

FACTORS CONDUCTIVE TO POOR STOCK-SCION UNION AND
VIRUS-LIKE SYMPTOM DEVELOPMENT IN DWARF PEACH

by

George N Agrios

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Plant Pathology

Approved:

Signature was redacted for privacy.

~~In Charge of Major Work~~

Signature was redacted for privacy.

~~Head of Major Department~~

Signature was redacted for privacy.

~~Dean of Graduate College~~

Iowa State University
Of Science and Technology
Ames, Iowa

1960

TABLE OF CONTENTS

INTRODUCTION	1
REVIEW OF LITERATURE	3
MATERIALS AND METHODS	23
SYMPTOMS AND OCCURRENCE OF DISEASED DWARF PEACH TREES IN THE NURSERY	27
CAUSE OF THE DISEASE	31
Observations of Elberta Peach on <u>P. tomentosa</u> and <u>P. besseyi</u> Rootstocks Transplanted in the Greenhouse and in the Field	31
Transmission Experiments	35
Attempts at transmission to peach and <u>P. tomentosa</u> from trees that had shown symptoms in the previous growth season	35
Attempts at transmission to peach from trees showing symptoms	38
Symptom Expression and Development on Trees with Poor Union between Peach Scion and <u>P. tomentosa</u> and <u>P. besseyi</u> Rootstocks	40
EXPERIMENTS ON THE POSSIBLE VIRUS EFFECT ON STOCK-SCION UNION	75
Virus Effect on Bud Take When the Bud or the Rootstock or Both are Infected with NRS Virus ..	75
Date-of-budding Effect on Bud Take When the Bud or the Rootstock or Both Carry the Virus ...	90
Bud Take of Healthy and Necrotic Ring Spot Virus-infected Peach Buds on Peach and Two Dwarfing Rootstocks	93
Growth of Peach Buds on Three Rootstocks in the Presence of Necrotic Ring Spot Virus	97

Survival of Peach Buds on <u>P. besseyi</u> and <u>P. tomentosa</u> 20 Days after Budding in the Presence of NRS Virus in Buds and Rootstocks	102
DISCUSSION	105
SUMMARY AND CONCLUSIONS	109
LITERATURE CITED	113
ACKNOWLEDGEMENTS	120

INTRODUCTION

In the last few years the production of peach trees on dwarfing understocks has become a commercial enterprise in Iowa nurseries. Standard procedure has been to bud standard peach variety scions on Sand cherry (Prunus besseyi) and Nanking cherry (P. tomentosa) seedling understocks.

During the summer of 1957, attention was directed to a peculiar malady of first year Elberta and Polly trees propagated on these two rootstocks. Appearance of many trees from July until leaf-drop in October was such as to suggest that the symptoms were induced by X virus. The potential destructiveness of X virus in peaches made it desirable to determine promptly whether or not it was the causal agent.

The several attempts to transmit X virus from afflicted trees, observations of subsequent growth of trees with and without symptoms in 1957, and observations of graft unions in trees with varying degrees of symptom expression, all tended to point to poor graft unions rather than virus as the basis for the malady.

Since, however, the presence of certain viruses in either the scion or the rootstock or both has been shown to interfere with bud take and to limit growth of a scion on a regularly compatible rootstock, it seemed desirable to know what would be the effect of a virus in a not-so-compatible combination, as seems to be the case with dwarf peach propagated on P.

tomentosa and P. besseyi rootstocks. Better budding results with early than with late budding has been reported (Brase and Way 1959) and was recognized by the nursery management.

Thus there was an opportunity to attempt an evaluation of three factors on bud take and subsequent tree development: 1. virus content of root and/or scion 2. date of budding, both with 3. compatible and relatively incompatible root-stock/scion combinations.

This thesis presents the results of experiments involving these three factors. They indicate that necrotic ring spot virus may reduce the bud take percentage if only the buds or the stocks, but not both, carry necrotic ring spot virus. There was a marked decrease in bud take with delay in budding after July and much better bud take on peach than on the relatively incompatible P. besseyi and P. tomentosa rootstocks. But there was no evidence of a cumulative or a counteracting effect of these three factors in these experiments.

REVIEW OF LITERATURE

Symptoms observed in 1957 and 1958 on peach trees propagated on Prunus besseyi and P. tomentosa rootstocks were similar to those reported in the literature as being induced by virus, particularly the X-disease virus. The symptoms of X-disease on peach, as summarized by Reeves et al (1951) are: irregular pale-green areas on the leaves, drop of the early infected leaves and yellowing or reddening of the leaves remaining on the trees later in the season. In late summer there are on the leaves tan to brown necrotic spots, usually surrounded by a reddish brown or purplish border. Leaves of young actively growing infected trees show a tendency to be rolled upward toward the midrib.

In 1937 Blodgett found in a nursery peach trees showing severe shotholing, reddish and purplish coloring of foliage and generally dull reddish color of bark. In almost all cases these trees, representing several varieties, were making poor growth, although their root system seemed to be normal. He considered this to be a virus disease, even though he was not able to transmit it (Blodgett 1939). Richards and Hutchins (1941) reported a disease on peach similar to western X-disease. When buds from diseased trees were grafted on healthy Elberta trees, symptoms were expressed in six weeks. Thomas in 1940 described a transmissible leaf casting yellows of peach and considered it similar to if not identical with dis-

eases of peach in other areas, including the X-disease of the northeastern states.

Bodine and Durrell (1941) found peach trees with X-disease in Colorado. Elberta peach trees budded with diseased buds on August 1, 1940, showed symptoms (60 per cent of the trees) next June or July. Zeller and Evans (1941) in trying to transmit Western X-disease and marginal leaf spot of peach, inserted buds on Lovell peach seedlings in the fall and got definite X-disease symptoms in fifty per cent of the cases the next spring. Hildebrand (1941) working with yellow-red virosis in peach got transmission of symptoms in a month by cutting the tops of the twigs and forcing new growth. In 1950 Richards and Wadley summarized the information then known about Western X-disease virus and concluded that Western X-disease virus causes Western X-disease in peach, red-leaf disease in chokecherry, wilt and decline and Western X-little cherry in sweet and sour cherries. They also reported that peach and cherry orchards in northern Utah often have 40 to 80 per cent infected trees. Schneider (1945), in studying the anatomy of Buckskin-diseased peach and cherry, found that in infected cherry trees on mahaleb stock, wound gum formation was very extensive even in mid-summer in the summer phloem. Ultimately the youngest sieve tubes were affected in the mahaleb stock just below the bud unions.

A shortening of internodes resulting in stubby growth accompanied with brittleness of infected twigs, red to

reddish-brown spots in some leaves, rolling and chlorosis of leaves similar to that of X-disease and yellow leaf roll were described by Wagnon et al (1958) as "stubby twig disease" caused by virus. Positive transmission was obtained in 80 per cent of the cases, involving eight peach varieties.

It is worthy of note that in all the cases recorded above, except one, transmission by bud grafts was successful.

Long before such symptoms were observed on peach, scion-rootstock incompatibility had been reported to cause symptoms suggestive of virus disease in plants other than peach. Thus Laubert (1914) described a leaf-roll and yellowing of lilac plants which he attributed to excessive pruning, complicated by numerous other possible environmental influences. Others later and chiefly Chester (1931) described the same disease and submitted evidence that the condition is caused by graft incompatibility. A devitalization of citrus trees on lemon rootstocks characterized by small leaves with dull, dingy hard appearance was described by Rhoads (1930). Schuster and Miller (1933) reported poor shoot growth and premature shedding of the leaves on a part or all of the tree of Persian (English) walnuts grafted on black walnut stocks. Suckers appeared from the rootstocks and the trees died three or more years after the first symptoms.

Proebsting and Hansen (1943) observed a leaf scorch and die back of apricots on Myrobolan rootstocks. Leaf symptoms varied in intensity from a cupping of the leaf to severe

marginal scorch, followed by excision of the dead marginal tissue. They treated the trees for K deficiency, alkali injuries, S, Ca, B, Mn, Mo, Zn, Th, Ba, W, Cr, Cd, and Co deficiencies without satisfactory results. Diseased trees when inarched, or when they developed scion roots, were always healthy.

Overholser (1947) suggested that the incompatibility reactions between sour orange rootstock and various citrus scions is probably directly related to the problem of tristeza (also known as quick decline or bud union decline) and so probably due to virus. He notes that perhaps the virus is present in a latent form in certain species or varieties and produces pathological conditions only when certain stock-scion combinations are made. Weeks (1948) studying the incompatibility between Spy 227 and certain apple varieties, has demonstrated that a toxic principle causing the death of Spy 227 can be transmitted by grafting from a lethal strain to a congenial strain, the principle not being manufactured in the leaves. He concludes "It would appear from the evidence at hand that the toxic principle is a virus. If it is a virus it is of a most peculiar nature, in that it only manifests itself when two comparable factors are brought together. There is no evidence that this virus has a vector." A similar case has been reported in relation to a known virus. When sweet cherries infected with "buckskin disease" are grafted on mahaleb stock, the stock becomes infected and dies

(Thomas and Rawlins, 1941). On the other hand, when the sweet cherry is on mazzard roots the tree is not seriously affected by the virus disease (Rawlins and Parker, 1934).

