyacetic acid.

## GENERAL METHODS AND MATERIALS

Selection of Plant Material - Tomato varieties differing in color, shape, size and number of locules of fruit were selected in fall, 1946. These were: John Baer (large, red), Stokesdale (large,red), Golden Queen (large, yellow), Italian Red Pear (small, red, pear-shaped), Italian Red Plum (small, red, plum-shaped), and White Beauty (large, white). The seeds of these varieties, which were guaranteed for purity of strains, were obtained from commercial seed companies. Incidentally, some of the varieties selected were found to be the same as those selected by Wilson and Withner (1946) whose work on stock-scion relationships in tomatoes was reported in December, 1946. In addition to the above recorded varieties, one line developed by Yeager and Purinton (1946) for high ascorbic acid content, was added to the collection in 1947 for studies on ascorbic acid synthesis.*

Procedures of Grafting - The method of splice grafting illustrated in figure I was used entirely in the first year of the investigations and mostly in the second year. The terminal portion of the plant (to be used as stock), in-

*The seed of tomato with high ascorbic acid content was obtained by Dr. S. H. Wittwer, Michigan State College, East Lansing, Michigan, through the courtesy of Dr. A. F. Yeager, University of New Hampshire, Durham, New Hampshire.

Fig. I

Technique of Grafting

Consecutive steps in the procedure when scions from three-week old plants were grafted onto six-week old stocks.


Figure I

Fig. II

Technique of Grafting

Consecutive steps in the procedure when scions with two cotyledons and two small leaves were grafted onto six-week old stocks.

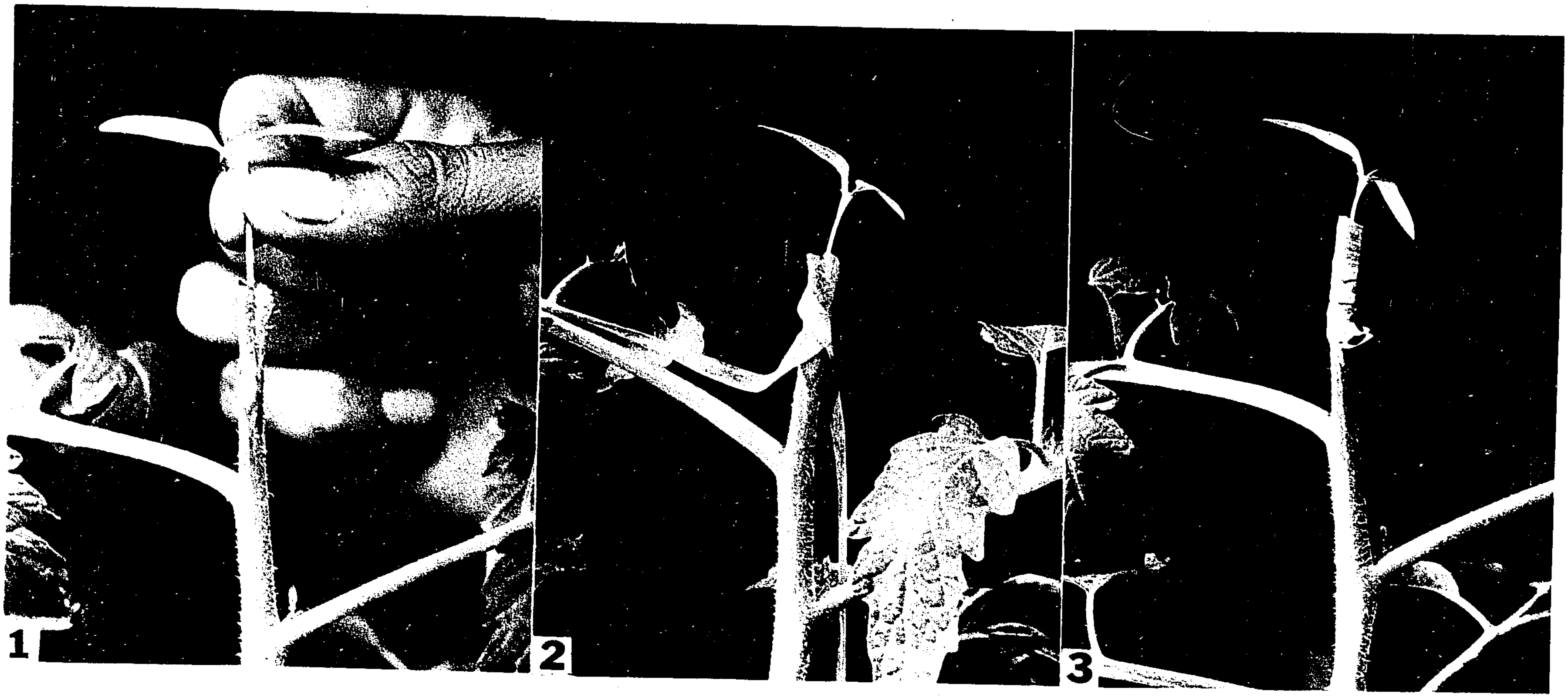


Figure II

cluding the apex and young leaves, was removed with an oblique cut. A similar cut was made on the terminal portion of the comparatively younger plant (to be used as scion). The cut surfaces of stock and scion were brought together and then held in place by wrapping them with a narrow strip of wet raffia fiber as shown in figure I (1-3). Immediately after grafting, the plants were placed in the humid atmosphere of chambers, each of which held thirty-six 4-inch pots. The plants were kept in the chambers until the grafts had begun to unite which usually took seven to ten days. In the meantime the humidity was kept high by occasionally sprinkling with water. However, too high a humidity and temperature always proved conducive to the development of non-specific fungi which destroyed the graft unions. Proper aeration helped to reduce the damage. The plants were taken out of the chambers when some healing had taken place and were transferred to the shaded section of the greenhouse. If scions showed dropping owing to lack of proper union, the plants were again placed in the chambers for another few days. When the union was completed, the raffia fiber was removed carefully by cutting it into small strips.

The method, illustrated in figure II, was used to a limited extent in the second year of the investigations, in order to study the stock-scion interaction when very young seedlings were used as scions. The terminal portions of plants to be used as stocks were decapitated and later split open by a cut as if for cleft grafting. The hypocotyl of the seedling, with two cotyledons and two small leaves, was given an oblique cut on one side only and inserted into the slit already made in the stock. A strip of raffia fiber was wrapped, as shown in figure II (2-3), to hold the seedling in place. Further steps taken, to secure a good union, were the same as discussed in the first method. It took about two weeks for the union to form sufficiently so that the plants could be removed from the moist chamber.

Determination of Total Soluble Solids, Acidity and Ascorbic Acid - For these determinations, four to eight representative fresh and fully ripe fruits from each plant were analyzed at one time. Two samples of 100 grams each were weighed separately from the longitudinally cut sectors of representative fruits. One sample was used for the estimation of total soluble solids and acidity and the other for ascorbic acid determination.

The sample meant for total soluble solids and acidity determination, was crushed in 100 ml. of distilled water by the use of a Waring Blendor and then filtered through a dry filter paper. The filtrate was tested for total solids by means of a refractometer. Acidity was determined by titrating a 20 ml. aliquot of the filtrate against standard sodium hydroxide using phenolphthalein as the indicator.

The method of crushing the fruit in a limited quantity of water, and then transferring the crushed material to a volumetric flask and making up to volume, was not adopted, because during the process of blending, introduction of unknown and unequal quantities of air hindered in the process of making up to the final volume.

Ascorbic acid determinations were made after the procedure of Lucas (1944). A fresh 100-gram sample of fruit was placed in 300 ml. of 2 per cent metaphosphoric acid in a Waring Blendor and crushed. The crushed material was filtered through dry filter paper. An aliquot of filtrate was titrated against standard sodium 2,6-dichlorobenzenone-indophenol solution and the amount of ascorbic acid was expressed as milligrams per 100 grams of fresh fruit.

# EXPERIMENTATION

## Stock-Scion Relationship with Reference to the Interaction Claimed by Lysenko

As has been stated in the "Review of Literature", geneticists have been rather critical about Lysenko's and his follower's ideas. Many of them, although strictly opposed to an acceptance of the conclusions drawn by Lysenko, have stressed the need for a repetition of some of the experiments described by the Russian school. The present study attempted to contribute a critical examination of the results of grafting experiments with tomatoes claimed by Avakian and Jastreb (1941) by repeating them as closely as the details given in their reports permitted. Their work was singled out because it provides, according to Lysenko, the most notable support for his hypotheses.

### Experiment (1947)

Procedure - Seeds of all the varieties previously mentioned were sown in separate flats in the greenhouse during January, 1947. Uniform seedlings of all varieties, eight to ten days old, were transplanted into 4-inch new pots. The plants were grafted during the last week of March and first two weeks of April, when they were 30 to 35 cm. tall. Reciprocal grafts were made between John Baer and White Beauty and between Italian Pear and White Beauty. In the case of the rest of the varieties, viz., Stokesdale, Golden Queen and Italian Plum, they were used as stocks with White Beauty as the scion in each case. The plants to be used as scions were grown later so that very small terminal parts without any flower primordia could be obtained and grafted onto old plants. Usually three-week old seedlings, 8 to 10 cm. tall, were used for obtaining terminal portions to serve as scions.

The numbers of total and successful grafts in 1947 are shown in table 1.

Table 1. Numbers of plants of varieties differing in fruit characters, which were grafted and which survived during 1947.

| Graft combinations | | | | Fruit character differences | | | |
|---|---|---|---|---|---|---|---|
| Stock | Scion | Number grafted | Number survived | Color | Shape | Size | No. of locules |
| John Baer | White Beauty | 36 | 31 | x | - | - | - |
| White Beauty | John Baer | 36 | 28 | x | - | - | - |
| Stokesdale | White Beauty | 36 | 31 | x | - | - | - |
| Italian Pear | White Beauty | 36 | 35 | x | x | x | x |
| White Beauty | Italian Pear | 36 | 30 | x | x | x | x |
| Italian Plum | White Beauty | 36 | 35 | x | x | x | x |
| Golden Queen | White Beauty | 36 | 34 | x | - | - | - |
| Total | | 252 | 224 | | | | |

x indicates a difference in the character between two varieties.

Most of the grafted plants were trained to two shoots, one from the stock and one from the scion, but some were limited to one shoot from the scion. The development of lateral branches was suppressed by regular and frequent removal of axillary buds. The flower buds were also removed until June 6, when the plants were transplanted into the field in rows 5 feet apart, the distance between plants also being 5 feet. Soon after transplanting, the plants were staked and the following experimental treatments started:

(a) Only one shoot of the scion was allowed to develop and bear fruit. The stock provided only roots and a small piece of stem. All buds developing below the graft unions were removed. This treatment provided conditions for studying the influence of the rootstock on the fruit of the scion.

(b) Two shoots, one from the stock and the other from the scion were trained. The scion shoot was kept defoliated and the flowers allowed to set fruit on it. Only two or three small leaves protecting the shoot apex, were left on the scion. They were also removed after two clusters had set fruit. The leaves on the shoot from the stock were left intact but the flower clusters were removed as soon as they appeared. The scion fruits in this treatment developed under the influence of stock leaves, but in the absence of stock fruit.

(c) This treatment was the opposite of treatment (b) and provided conditions for studying the development of stock fruit under the influence of scion leaves but in the absence of scion fruit.

(d) Both stock and scion shoots were allowed to bear fruit. No leaves were removed. This treatment served as a grafted control.

The number of plants under each treatment from different graft combinations is shown in table 2. In addition, twelve plants of each variety were grown on their own roots for the purpose of comparison of fruit characters, and to serve as controls. Out of these, six plants were trained to a single stem and allowed to set fruit (treatment (e)). The remaining six plants were trained to two shoots. One of these shoots was defoliated and the fruit allowed to set on it; on the other shoot, leaves were left intact but no fruit was permitted to set (treatment (f)).

Table 2. Number of plants of different graft combinations under various treatments employed during 1947.

| Graft combination | | Number of graft symbionts under various treatments | | | | |
|---|---|---|---|---|---|---|
| Stock | Scion | (a) | (b) | (c) | (d) | Total |
| John Baer | White Beauty | 5 | 8 | 6 | 4 | 23* |
| White Beauty | John Baer | 4 | 10 | 8 | 6 | 28 |
| Stokesdale | White Beauty | 5 | 10 | 10 | 6 | 31 |
| Italian Pear | White Beauty | 6 | 12 | 11 | 6 | 35 |
| White Beauty | Italian Pear | 5 | 12 | 8 | 5 | 30 |
| Italian Plum | White Beauty | 6 | 12 | 11 | 6 | 35 |
| Golden Queen | White Beauty | 6 | 12 | 10 | 6 | 34 |
| Total | | 37 | 76 | 64 | 39 | 216 |

*The number varies from table 1 because eight of the successfully grafted plants died soon after transplanting in the field.

<u>Results and Discussion</u> - The development and maturity of fruits on defoliated shoots of treatments (b) and (c) were normal. Obviously, the fruit on the defoliated shoots drew elaborated food materials from the other symbiont.

No changes in visible characters of fruits from grafted plants, were produced under any experimental treatment employed. Defoliation and defloration of either stock or scion did not result in changes in color, shape, size or number of locules of fruit, which could be attributed to the treatments.

The plants under treatment (a), where the influence of stock roots alone on scion fruit was studied, produced normal fruits of the scion variety. Similarly, the fruits of grafted control plants (treatment (d)) developed normally and showed no modifications in characters. Each graft symbiont bore fruit characteristic of its own variety.

These results are in perfect agreement with those reported by Wilson and Withner (1946) but fail to confirm the changes in fruit characters of graft symbionts reported by Avakian and Jastreb (1941). Wilson and Withner (1946) used similar varieties to those used in the present investigation. However, the number of plants grafted and employed by them in each treatment was too limited to draw any solid conclusions. In the present investigations the number of grafted plants under each treatment was sufficient to give a high significance to the results.

Of the six varieties used White Beauty and Golden Queen were late-ripening. The variety White Beauty was not appreciably influenced with regard to the time of maturity of fruit when grafted on the other varieties. However, effects on the time of fruit ripening, in grafts between early and late varieties of fruit trees, have been reported quite often and explained on nutritional basis.

The variety White Beauty was found vigorous and very prolific in fruit bearing. It was highly compatible with the other varieties employed, and no difficulty was experienced while grafting it with other varieties, as is evidenced by the number of successful grafts in table 1. In contrast, Wilson and Withner (1946), who did not find appreciable differences between the vigor of varieties used, considered Crystal White a poor grafting variety. However, the recent work of Nickell (1948) on heteroplastic grafts clearly indicates that even grafts between unrelated plants can be made with considerable success.

The analysis of fruit from control plants of all the varieties under study, showed that maximum differences were exhibited between John Baer and White Beauty with regard to total soluble solids, titratable acidity and ascorbic acid. Hence a study of the fruit composition, of the reciprocal grafts of only these two varieties, under different treatments, was undertaken in the first week of September, 1947. Representative samples of fruit from four plants of each treatment, were analyzed separately. The results of analyses are presented in tables 3, 4 and 5. The total soluble solids, acidity and ascorbic acid content of fruits from different treatments of graft symbionts, did not show significant differences, when compared with the controls.