Armstrong and Brison (1949) reported a delayed incompatibility of a live oak-post oak graft union. It was shown that the graft union was normal until 2 or 3 inches in diameter, but 6 or 7 years after the grafting, breaks in the union between stock and scion began to occur. Schneider (1957) described a chronic decline, a tristeza-like bud-union disorder of orange trees characterized by excessive numbers of fiber sheets immediately below the bud union, where also the bark sometimes becomes abnormally thick.

Few cases of incompatibility involving peach have been reported. Chang (1938) mentions that a Chinese publication in the year AD 500 states that plum can be successfully grafted on peach whereas the peach usually fails to grow on plum stocks. Mason and Hall (1897) reported that peaches on plums early began to show signs of failing and developed a trunk with strongly marked enlargement just above the stock. All 44 peach trees on Myrobolan were either dead or in very poor condition - conclusive evidence of incompatibility between Myrobolan stock and peach scion. This is in accordance with Schneider's (1945) observation that peach scions on Myrobolan rootstock often grow normally at first, but later exhibit yellow, rolled leaves with swollen veins and some premature defoliation. Such stems contained necrotic sieve

tubes with wound gum. Happner and McCallum (1927) also found that peach was a good stock for plums but peaches and almonds generally failed to grow on plum rootstocks.

Overholser et al (1943) reported that peach as a rule is not satisfactorily grown as a dwarf tree. Apricot seedlings exhibit some uncongeniality with the peach, resulting in a dwarfing effect. The Myrobolan plum (Prunus cerasifera), Marianne peach (P. cerasifera X P. munsoniana), St. Julien plum (P. domestica var. insititia) and the western sand cherry (P. besseyi) all give a dwarfing effect when used as understocks for peaches.

Buds of Hale's Early peach inserted, at weekly intervals from June 24 to August 26, into a compatible rootstock (Brompton) and an incompatible rootstock (Myrobolan B) with that variety all gave rise to trees typical of compatible and incompatible stock-scion combinations respectively (Garner and Hammond, 1938). The percentage "take" of grafts was 55 per cent on Myrobolan B and 81 per cent on Brompton. The symptoms of incompatibility in the peach-Myrobolan B combination were a sudden cessation of growth in mid-summer. Time of budding did not have any lasting effect, if any at all, upon stock-scion compatibility.

In the experiments of Herrero (1951), in September of the first growth season the leaves of Hale's on Myrobolan B showed a tendency to curl upwards at the margins and developed a reddish color starting at the edges, first on the leaves at the

apex of the shoot and then spreading downwards. On October 28, 80 per cent of the leaves of Hale's on Myrobolan B had fallen on the ground compared to 5 per cent of those on Brompton. During the next winter a few trees on Myrobolan B died, the death of the scion preceding that of the stock. In the second year small growth developed and typical curling and red discoloration of the leaves appeared in August. Overgrowth of the scion at the union of Hale's on Myrobolan B was evident and in some places where the edges of the stock and scion were not matched the ingrowing bark of the scion was pushing the components apart. However, the actual amount of vascular continuity necessary for healthy growth of crown is very small. A union may be discontinuous in many parts and have little effect on the general behavior of the tree. Herrero examines the possibility of a virus being involved in this incompatibility, but, he states that if a virus is involved it shows only when these two varieties are grafted together. He observed that while in the pear-quince combinations studied by him abnormalities occurred mainly in the graft union, in the peach-plum combinations there were in addition restricted vegetative growth, signs of ill health in the leaves accompanied by early defoliation, premature degeneration of the phloem and abnormal distribution of starch.

Mosse (1955) studied the behavior of two trees of Hale's Early on Brompton with a ring of Myrobolan B tissue in the Brompton rootstock stem (6-9 inches below the peach bud).

Because incompatibility symptoms developed in the Hale's scion in spite of extensive bridging of the Myrobolan B rings and because there were certain abnormalities in the structure of the ring tissues, she suggested that probably this is a genuine example of the transmission of incompatibility symptoms to an otherwise compatible stock-scion combination. Myrobolan B trees on Brompton rootstock were perfectly compatible with no sign of incompatibility at the union or any cambial disorder in the Myrobolan B. Therefore, she attributed the abnormal growth of the Myrobolan B ring graft tissues to the presence of the Hale's scion, which also showed some die-back of shoots and buds and later definite symptoms of incompatibility, although substantial bridges of Brompton tissue formed across the split Myrobolan B bark ring during the year. The roots of the ring-bark grafted trees contained no starch, and on the foliage most of the starch was accumulated in the more severely affected branches. In healthy trees there was much starch in the roots and uniformly small amounts in the stems.

Sax (1956a) reported peach interstocks used as a compatibility bridge of apricots on Prunus tomentosa rootstocks. P. tomentosa seedlings were first budded with a compatible peach variety and the following year the peach was budded about 6 inches above the graft union, with apricot. Sax (1957) also described the Nanking cherry (P. tomentosa) as an excellent dwarfing stock for ornamental peaches and plums.

He noted that P. besseyi rootstock produces dwarf, productive peach and plum trees, but the trees in the nursery often tip on their side and P. besseyi suckers badly from the roots. The Nanking cherry is not as compatible with so many varieties. Individual seedling rootstocks may vary largely in their compatibility with a given scion variety. Only about 10 per cent of the P. tomentosa seedlings were compatible with Elberta peach (although he notes that other propagators reported a good "take" of Elberta on P. tomentosa). Holmes (1957) describes the use of P. besseyi and P. tomentosa seedlings as understocks for dwarf peach budding. He states that the "take" to peach buds varied from 78 to 91 per cent.

Several investigators have attempted to examine the nature and the cause of incompatibility. In many cases the scion has grown but the union is bad, that is, mechanically weak (Waugh, 1904). When a branch breaks at the point of graftage it is adequate evidence that that was the weakest point in the particular branch. He suggested that because of some physiological irritation the wound does not heal readily, considerable quantities of loose meristematic tissue are deposited and fill the space, whereas in a successful graft the long parenchymatous cells and ducts interlace. Loose corky tissue, filling in between the wood and the bark, forms the large swellings at the point of junction.

Poor unions were thought to be brought about by excessive production of callus tissue (Bailey: 1923, Dirks: 1925,

Fisk: 1927), cork tissue formation between the stock and scion (Dirks: 1925), failure of cambiums to unite or to maintain cambium continuity (Dirks: 1925, Bradford and Sitton: 1929) or failure of conduction tissues to be formed (Dirks: 1925, Countryman: 1931). Roberts (1949) however, considers incompatibility any abnormal reaction between stock and scion and not only the effects arising directly from the union. According to Argles (1937) the form or type of failure is governed or influenced by environment and treatment, but the term incompatibility should not be applied to failures that are caused by environment and treatment.

Roberts (1929) observed that the stock effect on the scion is localized in the stem portion of the stock. His observations indicated that a direct grafting upon a piece of root will respond differently than upon the stem of a seedling or upon a rooted stem cutting. He suggests that stock effects are problems of transport and in some cases the carbohydrate materials seem to be involved in this particular influence.

Warne and Raby (1939) point out that certain growth characters of trees on dwarfing rootstocks are features that might be expected if the water supply to the shoots was somewhat restricted. Sax and Dickson (1956) observed that the inversion of a ring of bark on the trunk of a tree results in checking phloem transport to the roots and dwarfing the tree.

On the other hand it has long been known (Cochran et al 1951) that the degree of success of the union between stock and scion is influenced by the presence of a certain virus or combinations of viruses or strains of viruses in the stock or in the scion or both. The same factors also influence the percentage of successful scion-stock unions and the subsequent growth and performance of the scion. This influence, however, was found to vary with the species used as stock or scion and it also varied with the time of budding in relation to, or regardless of, the time of infection with the virus.

Working with oriental flowering cherry (Prunus serrulata), Milbrath and Zeller (1945) found that its virus-free buds failed to take when they were placed on mazzard seedlings or cherry varieties containing the ring spot virus. However, no appreciable difference was noted in the number of failures when ring spot virus-infected buds of apricot, almond and plum were placed on Lovell peach than when healthy buds of the same species were budded on the same peach stock. They suggested that the bud failure is due to the killing of newly invaded cells of the stock adjacent to the diseased bud shield, which prevents union of bud and stock.