Table 3. Effect of different treatments on the total soluble solids content of fruits, 1947.

| Graft combination | | Treat-ments* | Fruit analy-zed | Per cent total soluble solids of fruits of 4 individual plants | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Stock— | Scion | | | 1 | 2 | 3 | 4 | Mean | | ± S.E. |
| JB | WB | (a) | WB | 4.4 | 3.6 | 4.0 | 3.8 | 3.9 | | .17 |
| JB | WB | (b) | WB | 3.8 | 4.0 | 4.6 | 4.0 | 4.1 | | .20 |
| JB | WB | (c) | JB | 5.2 | 6.0 | 5.4 | 5.6 | | 5.5 | .17 |
| JB | WB | (d) | JB | 6.2 | 5.6 | 6.0 | 5.8 | | 5.9 | .13 |
| | | | WB | 4.6 | 4.0 | 4.0 | 3.8 | 4.1 | | .17 |
| JB | | (e) | JB | 5.4 | 5.8 | 6.2 | 5.6 | | 5.7 | .17 |
| JB | | (f) | JB | 5.2 | 5.0 | 6.0 | 5.4 | | 5.4 | .22 |
| WB | JB | (a) | JB | 5.8 | 5.0 | 6.2 | 5.6 | | 5.6 | .25 |
| WB | JB | (b) | JB | 5.2 | 4.8 | 6.0 | 5.4 | | 5.3 | .25 |
| WB | JB | (c) | WB | 4.4 | 4.0 | 3.4 | 3.8 | 3.9 | | .21 |
| WB | JB | (d) | WB | 4.6 | 4.4 | 4.0 | 3.8 | 4.2 | | .18 |
| | | | JB | 5.4 | 5.6 | 6.0 | 5.0 | | 5.5 | .21 |
| WB | | (e) | WB | 4.4 | 3.6 | 4.2 | 3.8 | 4.0 | | .18 |
| WB | | (f) | WB | 3.6 | 4.0 | 4.0 | 3.4 | 3.7 | | .15 |

*For tables 3, 4 and 5.

JB and WB indicate John Baer and White Beauty varieties respectively.

Treatments:

(a) One shoot from scion, with fruits and leaves.
(b) Two shoots; one from scion, defoliated, with fruits; one from stock deflorated, with leaves.
(c) Two shoots; one from scion, deflorated, with leaves; one from stock; defoliated, with fruits.
(d) Two shoots; one from scion and one from stock; both with leaves and fruits.
(e) One shoot with fruits and leaves (ungrafted control).
(f) Two shoots; one defoliated with fruits, the other deflorated with leaves (ungrafted, control).

Table 4. Effect of different treatments on the titratable acid content of fruits, 1947.

| Graft combination | | Treat-ments* | Fruit analy-zed | Acidity in terms of ml. of 0.1 N NaOH used to titrate a 20 ml. aliquot | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Stock - | Scion | | | 1 | 2 | 3 | 4 | Mean | ± S.E. |
| JB | WB | (a) | WB | 6.4 | 7.0 | 6.8 | 5.7 | 6.5 | .29 |
| JB | WB | (b) | WB | 6.2 | 6.4 | 5.8 | 6.8 | 6.3 | .21 |
| JB | WB | (c) | JB | 9.2 | 7.5 | 8.1 | 9.0 | 8.4 | .27 |
| JB | WB | (d) | JB | 8.0 | 8.6 | 9.0 | 7.8 | 8.3 | .28 |
| | | | WB | 5.6 | 6.2 | 6.4 | 6.8 | 6.2 | .25 |
| JB | | (e) | JB | 9.2 | 9.0 | 8.7 | 8.3 | 8.8 | .20 |
| JB | | (f) | JB | 8.4 | 8.8 | 9.1 | 7.6 | 8.5 | .33 |
| WB | JB | (a) | JB | 7.8 | 9.0 | 9.2 | 8.7 | 8.7 | .31 |
| WB | JB | (b) | JB | 9.1 | 7.2 | 8.7 | 8.1 | 8.3 | .41 |
| WB | JB | (c) | WB | 5.6 | 5.8 | 6.2 | 6.6 | 6.0 | .22 |
| WB | JB | (d) | WB | 5.2 | 6.0 | 5.8 | 6.8 | 5.9 | .33 |
| | | | JB | 8.5 | 9.0 | 9.2 | 7.9 | 8.6 | .29 |
| WB | | (e) | WB | 6.3 | 6.4 | 5.5 | 6.2 | 6.1 | .20 |
| WB | | (f) | WB | 6.4 | 6.7 | 6.0 | 5.8 | 6.2 | .20 |

*See footnote to table 3.

Table 5. Effect of different treatments on the ascorbic acid content of fruits, 1947.

| Graft combination: Stock | Scion | Treatments* | Fruit analyzed | Ascorbic acid, mg/100 gm. of fruit, of 4 individual plants: 1 | 2 | 3 | 4 | Mean | ± S.E. |
|---|---|---|---|---|---|---|---|---|---|
| JB | WB | (a) | WB | 30.0 | 29.2 | 27.6 | 28.4 | 28.8 | .52 |
| JB | WB | (b) | WB | 28.4 | 28.8 | 28.0 | 27.6 | 28.2 | .26 |
| JB | WB | (c) | JB | 26.0 | 24.4 | 28.0 | 23.6 | 25.5 | .97 |
| JB | WB | (d) | JB | 25.2 | 24.0 | 26.0 | 24.0 | 24.8 | .49 |
| | | | WB | 27.2 | 28.0 | 30.0 | 26.0 | 27.8 | .83 |
| JB | | (e) | JB | 24.0 | 26.0 | 23.6 | 27.2 | 25.2 | .85 |
| JB | | (f) | JB | 24.8 | 24.0 | 26.0 | 25.2 | 25.0 | .42 |
| WB | JB | (a) | JB | 24.0 | 26.8 | 22.8 | 25.2 | 24.7 | .85 |
| WB | JB | (b) | JB | 27.2 | 24.4 | 24.0 | 26.0 | 25.4 | .74 |
| WB | JB | (c) | WB | 28.0 | 29.0 | 30.0 | 27.6 | 28.6 | .54 |
| WB | JB | (d) | WB | 30.0 | 28.4 | 31.6 | 26.0 | 27.6 | 1.20 |
| | | | JB | 24.0 | 26.4 | 23.6 | 26.8 | 25.2 | .82 |
| WB | | (e) | WB | 29.6 | 27.2 | 26.0 | 30.0 | 28.2 | .92 |
| WB | | (f) | WB | 30.0 | 27.2 | 28.0 | 29.6 | 28.7 | .65 |

*See footnote to table 3.

The variety White Beauty was found to be significantly lower in total soluble solids and titratable acidity, and significantly higher in ascorbic acid content than John Baer, as is evidenced from the results under treatments (e) and (f), the ungrafted controls. The values for these different composition factors for fruits obtained from the scions of plants under treatment (a) compared closely with that of the fruit of the scion variety used. This shows that the influence of the rootstock was negligible. The fruits from the scion shoot, which developed under the influence of stock leaves (treatment (b)), had more or less similar composition to that of the fruit from the scion variety. The stock fruit from treatment (c), which apparently obtained its elaborated food materials from scion leaves, contained total solids, acids and ascorbic acid comparable to the fruit from control plants for treatment (f). In other words, the defoliation of scion and stock, and the development of fruit in each case under the influence of the other symbiont, did not result in an appreciable change in fruit composition, which could be associated with either stock or scion influence. The shoots of grafted plants, in treatment (d), produced fruit on each graft symbiont with the quality characteristic of its own variety. These results show that the composition of fruit was not changed by any of the experimental treatments.

Instances, in fruit trees, of the influences of stock on the quality and composition of fruit borne by the scion, are quite common in horticultural literature. The observed effects have been explained either on the basis of direct influence of stock on the vigor of the scion or the indirect influence brought about by the degree of compatibility of stock and scion. As discussed earlier, the varieties used in these investigations were perfectly compatible with each other and the grafted plants grew without any signs of change in vigor. It appears that in this experiment, the fruits of one of the graft symbiont, although developed under the influence of the other symbiont maintained the metabolic activity of that variety.

## Experiment (1947-48)

Procedure - At the conclusion of the first year's work, it was thought desirable to limit the number of varieties used and pursue the studies under more controlled conditions in the greenhouse. It was also considered necessary to increase the scope of the problem by attempting to graft the plants at different stages of growth. Particular emphasis has been placed by Lysenko on the age of the scion. He has mentioned that the younger the plant, the characters of which are desired to be changed, the more successful will be the experiment. On the other hand, he has claimed that the plants from which it is desired to obtain a certain property or character must be older; it is best if they are middle aged.

The work was started during fall, 1947, in the greenhouse, with the object of studying the interaction of very young scions and comparatively older stocks. Two varieties which are stable for different characters, John Baer and White Beauty, were selected. A number of attempts were made to graft very young seedlings, but without much success at first. The failure was mostly due to inability to exercise proper controls as far as humidity, temperature and aeration were concerned. However, constant and vigilant efforts did help to develop the technique and secure conditions favorable for establishing the graft unions.

During January, 1948, twenty successful reciprocal grafts of John Baer and White Beauty were made, using the method illustrated in figure II. The stock plants in each case consisted of six-week old tomato plants with flower buds, which were removed just at the time of grafting. The scions consisted of small seedlings with two cotyledons and two small leaves.

The scions in all these grafts grew, but the leaves of the developing scions presented peculiar symptoms. They were thick, curled and deformed. The causes of these abnormalities were not accounted for, and the plants were rejected. Another attempt to graft younger seedlings met the same type of results and had to be given up in favor of the method followed during the previous year, with a view to repeat once again the experiment of Avakian and Jastreb (1941).

During the last part of April, 1948, eighty successful reciprocal grafts of John Baer and White Beauty were made, using the technique followed during the previous year. The plants used as stocks were six weeks old and 25 to 30 cm. in height. The scions were obtained from three weeks old plants. These grafted plants were set in the greenhouse in five rows during the first week of June. The rows were 3 feet apart and the plants in the rows were spaced 1½ feet apart.

In addition to the grafted plants, five plants of each variety, of the same age as those used for stocks, were transplanted for the purpose of comparing the fruit from the graft symbionts with fruit from ungrafted plants. Soon after the transplanted grafts showed signs of establishment, treatments were started. The grafted as well as the control plants were trained to two shoots on trellises. These trellises were especially set up in the greenhouse for the purpose of providing uniform light conditions which could not be procured otherwise because of close planting. In the case of grafted plants, one shoot from the scion and one from the stock were allowed to grow. The different treatments adopted consisted of the following types of training:

(a) In the case of ungrafted control plants, one shoot was defoliated and the fruit allowed to set and develop under the influence of the leaves of the other shoot, which was deflorated.

(b) The shoot from the scion was defoliated, the flowers were allowed to set fruit and mature under the influence of stock leaves but in the absence of stock fruit.

(c) The scion shoot was defoliated and the fruit was allowed to set and develop on both stock and scion shoots. In other words, the fruit on the scion shoot developed under the influence of leaves and fruits of the stock.

(d) This treatment consisted in defoliation of the stock shoot and allowing it to set and develop fruit under the influence of the scion which was not allowed to set fruit, but on which the leaves were kept intact.

(e) The stock was defoliated and allowed to set and develop fruit under the influence of scion leaves and fruits.

In each of the above treatments only the first cluster of flowers was allowed to set fruit on the defoliated shoot. All vegetative growth was supressed after the fruit had set.

The number of plants under different treatments are presented in table 6.

Table 6. Number of ungrafted and grafted plants under different treatments employed during 1948.

| Graft combination | | No. of ungrafted plants | Number of graft symbionts under different treatments | | | |
|---|---|---|---|---|---|---|
| Stock - | Scion | (a) | (b) | (c) | (d) | (e) |
| John Baer | White Beauty | 5 | 20 | 5 | 10 | 5 |
| White Beauty | John Baer | 5 | 20 | 5 | 10 | 5 |
| Total | | 10 | 40 | 10 | 20 | 10 |

Results and Discussion - The fruits of graft symbionts under different treatments developed normally. No changes in the characters under study were exhibited by fruit from grafted plants.

The fruits under treatment (b), where the influence of stock leaves in the absence of its fruits on the development of scion fruits was studied, were typical of the scion variety used. The fruits from the defoliated shoots of the scion, which developed under the influence of stock fruits and leaves, treatment (c), were normal and compared in form and color exactly with the scion variety used.

The influence of scion on stock fruit was studied in treatments (d) and (e). The stock fruit developed under the influence of scion leaves but in the absence of scion fruit in treatment (d) and under the influence of scion leaves as well as fruit under treatment (e). The stock fruit which derived elaborated food materials mostly from the scion leaves did not present any change in the characters under study.

These results which were obtained under carefully controlled conditions and strict observation of experimental treatments, confirmed the findings of the first year.

### Stock-Scion Relationship With Reference to Synthesis of Ascorbic Acid and its Relation to Total Soluble Solids and Acidity

Tomato varieties are known to differ considerably in the ascorbic acid content of their fruits. Is this difference in ascorbic acid content the expression of a character entirely under genetic control? Can the ascorbic acid content of fruits of a particular variety be changed by providing roots or photosynthesizing parts of another variety? To study these questions, an experiment was designed in which reciprocal grafts were made of plants of two varieties differing greatly in the ascorbic acid content of their fruits. Various treatments, to be discussed later, were given to secure the conditions for investigating the problem.

It was considered that the experiment might furnish some additional information in support of the studies previously reported.

## Procedure

Two varieties of tomatoes, John Baer and a selected line of high ascorbic acid tomato developed by Yeager and Purinton (1946), hereafter called "Yeager", were chosen for the experiment. The seeds of these varieties were grown in flats in the greenhouse during the beginning of March, 1948. The seedlings were transplanted into 4-inch pots a week after their emergence. A uniform and well mixed greenhouse potting soil was used to fill the pots. The plants of the two varieties attained a height of 25 to 30 cm. by the second week of May, 1948. They were then paired according to size. Twenty successful reciprocal grafts were obtained by using the splice method of grafting illustrated in figure 1. In addition, ten plants, five of each variety, were successfully grafted onto their own rootstocks. Ungrafted plants, ten in number, five of each variety, were selected to serve as controls.

The thirty grafted and ten ungrafted plants were transplanted in the greenhouse during the first week of June, 1948, in five rows for experimental treatments. In addition, one buffer plant was set at the end of each row. Each row contained eight experimental plants which included four reciprocal grafts of the two varieties; also two plants, one of each variety, grafted onto their respective rootstocks, and two ungrafted plants one of each variety. The rows ran North-South. They were 3 feet apart, and the plants were set 1½ feet apart in the rows. The soil conditions in the greenhouse were uniform.

In order to have a comparison of greenhouse and field grown tomatoes, ten ungrafted plants, five of each variety, of the same age as used in the greenhouse, were transplanted into the field during the first week of June, 1948. They were trained to a single stem and staked. The greenhouse tomatoes were trained on trellises to provide uniform light conditions.

The following treatments provided conditions for the study of the influence of rootstock on the ascorbic acid content of scion fruits in the two graft combinations:

(a) Ungrafted plants of each variety were trained to a single stem and the flower clusters were allowed to set and develop fruit. This treatment served as control.

(b) Two shoots, one from the stock and the other from the scion, were trained. The scion shoot, in each graft combination, was kept defoliated, and the fruits permitted to set on the first two clusters. Later, the shoot was decapitated just above the second cluster. The scion fruits developed under the influence of the stock shoot, on which no fruit was allowed to set but the leaves of which were kept intact.