The very indexing of stone fruits on Shirofugen for the ring spot virus (Milbrath and Zeller 1945, 1948) is based on the principle of reaction of a virus-infected bud when placed on a virus-free Shirofugen plant. When trees are inoculated with diseased buds the virus moves slowly from these diseased

buds into the surrounding tissue, while the bud dies. The tissue invaded becomes necrotic, and if left unimpeded the virus will eventually move down the stem, killing the whole plant.

When peach buds infected with ring spot virus are budded on healthy Lovell peach seedlings or other peach variety trees, the buds usually die without making any perceptible union, yet a high percentage of the seedlings or trees become infected (Cochran et al 1951). Healthy buds of a variety on a virus-infected rootstock also often fail to live. Insertion of virus-infected buds on virus-infected rootstocks results in normal bud union and no abnormal number of bud failures. They state that bud failure of virus-infected buds on healthy stock varies, depending on the relative severity of the virus strain on severely damaged varieties and the relative susceptibility of the seedling or variety understock to injury. A greater percentage of peach buds carrying the severe virus strain fail on all peach varieties than of buds carrying only mild virus strain. But even buds carrying only mild strains sometimes fail on peach varieties which are strong reactors. Some varieties such as Lovell are only mildly affected by forms which severely injure J. H. Hale.

In nursery plantings the percentage of bud take is often poor when the buds used for propagation are infected with virus (Gilmer et al: 1957). Even when bud take is successful, growth of the produced budlings may be abnormal. These in-

investigators report 72.3 per cent bud take with Montmorency buds infected with necrotic ring spot virus, compared to 84 per cent bud take with virus-free Montmorency buds. How much the bud take and the growth of the infected budlings will be reduced depends a great deal on the particular virus or virus strain involved (Millikan, 1955).

The behavior of various rootstock-scion combinations following inoculation with mild and severe strains of a virus was studied by Costa et al (1954) in citrus trees. They found that on non-tolerant sour orange rootstock, all varieties inoculated with the severe strain of tristeza virus, were dead or made extremely poor growth. When inoculated with the mild strain of the virus the average growth was 50 to 70 per cent of the normal. On the virus-tolerant sweet orange rootstocks, the sweet orange and tangerine tops had very good growth (average 80 to 100 per cent) when inoculated with the severe strain or with the mild strain. The tristeza virus, however, has some detrimental effects on grapefruit tops even when they are grown on tristeza-tolerant rootstocks, on which the severe strain limited growth to 60 per cent, the mild strain to 82 per cent of the normal. On non-tolerant rootstock the severe strain limited growth to 14 per cent, the mild to 70 per cent of the growth of healthy trees. Olson (1958) grafted lime scions carrying a mild virus strain on a rootstock carrying the severe strain. The scion tops during the next 20 months developed no symptoms of the severe tristeza strain. Upon

indexing, however, 25 of the 37 index plants showed definite severe symptoms and 12 showed only symptoms of the mild strain, which indicates that the severe strain moved from the rootstock to the mild-strain-infected growth resulting from the scion, even though the top showed only mild symptoms during the 20-month observation period. When an attempt was made to grow similar plants with a severe-strain-infected top and a mild-strain-infected rootstock the results were unsuccessful because shoot growth did not develop from the scions; sucker growth was abundant from the rootstock.

Milbrath (1950) found the differences in growth of the ring spot virus-free cherry trees and the regular nursery stock to be quite apparent without comparative measurements (8-14 inches taller). When mazzard seedlings were budded in August with buds of Royal Anne, Bing and Lambert containing no virus, a mild strain, or a severe strain of ring spot virus, and the budlings were measured at the end of one growing season, the mild strains of ring spot did not limit growth as much as the severe strains. He also reports that the stand of buds in a row of Montmorency nursery stock is reduced as much as 50 per cent by ring spot virus in the scion wood.

Buds collected from random Montmorency trees of a nursery block and budded into P. mahaleb seedlings, in the next season produced 30 per cent less growth than buds from ring spot virus-free Montmorency trees (245 inches to 186 inches) (Millikan 1955b). Comparison of the growth of ring-spot-

affected and ring-spot-free Montmorency trees indicated that certain strains of ring spot virus caused a significant reduction in growth. This reaction, however, was apparent only in the first part of June and disappeared during the last part of the growing season.

A rootstock effect was noticed by Hildebrand (1953) when certain strains of ring spot virus were inoculated into Montmorency trees and much more conspicuous and severe symptoms were produced in trees on mazzard than on mahaleb roots. In the case of cherry ring spot virus, before uninspected orchard budwood was prohibited, skips were occasionally observed in nursery plantings. On closer inspection the surviving mahaleb rootstocks showed leaf symptoms of a line pattern character. In subsequent experiments Montmorency was budded to some of the diseased mahaleb rootstocks with fair success. However, reciprocal experiments with diseased Montmorency budwood on healthy mahaleb consistently resulted in bud failure. It was suggested that mahaleb rootstock is an obstacle to the introduction of necrotic ring spot into the nursery from orchard sources. When the mazzard rootstock was employed there was a low but consistent percentage of sweet cherry bud survival.

The time of infection with a virus and its relation to the symptoms was reported by Davidson and George (1957) in an experiment in which virus-free Montmorency cherry trees were inoculated monthly with an isolate of necrotic ring spot virus.

Shock or etch symptoms were the only manifestations and the only variation in the shock symptoms was their location in relation to the point of inoculation; therefore, unless the point of infection was known the progress and location of shock was of no value in determining the time of infection. Working with the ring spot virus complex in sweet cherry, Millikan (1955) observed that the production of severe symptoms apparently is limited to inoculation at bud break or shortly thereafter. Field inoculations made about six weeks after bud break, failed to produce symptoms on the host while the necrosis caused by the bud break inoculations was replaced by leaf symptoms diminishing in severity. Mild symptoms of ring spot appeared in the next year on most of the trees inoculated after bud break the previous year.

Fink (1950) introduced Prunus tomentosa as an index plant for necrotic ring spot virus. He showed P. mahaleb to be a carrier and source of virus introduction into Iowa nurseries and showed many species of cultivated and wild Prunus to be reservoirs of necrotic ring spot virus and play a role in the contamination of virus free sour cherry trees. The introduction and spread of necrotic ring spot virus in sour cherry nursery blocks were studied by Hobart (1954, 1956) and Hobart et al (1955). Increases of 11, 9, and 4 per cent within 30 days were observed in three nursery blocks when 100 trees in each were indexed. The pattern of spread shows that some of the newly affected trees are adjacent to already infected

trees, others in groups of newly infected trees and some single trees completely isolated from other infected ones. Necrotic ring spot (NRS) virus was transmitted in all artificial root grafts between intra- or inter-specific pairs of P. americana, P. mahaleb, P. avium, and P. persica. In nature, however, root contacts, but no naturally occurring root grafts, were found in the field. There was evidence of virus passage from scion to rootstock, thence via root graft to another rootstock and to its scion, in seven root grafted pairs of cherry and eight pairs of peach nursery trees (Hobart 1956).

The contact periods in graft transmission of peach viruses was first reported by Kunkel (1938). Yellow virus did not pass from diseased buds into tissues below the points at which the trees were cut in as short a period as 4 days, but it sometimes passed in 5 days and it usually passed in 6 or 7 days. When the inoculation buds were removed the contact periods necessary for transmission varied with the season of the year and with the age and condition of the trees inoculated. The shortest periods (3-7 days) were obtained in early spring when trees were growing fastest. As the season advanced, the periods lengthened (8-14 days).

Peterson (1958) found that contact periods of more than four days were required for transmission of NRS virus to P. tomentosa seedlings inoculated at bud break with dormant P. tomentosa buds and to Gilbert Montmorency trees inoculated at

bud break with dormant fruitmorency buds. Many P. tomentosa plants which had been inoculated once or twice did not express symptoms the second time, and on those which did, the symptoms were mild. When P. tomentosa seedlings were inoculated with NRS virus in winter, then returned to cold storage and set in the field in spring, all expressed symptoms. Frindlund (1959) also studied the time and temperature requirements for the transmission of the necrotic ring spot virus of Prunus. He found that when P. tomentosa seedlings grown at 26 degrees C. were inoculated with virus cultures #2 and #7 from P. tomentosa or P. mahaleb and were then transferred to temperatures of 30, 26, 22 and 18 degrees C., all plants at 30 degrees C. became infected after contact of 72 hours, while those at 18 degrees required 168 hours. Culture #7 infected a few more plants than #2, and cultures #2 and #7 from P. tomentosa were transmitted slightly more often than from P. mahaleb. He concluded that a more severe reacting virus and a compatible bud-host combination at a given temperature and with a given time of contact result in slightly more transmission than a milder reacting virus or a non-compatible combination.