(c) Only the scion shoot was allowed to grow and bear fruits. The stock provided roots and a small piece of stem.

(d) Two shoots, one from the stock and other from the scion of plants grafted on their own rootstocks, were trained. The scion shoot was defoliated and permitted to bear fruits on the first two clusters which were nourished by the stock shoot without fruits. This treatment served as control for comparison with treatment (b).

(e) Ungrafted plants of each variety grown in the field, were trained to single stems, and allowed to set and develop fruit under field conditions.

Roofs and walls of the greenhouse were whitewashed during the month of July, in order to keep the temperature low. This lowered the intensity of light and provided conditions for better and uniform distribution of light to the plants. Water was applied uniformly, whenever needed, to all plants during the season. However, these factors were not under control in the case of outdoor grown tomatoes.

A sufficient number of fruits started ripening in the last week of August to permit the analytical work on fruits to be done at that time. The ripe fruits, usually numbering four to eight, obtained from the first and second clusters of each plant under different treatments, were collected on August 27 and 28, 1948. These fruits were harvested around ten o'clock in the morning and placed in cold storage at 40°F. They were removed from the storage after 2 hours and analyzed for ascorbic acid, total soluble solids and titratable acidity. The methods used for these estimations have been already described. The data were statistically analyzed. Standard errors were calculated for each mean and the significance of the difference between two means was tested by calculation of Student's "t". Odds greater than 19:1 were considered significant.

## Results and Discussion

Ascorbic Acid Content - The ascorbic acid content of scion fruits from treatment (c) was not influenced by the rootstock (table 7). The variety John Baer, when provided with roots of Yeager, bore fruits which contained ascorbic acid comparable to the fruits from ungrafted control, treatment (a). Likewise, the Yeager scions (treatment (c)) yielded fruits which had more or less the same ascorbic acid content as the fruit from ungrafted Yeager plants of treatment (a), table 7. These results show that the synthesis of ascorbic acid proceeded in the green assimilating parts of the plant regardless of the root system. Harding et al (1938, 1942) found some differences in the vitamin C content of oranges which were associated with particular rootstocks.

The selective absorption from the soil, of particular mineral elements which are known to play some part in ascorbic acid synthesis, may explain those results. However, the results of the present investigation point out that the rootstock did not play any significant part, directly or indirectly, in the synthesis of ascorbic acid by tomato fruits.

The ascorbic acid content, of fruits of John Baer, which developed under the influence of the leaves of Yeager (treatment (b)) was significantly higher than the fruits from treatment (d), where the fruits of John Baer scion were furnished with elaborated food materials from the leaves of John Baer stock. On the other hand, the fruits of Yeager in treatment (b) had slightly lower ascorbic acid content, though not significantly so, than the fruits of Yeager in treatment (d). It appears that the leaves played some part in the synthesis of ascorbic acid of fruits either directly by supplying the synthesized ascorbic acid which was transmitted through graft unions, or indirectly by supplying the precursor or substrate to the fruits. Rubin and Spiridonova (1940) considered that, in tissues of fruits, where no correlation exists between ascorbic acid content and enzyme activity, ascorbic acid is a product of storage and has been synthesized by other tissues. The results of the present studies are insufficient to support their views. If ascorbic acid is considered a mere product of storage in fruits, which has been synthesized in the leaves and then translocated into fruits, there should have been wider differences in the ascorbic acid content of fruits from graft symbionts under treatment (b).

The ascorbic acid content of fruits of John Baer nourished on Yeager leaves, treatment (b), was significantly lower than that of the fruits from field grown tomatoes of the John Baer (treatment (e)). This further raises the question whether the increase in ascorbic acid content of John Baer fruits from treatment (b), over other treatments in the greenhouse, is sufficient to draw any conclusions. It would have been possible to draw final conclusions only if the ascorbic acid content of fruits from scions of graft symbionts under treatment (b), had approached very closely that of the respective rootstocks which supplied the elaborated foods to the scion fruits. The results of present findings suggest that there may be a mechanism in the fruit of a variety to prevent the accumulation (if ascorbic acid is synthesized in the leaves and translocated to the fruits for storage) or synthesis of ascorbic acid beyond certain limits. Kelley and Somers (1948), from their studies on the reciprocal grafts between potato varieties differing in ascorbic acid content of tubers, have concluded that it was regulated by the genetic constitution of the underground portion of potato plants regardless of the genetic constitution of the aerial parts of the plant. However, they have stated that the aerial portion of the potato plant is not entirely without influence. It was observed by them and Smith and Gillies (1940) that the ascorbic acid content of potato tubers decreased soon after the tops died. On the basis of these observations, they proposed that the living leaves and stems are essential for maintenance of a certain level of ascorbic acid in the tubers, which is determined by the nature of the underground portion of the plant.

The field grown tomatoes of the two varieties had significantly more ascorbic acid content than the greenhouse tomatoes of the respective varieties.

<u>Total Soluble Solids</u> - There were no significant differences among different treatments of any graft combination as far as total soluble solids of fruits of greenhouse tomatoes were concerned (table 8). The field grown tomatoes of two varieties had significantly higher total soluble solids than the greenhouse tomatoes of the respective varieties. It may be remembered that the field tomatoes also contained more ascorbic acid than the greenhouse tomatoes. It appears that the ascorbic acid content was correlated with the total soluble solids of the fruits. This correlation may hold only under certain environmental conditions. Harris (1941) observed that vitamin C was correlated with the sugar content

of tomatoes. He found that the tomatoes grown in the warm areas with much sunshine were also rich in vitamin C. However, McCollum (1944) did not notice consistent relationship between ascorbic acid and total solids and sugar content.

The increase in ascorbic acid content of John Baer fruits treatment (b) which were supplied with food materials by the leaves of Yeager, was not associated with an increase in total soluble solids. This shows that no relationship between ascorbic acid and soluble solids existed under the conditions of this treatment.

When the total solids and ascorbic acid contents of the two varieties, either under greenhouse or field conditions, are considered together, it becomes evident that the low total soluble solids content of Yeager was associated with a high ascorbic acid content whereas a high total soluble solids content of John Baer was coupled with a low ascorbic acid content. These varietal differences, with regard to the relationship of total solids and ascorbic acid, can be explained on the basis of two assumptions, (1) that the capacity of the two tomato varieties to synthesize and accumulate soluble carbohydrates (sugars) in their fruits was more or less alike; (2) that part of the ascorbic acid in the fruits was synthesized from the sugars under genetic control. Under the above assumptions, it may be said that a greater part of the total soluble solids was changed to ascorbic acid, under the control of genetic factors in fruits of Yeager, with the consequent result that a comparatively lower quantity of sugars accumulated in the fruits. On the other hand, a greater amount of sugars accumulated in the fruits of John Baer probably because the genetic constitution did not allow the conversion of sugars into ascorbic acid beyond certain limits.

Titratable Acidity - The differences with regard to titratable acidity of John Baer fruits from any two treatments were non-significant (table 9). The fruits of Yeager from different treatments of greenhouse grown tomatoes also showed no significant differences. However, the acid contents of fruits of Yeager from defoliated scions of treatments (b) and (d) were significantly higher than the field grown tomatoes.

The lack of definite evidence of increase or decrease of acidity in the fruits of either of the varieties of field grown tomatoes as compared with greenhouse tomatoes, and the presence of strong evidence that field grown tomatoes contained greater amounts of ascorbic acid and total soluble solids, suggest that there was no relationship between acidity and ascorbic acid or total soluble solids. Yarbrough and Satterfield (1939) and Harris (1941) also found no correlation between acidity and vitamin C content.