Willison et al (1948) note that a greater percentage of trees are likely to be infected with necrotic ring spot than with yellows when an orchard is set out. The rates of spread are largely determined by initial incidence and by the relative position of affected and healthy trees at planting.

After Cochran (1946) noted the passage of the ring spot

virus through mazzard cherry seeds, Cation (1949) followed with the demonstration that more than 10 per cent of mahaleb cherry seedlings grown from commercial seed carry the ring spot virus. Cochran again (1950) reported the passage of the ring spot virus through Lovell peach seeds, while Gilmer (1955) found that 16 per cent of the seedlings from "French-imported" mahaleb seed were NRS virus-infected. A most interesting paper came from Way and Gilmer (1958) who demonstrated transmission of the necrotic ring spot virus in cherry through pollen. In their experiments a healthy English Morello plant was pollinated with pollen from NRS virus-infected and yellow-infected Montmorency trees. When 18 of the resulting seedlings were indexed on cucumber, 5 of them were found NRS virus-infected. They called attention to the point that NRS virus transmitted through pollen to seeds developed on virus-free trees may be translocated back into mother tree - although this has not been proved yet. This may be one way of spreading of NRS virus in an established orchard. Another point is that seeds, even though produced on a virus-free tree, may carry NRS virus, which came from the pollen parent.

Gilmer and Brase (1960) advocate that the probability of virus infection of a nursery tree at propagation depends on three factors:

V_s = virus incidence in the scions
 V_r = virus incidence in the rootstocks
 K = effect of presence of the virus on scion-stock union

The values of K depend on both: 1. The individual virus and 2. the scion-rootstock combination. The total virus incidence $V_t = K(V_s+V_r-V_sV_r)$. When $V_r = 0$, then $V_t = V_sK$, which means that irrespective of the number of successive propagations, virus incidence remains at the level originally established in the initial propagation. When $V_s = 0$ but $V_r > 0$ because of the commercial propagating practices, the formula $V_t = V_rK$ becomes $nV_t = K[1-(1-V_r)^n]$, where n is the number of serial propagations. This means that when infection is derived from rootstocks, an increment of virus infection is added with each successive propagation. So, in nursery certification programs, increased emphasis should be placed on virus-free rootstock procurement.

Millikan (1959) has found NRSV-infection to vary from 8 per cent in one peach orchard to over 20 per cent in the case of a newly established scion block. Random Lovell seedlings showed about 15 per cent infection, suggesting that these are a major source of inoculum. The vigor of the trees found to be infected, however, does not seem to be visibly impaired and there appears to be no difficulty in locating disease free clones in any one peach variety. According to Wagnon et al (1960), though, 106 peach seedlings which showed no evidence of virus infection by visual inspection and the Shirofugen tests averaged 54.77 inches in height, whereas 43 seedlings which gave a positive test on Shirofugen averaged 46.58 inches in height (highly significant in 0.05 level).

MATERIALS AND METHODS

Diseased and healthy Prunus persica variety Elberta trees grafted on P. besseyi and P. tomentosa rootstocks were furnished by the Mount Arbor Nurseries, Shenandoah, Iowa. Those were taken from stocks of one-year salable trees grown in 1957. The P. besseyi rootstocks were from seed that came from E. C. Moran, Stanford, Montana. The P. tomentosa rootstocks were grown from seed purchased of Seitaro Arai & Co., Ltd., I Onsecho, Naka-ku, Yokohama, Japan.

Prunus persica varieties Elberta, Golden Jubilee, Halehaven, and Polly, propagated on P. tomentosa rootstocks were growing in a field block in Mount Arbor Nurseries in 1958.

Prunus persica variety California Lovell seedlings growing in the fields of Mount Arbor Nurseries were made available for transmission experiments in 1957 and 1958. In 1959, a nursery block of 2800 California Lovell peach seedlings, half of which had been inoculated with necrotic ring spot virus, were used as understocks for Elberta peach buds, half of which were also infected with NRS virus.

Prunus persica variety Elberta buds free of NRS virus or infected with NRS virus were obtained from 60 nursery block, one-year-old peach trees, growing in the Mount Arbor premises in 1959 and 1960.

Prunus persica variety Elberta on peach seedling understocks were from a commercial lot and furnished by Mount Arbor

Nurseries.

Prunus tomentosa Thunb. seedlings for transmission experiments, and also as rootstocks for peach propagation, were obtained from Plumfield Nurseries, Fremont, Nebraska. One batch of 1800 seedlings were planted outdoors in 1959 and half of them were inoculated with NRS virus. Later on all seedlings were budded with virus-free or virus-infected Elberta peach buds. Another batch of 1100 seedlings, while in cold storage in February 1960, were budded half with dormant cherry buds carrying the NRS virus and the other half with NRS virus-free buds and then were put in storage again until the temperatures became favorable and the seedlings were planted outdoors. They were budded again with virus-free or virus-infected peach buds on August 3, 1960.

Prunus besseyi seedlings for rootstocks were received from Plumfield Nurseries, Fremont, Nebraska. They were treated in exactly the same way as the *P. tomentosa* seedlings that were inoculated while dormant, as described above.

Prunus serrulata variety Shirofugen grown in the Interstate Nurseries, Hamburg, Iowa, was used for all field indexings of peach bud sources and necrotic ring spot virus sources.

Necrotic ring spot virus was obtained from infected Fruitmorency trees grown in Lake's Nurseries, Shenandoah, Iowa. Part of the virus-infected buds that were used to inoculate P. besseyi and P. tomentosa seedlings in 1960 was obtained from a Special Montmorency tree on the Botany and Plant

Pathology plots at Ames.

All trees used in the greenhouse were grown in unsteamed compost soil in clay pots. The healthy and diseased Elberta trees on P. besseyi and P. tomentosa rootstocks were pruned to a height of about 80 cm. Branches were pruned by cutting them at lengths up to 15 cm or removing them completely. Roots were also pruned so that the trees could be placed in 25 cm pots. The potted trees were placed on the walks along the North and West walk of a greenhouse unit.

The P. tomentosa seedlings were placed in 12 cm pots, were pruned so that only a whip about 45 cm tall remained and were either placed on greenhouse benches for immediate use or stored out of doors until needed. The Elberta trees used for inoculations received exactly the same treatment as the P. tomentosa seedlings. The night temperatures of greenhouse rooms ranged from 21 to 24 degrees C. and the rooms were kept near to these temperatures during the day.

In summer transmission experiments California Lovell seedlings were budded in place on the premises of Mount Arbor Nurseries where most of the field observations were also made.

The Prunus tomentosa seedlings and the Elberta peach trees were inoculated 6 to 8 cm above the soil line. The inoculations were made by removing a shield-shaped piece of bark about 2 cm long from the bark of the tree being inoculated and replacing with a bud on a piece of bark of the same shape and size taken from the tree to be tested. When the bud had been

placed it was wrapped with a rubber band so that only the bud itself was not covered.

Peach seedlings in the field were inoculated 5 to 10 cm above the soil line, by inserting the bud under the bark through a T-shaped split. In some cases two buds were inserted on each seedling.

For the statistical test of differences between the proportion (P_1) of trees responding to one treatment and the proportion (P_2) of another group of trees responding to a comparable treatment, the equation

$$U = \frac{P_1 - P_2}{\sqrt{\hat{p} \hat{q} \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}}$$

was employed, in which

n_1 = number of trees subjected to treatment I.

n_2 = number of trees subjected to treatment II.

$$\hat{p} = \frac{n_1 P_1 + n_2 P_2}{n_1 + n_2}$$

$$\hat{q} = 1 - \hat{p}$$

The value of U obtained from the above equation was then compared with the values at the 0.05 and 0.01 level of significance in the normal distribution table and for infinite degrees of freedom.

SYMPTOMS AND OCCURRENCE
OF DISEASED DWARF PEACH TREES IN THE NURSERY

Symptoms on one-year peach trees (Prunus persica), varieties Elberta and Polly, on P. tomentosa and P. besseyi rootstocks in 1957 and varieties Elberta, Polly, Halehaven and Golden Jubilee on P. tomentosa rootstocks in 1958, were striking and characteristic and were considered to be similar to those of the virus-induced X-disease of peach. These symptoms were as follows:

Throughout the spring and early summer of the year following the grafting procedure, growth of the bud into a whip and thence into a nursery tree was rapid and foliage was normal. Through June and early July, peach trees grafted on dwarfing stocks attained a size almost equal to that of peach trees grafted on non-dwarfing stocks (seedling peach).