Table 7. Ascorbic acid content of ripe fruits of tomatoes from different treatments of two graft combinations, 1948.

| Treat-ment* | Graft combination Stock - Scion | | Ascorbic acid mg/100 gm of fruits from 5 plants analyzed separately 1 | 2 | 3 | 4 | 5 | Mean | ±S.E. |
|---|---|---|---|---|---|---|---|---|---|
| (a) | JB | | 16.0 | 15.6 | 16.0 | 15.6 | 16.7 | 16.0 | .20 |
| (b) | Y | JB | 17.3 | 16.0 | 19.4 | 17.5 | 20.5 | 18.1 | .80 |
| (c) | Y | JB | 15.2 | 15.2 | 16.7 | 15.6 | 17.1 | 16.0 | .40 |
| (d) | JB | JB | 14.8 | 16.3 | 16.0 | 14.8 | 16.3 | 15.6 | .35 |
| (e) | JB | | 22.4 | 20.9 | 20.9 | 22.4 | 19.8 | 21.3 | .50 |
| | | | | | | | | | |
| (a) | Y | | 28.5 | 33.3 | 29.6 | 32.7 | 30.8 | 31.0 | .91 |
| (b) | JB | Y | 28.5 | 27.7 | 25.6 | 26.6 | 27.7 | 26.8 | .86 |
| (c) | JB | Y | 27.7 | 32.7 | 30.4 | 29.7 | 31.9 | 30.5 | .87 |
| (d) | Y | Y | 30.4 | 26.6 | 28.5 | 31.2 | 32.3 | 29.8 | 1.01 |
| (e) | Y | | 41.8 | 53.2 | 51.3 | 53.2 | 50.2 | 49.9 | 2.11 |

*Footnote for tables 7, 8 and 9.

JB and Y indicate John Baer and 'Yeager' varieties respectively.

Treatment:
- (a) One stem with fruits; ungrafted plant (in greenhouse)
- (b) Two shoots, one from scion and one from stock; scion defoliated and its fruits developed under the influence of stock leaves (other variety).
- (c) One shoot from scion with fruits; stock provided roots only.
- (d) Two shoots, one from scion and one from stock; scion defoliated; its fruits derived food materials from stock leaves (same variety).
- (e) One stem with fruits; ungrafted plant (in field).

Note: (a), (b), (c), and (d) treatments performed in greenhouse and (e) in field.

Table 8. Total soluble solids content of ripe fruits of tomatoes from different treatments of two graft combinations, 1948.

| Treat-ment* | Graft combination | | Per cent total soluble solids of fruits of 5 individual plants | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Stock - | Scion | 1 | 2 | 3 | 4 | 5 | Mean | ± S.E. |
| (a) | JB | | 5.0 | 5.6 | 4.8 | 5.2 | 5.4 | 5.2 | .14 |
| (b) | Y | JB | 5.0 | 5.4 | 5.2 | 5.0 | 4.8 | 5.1 | .10 |
| (c) | Y | JB | 5.2 | 4.8 | 5.4 | 5.6 | 5.0 | 5.2 | .14 |
| (d) | JB | JB | 5.2 | 5.0 | 5.0 | 4.8 | 5.0 | 5.0 | .02 |
| (e) | JB | | 7.8 | 6.8 | 6.0 | 6.4 | 7.0 | 6.8 | .30 |
| | | | | | | | | | |
| (a) | Y | | 4.0 | 4.4 | 4.2 | 4.0 | 4.4 | 4.2 | .09 |
| (b) | JB | Y | 4.2 | 3.6 | 4.4 | 4.6 | 3.8 | 4.1 | .19 |
| (c) | JB | Y | 4.4 | 3.6 | 3.6 | 4.0 | 4.5 | 4.0 | .19 |
| (d) | Y | Y | 4.4 | 3.8 | 4.0 | 4.0 | 3.8 | 4.0 | .11 |
| (e) | Y | | 6.0 | 6.0 | 5.6 | 4.4 | 5.0 | 5.4 | .31 |

*See footnote table 7.

Table 9. Titratable acid content of ripe fruits of tomatoes from different treatments of two graft combinations, 1948.

| Treat-ment* | Graft combination Stock | - Scion | Acidity in terms of ml. of 0.1 N NaOH used to titrate a 20 ml. aliquot 1 | 2 | 3 | 4 | 5 | Mean | ±S.E. |
|---|---|---|---|---|---|---|---|---|---|
| (a) | JB | | 10.4 | 9.2 | 8.7 | 9.0 | 10.0 | 9.5 | .32 |
| (b) | Y | JB | 8.7 | 9.5 | 10.0 | 9.3 | 10.3 | 9.6 | .28 |
| (c) | Y | JB | 9.7 | 10.4 | 9.1 | 10.2 | 8.7 | 9.6 | .32 |
| (d) | JB | JB | 8.8 | 10.7 | 10.2 | 10.0 | 9.6 | 9.9 | .32 |
| (e) | JB | | 11.0 | 11.5 | 9.0 | 11.3 | 10.5 | 10.7 | .45 |
| | | | | | | | | | |
| (a) | Y | | 7.7 | 7.4 | 8.0 | 7.1 | 8.2 | 7.7 | .20 |
| (b) | JB | Y | 7.5 | 8.0 | 8.6 | 7.4 | 9.0 | 8.1 | .31 |
| (c) | JB | Y | 7.5 | 8.1 | 6.1 | 8.2 | 8.0 | 7.6 | .39 |
| (d) | Y | Y | 7.5 | 7.1 | 8.8 | 8.6 | 8.5 | 8.1 | .34 |
| (e) | Y | | 5.9 | 6.6 | 7.5 | 6.5 | 7.0 | 6.7 | .27 |

*See footnote table 7.

## Stock-Scion Relationship in Connection With Studies on Reciprocal Grafts of Normal Plants and Plants Modified Through the Action of 2,4-dichlorophenoxyacetic Acid

The malformations produced by phenoxy compounds, with their similarity to virus manifestations and induced chromosomal disturbances (Stein 1939), have been of interest ever since their observation. The mechanism by which the phenoxy compounds act and produce these physiological and morphological disturbances in plants is yet unknown.

The transmission of certain viruses through graft unions has been shown by various workers such as Vasudeva and Sam Raj (1948). Stein (1939) observed that grafting of a tomato mutant, with dwarf character and chlorophyll deficiency (radium induced variations which resembled 2,4-D induced changes), onto a normal tomato plant caused its elongation. However, other morphological characters of the grafted mutant remained unchanged. The object of the present investigation was to study how the plants, in which typical morphological changes had been induced by the use of 2,4-D, would react when grafted with normal plants.

### Procedure

Plants of the John Baer variety, five weeks old and 30 to 40 cm. in height, were treated by immersing their third basal leaves in a 1000 p.p.m. aqueous solution of sodium 2,4-dichlorophenoxyacetate during the first week of November, 1947. The necessary precautions, to prevent contamination of other parts of the plants, were taken by covering the treated leaves with cellophane bags.

After displaying the usual nastic effects, all plants developed malformations on their terminal parts. Three weeks after the treatment, when those effects were well established, reciprocal grafts were made with untreated plants. The method of splice grafting, illustrated in figure I, was used. Before grafting the treated leaves were removed. Twelve successful grafts of each combination were obtained. In addition, twelve plants each of the treated and untreated groups, were allowed to grow on their own roots. The graft unions were found established seven to ten days after grafting. Axillary shoots were not allowed to develop from the stock during this period and for two weeks afterwards. However, subsequently the plants were allowed to grow without being disturbed.

## Results and Discussion

Three weeks after the unions had developed, scions started growing appreciably. At that time signs of formative influences were noticed on some normal scions grafted on stocks in which morphological changes had been induced by treatment with 2,4-D. The symptoms evident on the plants three months after grafting were recorded. Quantitatively, there were considerable differences in the response of individual plants of the graft combination where normal scions were grafted on treated stocks. Qualitatively, however, a definite trend was noticed. Typical plants of each group are shown in figures III and IV.