By mid-summer, however, peach trees on dwarfing stocks began to exhibit a slight curling of the leaves and a chlorosis of the leaf margins and the larger inter-veinal areas of the leaf lamina. This curling and chlorosis became more evident as the summer progressed; ultimately the yellowish color of the leaves turned to a light red. At this stage small spots, 3-6 mm in diameter, made their appearance, mostly along the margins of the leaf. These spots became red or brown, soon became necrotic and were surrounded by a reddish-purple ring. The entire leaf seemed to become thicker and more

brittle as the summer progressed. Likewise yellowing and reddening of the leaf margins and the interveinal areas became progressively more intense, though there were still green or yellow-green areas along the main vein. The leaves gradually became more and more curled and puckered and sometimes distorted at the margins. Defoliation followed in some cases, but in others the leaves, yellow, red or brown, remained attached long into the fall.

By this time the twigs had taken on a red color; they remained short and slender with buds poorly developed. Late summer and early fall growth of affected trees was limited.

The first symptom expression was by leaves at the tip of the topmost branches and proceeded centripetally. Occasional trees were dead by the end of the growing season.

Though all four peach varieties examined showed the same pattern of symptoms, there seemed to be an earlier and faster expression of symptoms of trees on P. besseyi than on P. tomentosa.

In late summer of 1957, when the disease was first observed in dwarf peach blocks in the nursery at Shenandoah, a count of diseased trees revealed that 45 per cent of the peach trees propagated on P. besseyi and 6 per cent of those propagated on P. tomentosa had shown characteristic symptoms. As a result of the large percentage of diseased trees on P. besseyi, the use of this species as an understock for propagation of peach trees has been discontinued.

In 1958 there were in the field four peach varieties propagated on P. tomentosa rootstocks. The peach varieties were: Elberta, Golden Jubilee, Halehaven and Polly. At the start of the growing season, only a small percentage of the peach buds survived a destructive late spring frost so that during the summer, growing trees were scattered in the nursery block.

About the middle of July a few of the growing peach trees started showing yellowing, reddening and upward rolling of leaves. During the remainder of the summer progressively more trees became diseased and symptoms became increasingly pronounced and severe. Most of the trees with distinct symptoms remained strikingly stunted.

Because of unusually large amounts of rainfall, trees in the lower portions of the peach block were in standing water or in very wet soil during the whole month of July. There were relatively more diseased trees and more severely diseased trees in these wet locations than in the well drained areas of the block. Counts of healthy and diseased trees of the four peach varieties in respect to the wetness of their location are given in the table I. It is of interest that the over all percentage (7.5 per cent) of diseased trees in the well drained area is substantially the same as that (6 per cent) of diseased trees observed the previous year on P. tomentosa rootstocks.

Table I. Relative abundance of diseased peach trees in wet and well-drained areas of a nursery block, Shenandoah, 1958.

Varieties	Well-drained areas			Wet areas		
	No. trees	Diseased trees	% diseased	No. trees	Diseased trees	% diseased
Elberta	1327	77	5.8	609	368	60.4
Polly	803	69	8.6	274	202	73.7
Golden Jubilee	265	45	17.0	83	32	38.5
Halehaven	236	7	3.0	96	39	40.5
	2631	198	7.5	1062	641	60.3

CAUSE OF THE DISEASE

Two main lines of investigation were followed in the search for the cause of the disease: 1. the determination whether or not the disease was caused by a virus and 2. examination of the possibility that propagation of peach trees on P. tomentosa and P. besseyi seedling rootstocks resulted in poor stock-scion unions.

Observations of Elberta Peach

on P. tomentosa and P. besseyi Rootstocks

Transplanted in the Greenhouse and in the Field

In January 1958, forty Elberta peach trees were brought from the nursery block in which the disease had been prevalent in 1957 and were planted in clay pots in the greenhouse. Twenty of the trees were on P. tomentosa and twenty on P. besseyi rootstocks. Ten trees on each rootstock had shown disease symptoms in the nursery block in 1957, while the other ten were apparently healthy. Before planting, dormant buds were removed from each tree and placed in cold storage (1°C) for subsequent use in transmission trials.

At the time of planting there were notable differences between diseased and healthy trees on both P. tomentosa and P. besseyi rootstocks. Diseased trees were smaller in caliper, with fewer and more intensely reddish colored twigs and smaller buds, and appeared to be somewhat desiccated. In most cases their root systems were smaller and less branched

than those of healthy trees. The mean diameters at the graft union, 3 cm above and 3 cm below the union are recorded in table II. The large diameter at the graft union is worthy of note, in the light of subsequent observations.

The trees started breaking bud and developing leaves in from two to five weeks after they were planted. However the diseased trees started growth more slowly than did the healthy trees and seven of the diseased trees on P. besseyi rootstocks failed to grow. On most trees, growth was rapid for about a month, after which growth stopped or was very slow. There was considerable variation between trees in extent of terminal growth, green twig diameter, twig maturation and development of axillary buds, but there was no correlation of these variables with rootstocks or previous condition of the tree (diseased or healthy).

The main objective in transplanting these trees in the greenhouse was to be able to observe closely for development of disease symptoms on new growth. However, although the trees were kept under observation until late summer, they did not at any time show symptoms comparable to those observed on the diseased trees in the nursery block in 1957.

For the same purpose, sixty more Elberta peach trees on P. besseyi and P. tomentosa rootstocks were transplanted in the field in Ames in early April 1958. Fifteen trees on each rootstock had shown disease symptoms in the nursery block in 1957, and fifteen were apparently healthy.

Table II. Mean diameters at, above and below the graft union of healthy and diseased Elberta peach trees on two rootstocks.

Kind of trees	Position of measurement		
	At graft union	3 cm above	3 cm below
Healthy Elberta on <u>P. besseyi</u>	2.65*	1.91	2.09
Diseased Elberta on <u>P. besseyi</u>	2.57	1.77	1.86
Healthy Elberta on <u>P. tomentosa</u>	3.47	2.11	2.13
Diseased Elberta on <u>P. tomentosa</u>	2.90	1.76	1.90

*Centimeters: average of ten trees.

Of the 60 peach trees 8 failed to grow and one "diseased" Elberta on P. tomentosa showed symptoms again. The other trees remained symptomless all through the growing season. On October 8, 1958, five healthy and five previously "diseased" peach trees on P. tomentosa and P. besseyi were broken at the graft union. Most of these trees would not break first at the union but usually at some other point and more often below the union. When the union was finally broken apart, the union surfaces of the previously diseased trees did not exhibit quite as striking necrosis as they did in similar trees at the end of the first growth season. Actually there was very little difference in the appearance of the union surfaces in healthy and previously diseased peach trees on each of the two rootstocks.

In the spring of 1960, a survey was made of the healthy and "diseased" peach trees on P. tomentosa and P. besseyi that were transplanted in the Botany Farm in Ames, Iowa, in the spring of 1958. Except for those trees cut down previously, most of the other trees (35 out of 40) were still living and were all looking healthy. Caliper measurements revealed that the trees that had never appeared diseased were slightly bigger than those that had expressed disease symptoms in the first growth season. Table III shows the average diameter for the four groups of trees.

Table III. Mean diameter at 10 cm above the graft union of healthy and diseased Elberta peach trees on two rootstocks two years after transplanting.

Kind of trees	No. of trees	Diameter (cm)
Diseased Elberta on <u>P. tomentosa</u>	8	2.31
Healthy Elberta on <u>P. tomentosa</u>	10	2.92
Diseased Elberta on <u>P. besseyi</u>	9	2.56
Healthy Elberta on <u>P. besseyi</u>	8	2.84

Transmission Experiments

To ascertain the possibility that the symptoms observed on the year old peach trees were virus-induced, a series of grafting experiments were performed to determine transmissibility of the disease.

Hildebrand (1941) succeeded in transmitting X virus to one-year-old Elberta peach trees and Richards and Hutchins (1941) did likewise with western X virus. In both cases, there was symptom expression within 4 to 6 weeks by almost 100 per cent of the trees inoculated. Fink (1950) introduced P. tomentosa as an index plant for sour cherry viruses and as an indicator of other viruses of cherry and peach.

Attempts at transmission to peach and P. tomentosa from trees that had shown symptoms in the previous growth season.

In late January and early February, 1958, forty P. tomentosa seedlings were inoculated with dormant buds taken from the 20 "diseased" and 20 "healthy" trees planted in the

greenhouse for observations of symptom development on new growth (preceding section). Budsticks from these trees had been stored at 1°C since late December. The P. tomentosa seedlings were inoculated at bud break, two seedlings for each peach tree. Ten uninoculated P. tomentosa seedlings served as checks. Unfortunately, for all transmission experiments no known source of X virus was available for diseased checks.