Plant 1. An untreated plant. No peculiarities were evident.

Plant 2. A treated plant. It showed extreme modifications of leaves and even fruits.

Plant 3. A normal stock was grafted with a scion which originally was the apical portion of a treated plant. The tag and arrow indicate the graft union. With the exception of those few leaves which were modified before the scion was removed from the original plant, no morphologically changed organs were recorded.

Plant 4. A treated stock was grafted with a scion which originally was the apical portion of a normal plant. The foliage of the scion exhibited morphological abnormalities which indicated that the formative stimulus had been transmitted through the graft union.

Fig. III

Plant 1. Ungrafted, untreated.

Plant 2. Ungrafted, treated.


Figure III

Fig. IV.

Plant 3. Scion from a treated plant grafted onto a normal stock.

Plant 4. Scion from a normal plant grafted onto a treated stock.

Arrow indicates the graft-union.


Figure IV

From the experimental results, it would appear that greater quantities of the stimuli were stored in the stock portion and from there could influence the normal scion. It seems that the normal scion, after it had united with the treated stock (Plant 4), came under the constant influence of the stimulus just like the terminal parts of the treated but ungrafted plant (Plant 2). The stimulus moved from the lower portion to the young growing parts which subsequently developed modified leaves. Zimmerman and Hitchcock (1942) noticed that the cut stumps of tomatoes treated with p-chlorophenoxyacetic acid produced many new shoots with modified leaves, 30 days after treatment. They mentioned that it was not known whether the growing tips were still under the influence of the chemical applied to the stump or whether genetic changes could have occurred such as were induced by colchicine. They further suggested that if the influence were of a direct chemical nature the substance must continue to move downward in the old stem and then upward to the tip of the new shoot. The present findings indicate that no genetic changes were induced since the modified scion when grafted on a normal stock developed into a normal top (Plant 3). The influence seems to be of direct chemical nature. The modified scions used for grafting (Plant 3) either did not carry any formative stimulus or it was too insignificant to express itself in the form of modifications in new growth.

No positive statement can be made about the form of the stimulus. As mentioned by Zimmerman and Hitchcock (1948), only a few cases have been recorded in which the applied chemical was detected or identified beyond the region of application. The presence of 2,4-D in plants has not been shown after its absorption. However, numerous workers have reported on absorption and translocation of 2,4-D deriving their conclusions from indirect evidences of growth responses. Mitchell and Brown (1946) have suggested that the transport of 2,4-D is associated with that of organic foods. Nothing is known about the fate of the substance after its absorption and translocation and particularly during the subsequent period when morphological changes take place. Under the assumption that 2,4-D in the present experiment was absorbed, translocated and retained as such for three weeks, the conclusion is permissible that it was 2,4-D that moved through the graft union and caused the modification of the leaves of the scion. However, until such an assumption can be proved conclusively, it is a matter of speculation whether the causative agent was 2,4-D or a derivative or even a reaction product.

Attempts were made to test the validity of the assumption under which 2,4-D as such could be the form of the stimulus which moved through the graft unions. Direct approaches, by trying to isolate and detect 2,4-D, were made to investigate whether 2,4-D could be absorbed, translocated and retained as such for sufficiently long periods. The results of those findings do not lie within the scope of the studies reported here and will be presented in a separate paper elsewhere. However, it may be stated that it has not been possible to find detectable quantities of 2,4-D in tomato plants after its application to the leaves in concentrations as used in the present investigations.

## GENERAL DISCUSSION

The studies on stock-scion interaction, the results of which have been reported in the previous sections, were started in order to analyze the vegetative hybridization concept since no other work had been reported. Wilson and Withner's (1946) paper appeared after this work had been iniated. The results of the first years work during 1947 confirmed Wilson and Withner's findings. No changes in fruit characters of grafted plants were noticed under any graft combinations tried. The fruits were not influenced with regard to their composition in terms of ascorbic acid, total soluble solids and titratable acidity. These findings indicated that the metabolic processes of the fruits were not disturbed by the experimental treatments, which consisted in defoliating one graft symbiont and allowing its fruits to develop under the influence of the other graft symbiont. However, these results were not considered to be conclusive enough to disprove the vegetative hybridization concept. Lysenko has claimed that the interaction between stock and scion occurs only under appropriate biological conditions. He has stated that the frequency of obtaining vegetative hybrids would depend upon the ability of the experimenter to force the scion to "assimilate" as many as possible of the "nutrient" materials manufactured by the variety, the properties of which are to be transmitted to the scion. He has further added that in making grafts, the scion should be as young as possible and the stock of medium "age". As mentioned by Hudson and Richens (1946), Lysenko's terminology of nutrients and assimilation is very vague. Nutrients, to him, may be either inorganic substances, environmental conditions, whole organisms such as gametes, or organic substances such as are supposed to pass from stock to scion in grafting. Similarly, he uses the term assimilation in connection with all four types of "nutrients". It seems that when he mentions the comparative ages of stock and scion he may be basing his terminology on his concept of "Phasic Development of Plants". Considering this, the complete analysis of the vegetative hybridization concept becomes difficult. Lysenko and his followers, as revealed by the English translation of their work, fail to supply detailed information on the technical aspects of experimentation. It would be desirable to know more about their experimental procedures before their claims can be tested more adequately.

Attempts made during 1948 to graft very young scions with two cotyledons and two small leaves onto old stocks (six weeks old, from which the flower buds were removed at the time of grafting), though successful, did not help in the study of interaction between very young scions and old stocks. The scions soon after their establishment, presented peculiar symptoms in growth resembling virus manifestations, and the plants had to be rejected.

A repetition of the experiment of 1947, during 1948, which consisted in grafting scions obtained from three-week old tomato plants onto six-week old plants, confirmed the results of the previous year. It can be stated on the basis of these results, that an interaction between stock and scion, of the type claimed by Lysenko, is unlikely when plants are grafted at the stages of growth mentioned. However, the results of the present studies are too scanty to justify a definite conclusion and any claim that the findings of the Russian scientists have been disproved. The proof of a scientific discovery has always been in the reproduction of its crucial experiments. It is only by careful additional repetitions of the experiments which are supposed to lend support to Lysenko's theories, that the controversy will be resolved.

Considering Lysenko's theory of "Phasic Development of Plants" which is the subject of some disagreement but not complete rejection, it would seem desirable to repeat some of the experiments on grafting by selecting the stock and scion on the basis of physiological or developmental ages of the plants. This new approach would involve considerable work because of the technical aspects of the problem. It is rather difficult to select plants of different developmental ages because these may not be associated with any morphological changes; and profound internal developmental changes may be revealed only under definite environmental conditions. The problem is further complicated by the fact that no easy methods exist to ascertain some of these internal qualitative changes. It would be necessary to carry out these experiments under strictly controlled environmental conditions which are an essential prerequisite for the determination of physiological age of plants.

The studies on the interaction of stock and scion may be extended by attempting to find out the physiological differences between the grafted and ungrafted plants as indicated by certain constituents, such as vitamins. On the basis of the findings of some workers, which have already been reviewed, it would be logical to assume that substances of an alkaloidal nature can be transmitted from stock to scion or vice versa. These substances may play some role in growth and developmental phenomena. Wilson and Withner (1946) found no evidence of transfer of thiamin, riboflavin and niacin from one symbiont to the other in grafted tomato plants. The results of the present investigations on the synthesis of ascorbic acid gave no strong evidence that the ascorbic acid content of a tomato variety could be changed by allowing its fruits to develop under the influence of the leaves of another variety.

Although Lysenko's hypotheses are open to argument, a need for thorough analysis of his grafting experiments has been suggested. It is considered that any facts revealed by further repetition of his experiments, could be incorporated into the rapidly progressing fields of developmental physiology and physiological genetics.