The inoculated P. tomentosa seedlings were frequently observed for symptom development. Three seedlings, two inoculated with buds from one peach tree and one with a bud from a different peach tree, showed necrotic ring spot symptoms. All the other trees remained symptomless until early April. At this time the new growth on all P. tomentosa seedlings was cut back and all leaves removed to force new growth. This new growth also manifested no symptoms, either on inoculated seedlings or on uninoculated checks.

In late March 40 one-year-old Elberta peach trees were inoculated with dormant buds from the same stored budwood. The Elberta trees were inoculated at bud break, one bud for each tree. Ten uninoculated Elberta trees were grown for checks. No virus symptom expression occurred either on the inoculated trees or on the uninoculated checks.

Transmission of the X-disease virus was reported by Reeves et al (1951) to depend upon the type of inoculum used, the season of inoculation, the vigor of the tree being

inoculated and the length of time the inoculum has been held after removal from diseased tree before transmission is attempted. High infection was observed with inoculum taken early in the season and relatively low or zero in late summer, early fall and the dormant season.

Since no symptoms were expressed on P. tomentosa seedlings inoculated with dormant buds, 32 P. tomentosa seedlings were inoculated with green buds taken from the 32 Elberta trees growing in the greenhouse, 13 of which had been "diseased" in the field. On a few of these trees bud development was virtually nil and green bark from small tender twigs was used for grafting. Two inoculated P. tomentosa seedlings ultimately showed necrotic ring spot symptoms but no symptoms similar to those observed in the field appeared on any of the seedlings.

Helton (1956) reported that several known viruses cause symptom development annually under certain types of environmental conditions but fail to do so under others, and that sometimes a change in the environment is enough to "unmask" a virus and cause it to produce symptoms in the host tree. Therefore all P. tomentosa and Elberta peach trees, that were inoculated and kept in the greenhouse, were transferred to the field by the middle of June. They were left there until late fall, but no symptom expression occurred at any time.

It is known (Gilmer et al 1957) that peach seedlings are often not satisfactory host plants in the greenhouse,

since under greenhouse conditions symptoms of virus infection are usually not pronounced.

Since all the previous transmission experiments were performed in the greenhouse and there were no virus symptoms expressed on the host plants, a number of grafting experiments were then conducted in the field.

Forty Elberta peach trees were inoculated in the field with buds taken from 20 "diseased" (in 1957) and 20 "healthy" trees, all planted in a nearby plot at the same time. The inoculations were made at bud break and the trees were observed until late fall. No virus symptoms developed in any of the budded trees.

Attempts at transmission to peach from trees showing symptoms.

In late July 1957, eight California Lovell seedlings were inoculated with buds taken from 8 Elberta peach trees, and four more Lovell seedlings were inoculated with buds taken from 4 Polly peach trees. Both Elberta and Polly trees had been propagated on P. besseyi and were showing characteristic symptoms of the disease. All 12 inoculated Lovell seedlings were observed for virus symptoms during the 1958 growing season. However they made normal growth and remained symptomless.

For rapid transmission of X-disease of peach Hildebrand (1941) suggested the cutting off of the tops of the inoculated plants. Gilmer et al (1957), for midsummer inoculations, suggested the pruning back of the inoculated trees to within

two to four nodes above the inoculating buds. In both cases symptoms developed on the new growth within a period of 4 or 5 weeks.

Infection as influenced by the number of buds per tree was studied by Hildebrand (1941) and he concluded that no important significance could be attached to using more than one bud when grafting peach on peach.

On August 1, 1958, ten California Lovell peach seedlings were inoculated each with two buds. The buds for 5 of the seedlings came from 5 Elberta peach trees and the buds for the other 5 seedlings came from 5 Polly peach trees. Both the Elberta and the Polly peach trees had been propagated on P. tomentosa and had just started showing characteristic symptoms. The tops of the ten inoculated Lovell seedlings were cut off immediately after inoculation, according to Hildebrand's method. New growth appeared within 2 to 3 weeks, but the new as well as the old growth remained symptomless well after two months following the inoculation.

On July 24, 1959, eighty California Lovell peach seedlings were inoculated with buds from 20 Elberta peach trees which had been propagated on P. tomentosa and had just started showing characteristic symptoms. At the same time 20 peach seedlings were budded with buds from 10 Elberta peach trees which had also been propagated on P. tomentosa, but had not expressed any symptoms up to that date. The tops of all budded Lovell peach seedlings were cut off immediately after

inoculation. No symptoms were observed in any of the 100 inoculated seedlings when new growth developed a few weeks later or during the entire growing season of 1960.

All the transmission experiments reported heretofore, aiming mainly at transmitting the X-disease virus, are summarized in table IV. As it can be seen from the table, no X-disease virus transmission was observed in these experiments. But in the greenhouse experiments three P. tomentosa seedlings, two inoculated with buds from a "diseased" peach tree on P. tomentosa and one inoculated with bud from a "healthy" peach on P. besseyi showed symptoms commonly caused by necrotic ring spot virus.

Symptom Expression and Development
on Trees with Poor Union between Peach Scion
and P. tomentosa and P. besseyi Rootstocks

By the end of July 1958, when many trees in the nursery block were already showing symptoms, no virus symptom expression had been observed in any transmission experiments. Furthermore, in one independent experiment, on all two-year peach seedlings which had been girdled at the base by label wires there were symptoms very much like those observed on diseased peach trees. All similar seedlings which had not been girdled were without symptoms. It began to be evident also that although the symptoms on dwarf peach were similar to those reported as being induced by X virus, they were not

Table IV. Results of attempts to transmit virus from dwarf peach trees with virus-like symptoms.

Test trees:	In the greenhouse				In the field			
	<u>P. tomentosa</u>		Elberta peach		Elberta peach		Lovell peach	
	No. of trees	Sympt.	No. of trees	Sympt.	No. of trees	Sympt.	No. of trees	Symp.
Diseased Elberta on <u>P. besseyi</u>	20	0	10	0	-	-	-	-
Healthy Elberta on <u>P. besseyi</u>	1	NRSV*	10	0	-	-	-	-
Healthy Elberta on <u>P. besseyi</u>	19	0	-	-	-	-	-	-
Diseased Elberta on <u>P. tomentosa</u>	2	NRSV*	10	0	-	-	-	-
Diseased Elberta on <u>P. tomentosa</u>	18	0	-	-	-	-	-	-
Healthy Elberta on <u>P. tomentosa</u>	20	0	10	0	-	-	-	-
Diseased Elberta on <u>P. besseyi</u>	10	0	-	-	10	0	-	-
Healthy Elberta on <u>P. besseyi</u>	3	0	-	-	10	0	8	0
Diseased Elberta on <u>P. tomentosa</u>	9	0	-	-	10	0	80	0
Healthy Elberta on <u>P. tomentosa</u>	10	0	-	-	10	0	25	0
Diseased Polly on <u>P. besseyi</u>	-	-	-	-	-	-	4	0
Diseased Polly on <u>P. tomentosa</u>	-	-	-	-	-	-	5	0

*NRSV = necrotic ring spot virus.

identical in all details.

Observations of characteristic symptoms on girdled trees led to examination of the lower portion of scions and roots of diseased trees in the nursery dwarf peach block of Elberta, Golden Jubilee, Halehaven and Polly peach varieties on P. tomentosa rootstocks.

Five diseased trees were pulled and their roots examined for lesions or other damage caused by any possible factor. Root growth in length and diameter was limited but the roots apparently were healthy.

It was noted that when trees with severe symptoms were pulled from the soil, they nearly always broke at the graft union. On one occasion, 16 of 18 trees with moderate symptoms did likewise. In fact, about one-third of the trees with moderate vigor but without symptoms also broke at the graft union when pulled; only the largest trees (without symptoms) remained intact. With vigorous bending, nearly all trees could be broken at the graft union.

In contrast, similar pulling and bending of peach trees grafted on seedling peach root stocks almost never resulted in breakage, and when breakage did occur, it was above the graft union.

Four healthy and 4 diseased trees of each of the varieties Elberta, Golden Jubilee, Halehaven, and Polly, all propagated on P. tomentosa were cut and their graft unions are shown in Fig. 2, 4, 6, and 8 respectively, and 4 peach

trees propagated on peach were cut and their graft unions are shown in Fig. 1, top. These trees were then split longitudinally at the graft union and photographed (Fig. 3, 5, 7, 9, and 1 lower, respectively). A similar group of trees of the same varieties propagated on the same root stocks were broken at the graft union and the union surfaces are shown in Fig. 10 - 14.

Fig. 1 Top: Exterior appearance of graft unions of commercial peach varieties propagated on peach.

Fig. 1 Lower: Longitudinal sections of the graft unions of the same four trees.

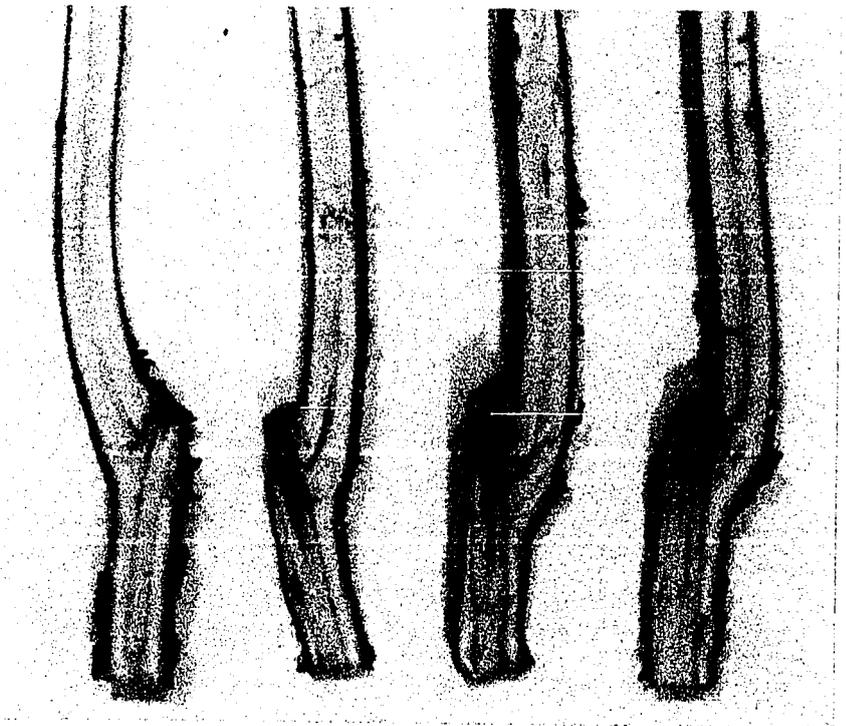
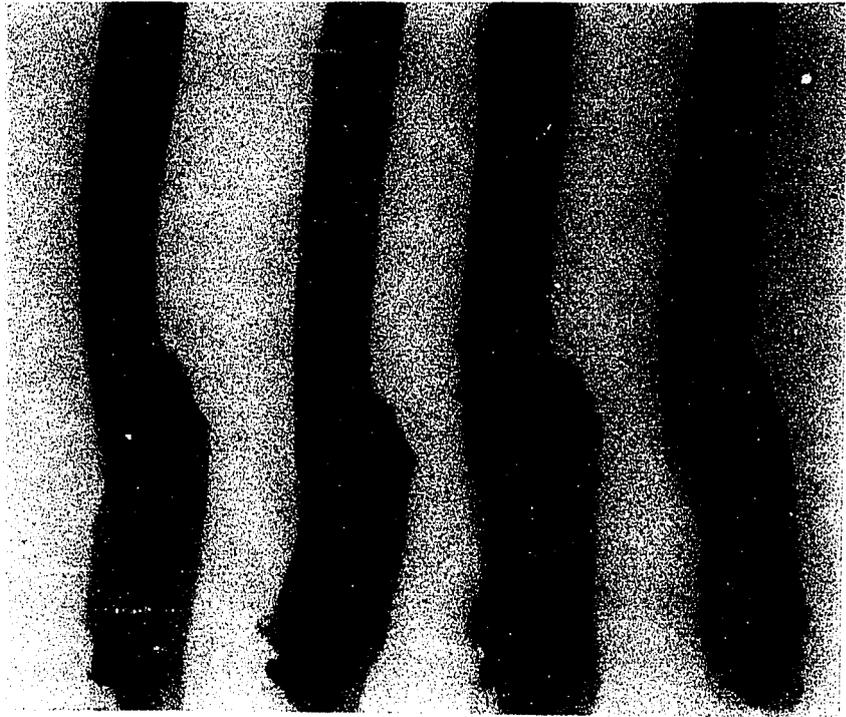


Fig. 2 Top: Exterior appearance of graft unions of "healthy" Elberta peach trees propagated on P. tomentosa.

Fig. 2 Lower: Graft unions of diseased Elberta on P. tomentosa.

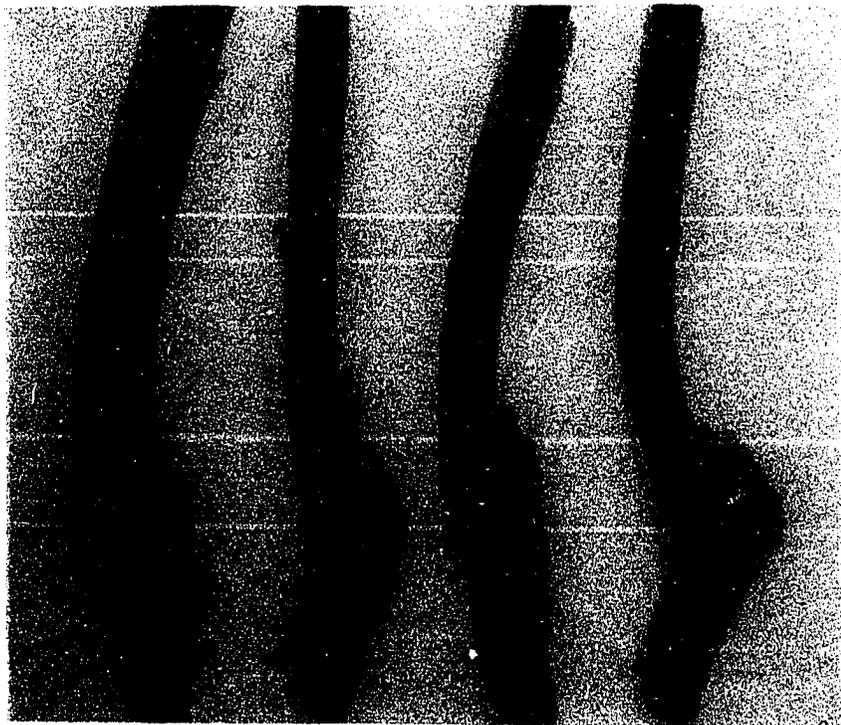
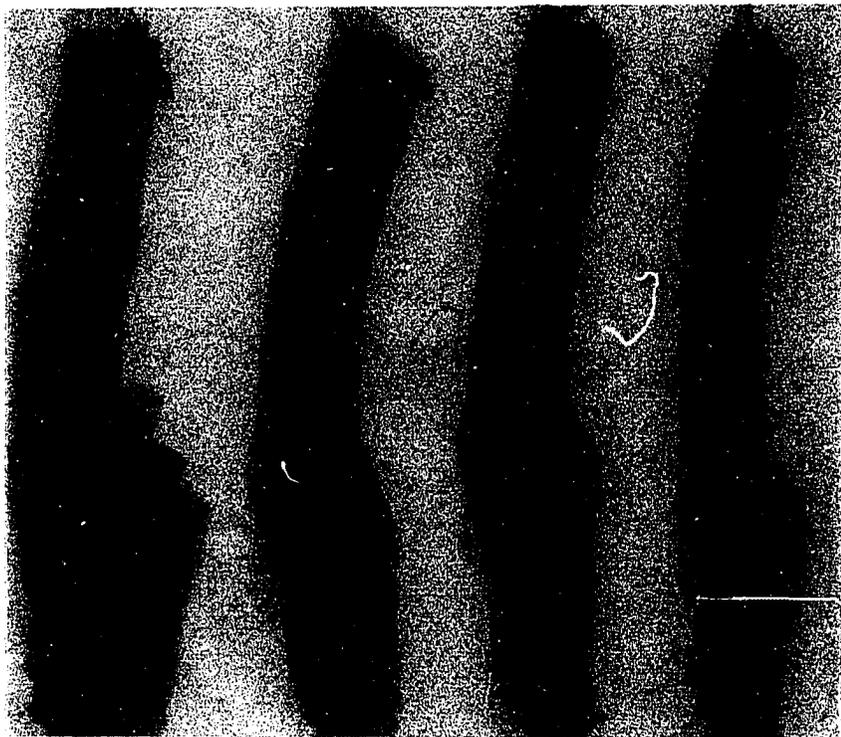


Fig. 3 Top: Longitudinal sections of the graft unions of "healthy" Elberta peach trees propagated on P. tomentosa (same four trees shown in fig. 2, top).

Fig. 3 Lower: Longitudinal sections of graft unions of diseased Elberta on P. tomentosa (same four trees shown in fig. 2, lower).