Fyfe (1947) has stated that the new theories of genetics proposed by Lysenko may be entirely wrong and may be disregarded as a contribution to biology, but that his experiments must be adequately and carefully tested, before a verdict is passed. Whyte (1946) seemed to appreciate the findings of the Russian workers in their grafting experiments. He has proposed a connection between these results and the research on grafting in relation to hormonal exchange to induce flowering (Hamner and Bonner 1938), and even speculates about the possibilities of the application of these results to crop production.

Went (1938), in investigations of the effect of stock on scion, using different varieties of garden pea, found that the growth of the scion was not merely a simple nutritional effect. He considered that the expression of many characters in the scion apparently depended on the presence of growth factors in the rootstock which he named "calines". It is quite possible that such substances of hormonal nature may be present in tomatoes and may influence the stock-scion reactions, resulting in morphological changes. The possibility of a carry-over of such morphologically changed characters into the next generation is doubtful. However, there is evidence that some of the effects induced by synthetic growth regulators are carried through seed into the following generations (Clark and Wittwer 1949). The question of how this influence is carried through is yet not decided.

Strasburger as quoted by Batchelor and Webber (1948) found protoplasmic connections (plasmodesms) between the cells of the stock and those of the scion in certain plants; it is thus possible that there is migration of idioplasm and plasm particles. It does not seem likely that plastids or organized protoplasmic bodies would pass from scion to stock or vice versa, but substances such as hormones and enzymes may be expected to do so and produce characteristic reactions. The results of the studies on the transmission of the formative stimulus induced by 2,4-dichlorophenoxy-acetic acid, through graft unions, suggest that other similar stimuli can be translocated across the graft union.

The modern concepts in physiological genetics about gene action cover a very wide range. Some of the results claimed by Russian workers may be easily explained on the basis of the "hormone-gene" hypothesis proposed by Goldschmidt (1938). However, Schmuck (1945) has mentioned that the theory of the "hormone-gene" is an inadequate explanation of stock-scion interaction, especially in view of his opinion that the actual existence of the genes is very doubtful. The conception that genes are not inert protein molecules but play an active role in the physiology of cells, probably by releasing substances of hormonal or enzymic nature, which control the processes of differentiation and morphogenesis, makes the biologist assume that the essential continuity within the living organism is a chemical continuity. Goldschmidt (1938, 1940) has put forward the view that the determination of regional characters in development is brought about by the emission of successive "hormones". Each of these "hormones" reacts in turn with that part of the organism which has reached the appropriate stage of maturity or competence. According to this notion, local differences in rates of maturation lie at the back of differential determination in the tissues; specific "hormones" are responsible for the details of the individuation. In the light of such conceptions it would be natural to assume that substances which govern the destinies of cells could diffuse from one cell to another and change its ontogenesis.

It is generally believed that the somatic cells are genetically identical throughout the body; and yet they suffer extreme divergences in differentiation. Wigglesworth (1948) has suggested that "there must be elements in the cytoplasm concerned in these cellular specializations, ---'that the differences in cell heredity that arise within a multicellular organism are cytoplasmic'." Darlington (1944) and

Wright (1945) developed the idea of self-reproduction in cytoplasmic constituents, which they named "plasmagenes". Darlington has linked up heredity and development by showing that nuclear genes and plasmagenes both act and interact. Waddington (1948) has stated that certain characters of some plants are dependent on self-reproducing bodies in the cytoplasm. If the importance of cytoplasmic factors of a self-reproducing nature in morphogenesis is accepted then the explanation of stock-scion interaction claimed by Soviet scientists becomes evident. The possibility of migration of plasma particles from stock to scion or vice versa has already been discussed.

Haldane (1940) suggested that Lysenko's results might be explicable through the invasion of either stock or scion by a non-pathological virus from the opposite member of the graft. Espinasse (1941) supported Haldane's explanation. Later, in 1944, Haldane suggested that Darlington's remarks on bud-grafting in roses which in some cases causes a reversion of climbing habit to bush habit, might lend some support to the results obtained by the Soviet workers. Darlington (1944) regarded that the causative agent transmitted through graft union was a plasmagene rather than a virus, since the bushy habit in roses could not be considered pathological. He concluded his article by stating that "The frontiers that exist between the studies of heredity, development and infection are thus technical and arbitrary and new possibilities of analysis and experiment will arise when we have learnt the passwords to take us across". These considerations, that no real distinction can be drawn between cytoplasmic inheritance and virus infection, make the elucidation of results of the Russian work on vegetative hybridization difficult.

It is also difficult to distinguish between vegetative hybridization and the induction of mutation in one member of a graft by the other. Kostoff (1929) observed physiological interaction between stock and scion of certain solanaceous species accompanied by morphological changes. He explained the results by postulating antibody production. The possibilities of induction of mutations by antibodies have been suggested by Sturtevant (1944) and Emerson (1944). Sturtevant considered that, "if a particular gene is responsible for the formation of a given antigen, there is a possibility that the antibodies induced by this antigen may react with the gene". He has advised that cases presented in support of views demonstrating the inheritance of acquired characters should be reexamined with the gene-antigen-antibody interaction possibility in mind.

The above mentioned considerations make the task of analysis of the vegetative hybridization concept very intricate. However, there is no other convincing way than to repeat the experiments with all possible modifications, under strictly controlled conditions, in order to arrive at definite conclusions.

## SUMMARY AND CONCLUSIONS

The results of studies on stock-scion relationships in tomatoes are reported mainly with the aim of analyzing recent claims of Russian scientists that they have demonstrated the genetic interaction between stock and scion. In addition, the results are presented of studies, with reference to stock-scion relationships, on the synthesis of ascorbic acid, its relation to total soluble solids and titratable acidity, and on the formative stimulus induced by 2,4-D.

1. Seven graft combinations of the six varieties selected for the study, were made during 1947. The effects on the development of fruits of defoliated shoots of one graft symbiont under the influence of the other graft symbiont, were observed. No changes in visible characters of the fruits of a variety which developed under the influence of leaves of another variety, were produced, which might indicate an interaction between stock and scion. Analyses of fruits from graft symbionts under different treatments, for total soluble solids, titratable acidity and ascorbic acid, did not reveal any changes in fruit composition. Only varietal differences were observed with regard to the composition factors under study.

Attempts were made during 1948 with the object of studying the interaction of very young scions and comparatively older stocks. Scions with two cotyledons and two small leaves were successfully grafted on six-week old stocks. The scion leaves consistently developed abnormalities and studies could not be continued.

2. Studies on the ascorbic acid content of fruits were made on reciprocal grafts of two varieties differing greatly in their capacity to synthesize ascorbic acid. It was demonstrated that the formation of ascorbic acid proceeded in the green assimilating parts independently of the root system. No rootstock influence was observed.

Ascorbic acid content of fruits of one variety which developed under the influence of the leaves of the other variety, was not changed appreciably. No definite conclusion could be drawn as to whether ascorbic acid was synthesized in the leaves and then translocated into the fruits, or was independently synthesized in the fruits, the substrate being supplied by the leaves.

No changes in the total soluble solids and acid contents of fruits which developed either under the influence of rootstock without leaves, or rootstock with leaves, were noticed.

The total soluble solids showed a relationship to the ascorbic acid content. No relationship was found between the titratable acidity and ascorbic acid.

3. The formative stimulus induced by 2,4-dichlorophenoxyacetic acid was transmitted through graft unions of tomato

plants from stock to scion. The stimulus seemed to be of chemical nature but its actual form was not determined.

4. The results of the investigations, with suggestions for additional work to analyze more thoroughly, the vegetative hybridization concept, have been discussed. The results reported have been considered inadequate for drawing final conclusions.

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