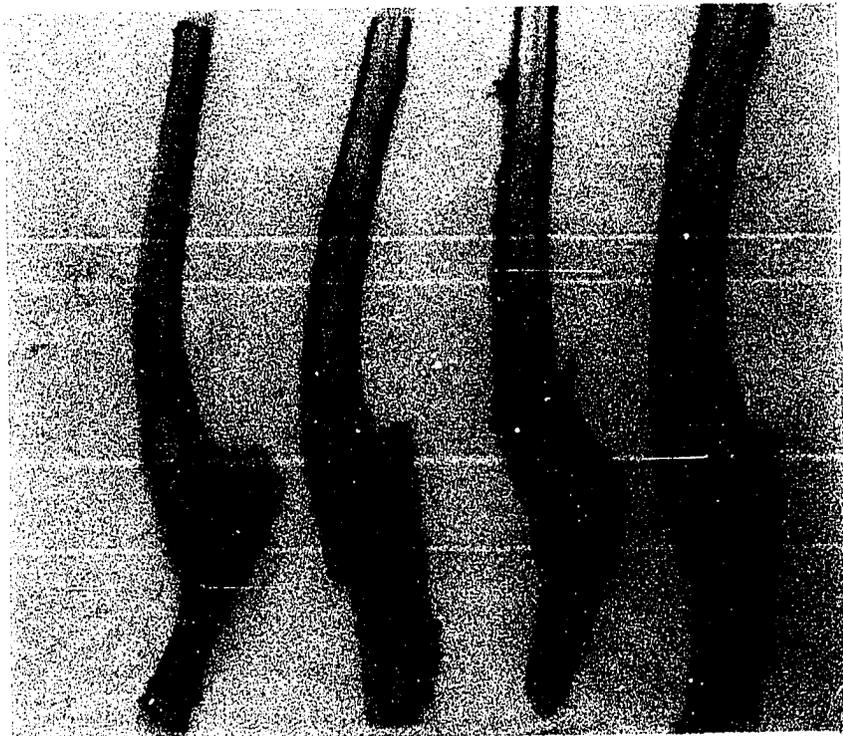
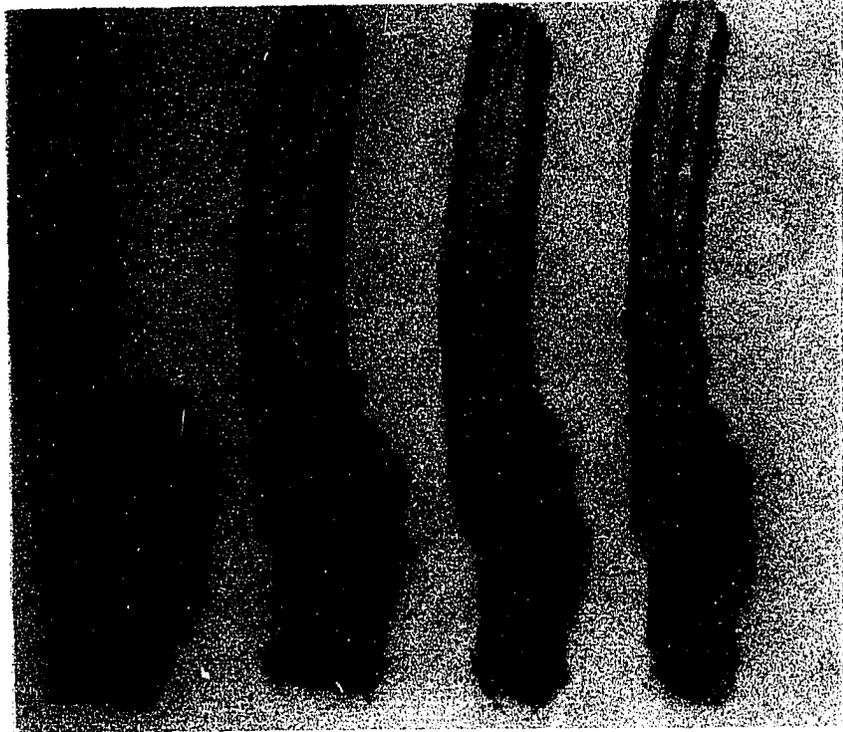


Fig. 4 Top: Exterior appearance of graft union of "healthy"
Golden Jubilee peach trees propagated on P.
tomentosa.

Fig. 4 Lower: Graft unions of diseased Golden Jubilee on
P. tomentosa.

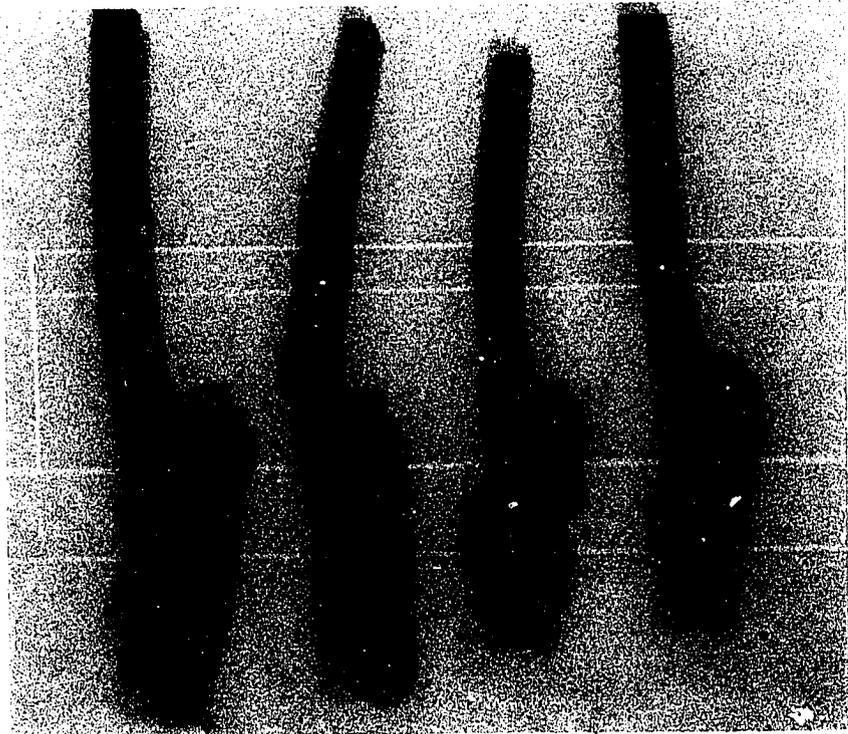
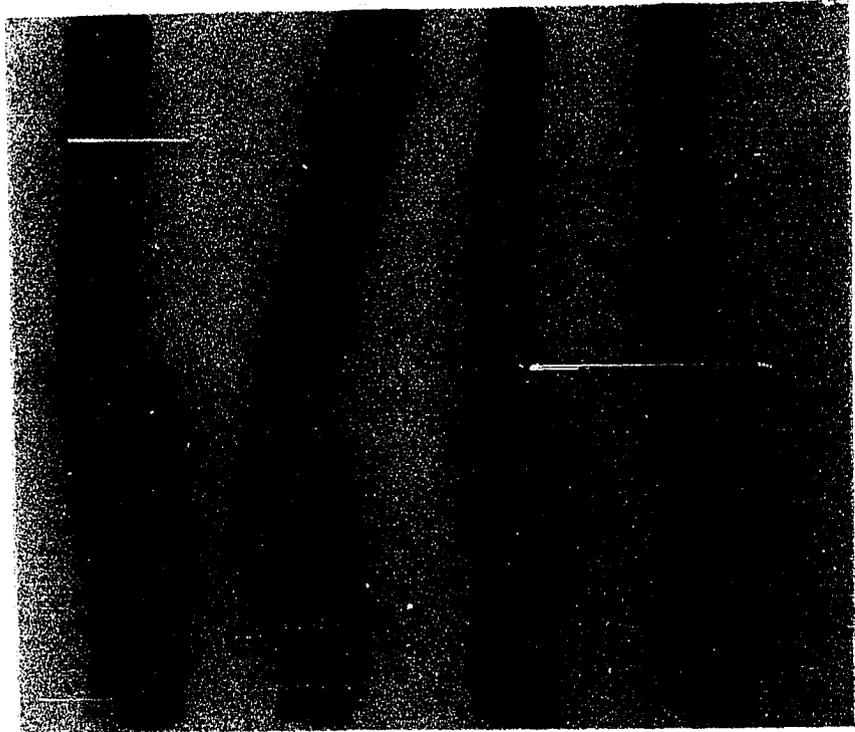


Fig. 5 Top: Longitudinal sections of the graft unions of "healthy" Golden Jubilee peach trees propagated on P. tomentosa (same four trees shown in fig. 4, top).

Fig. 5 Lower: Longitudinal sections of graft unions of diseased Golden Jubilee on P. tomentosa (same four trees shown in fig. 4, lower).

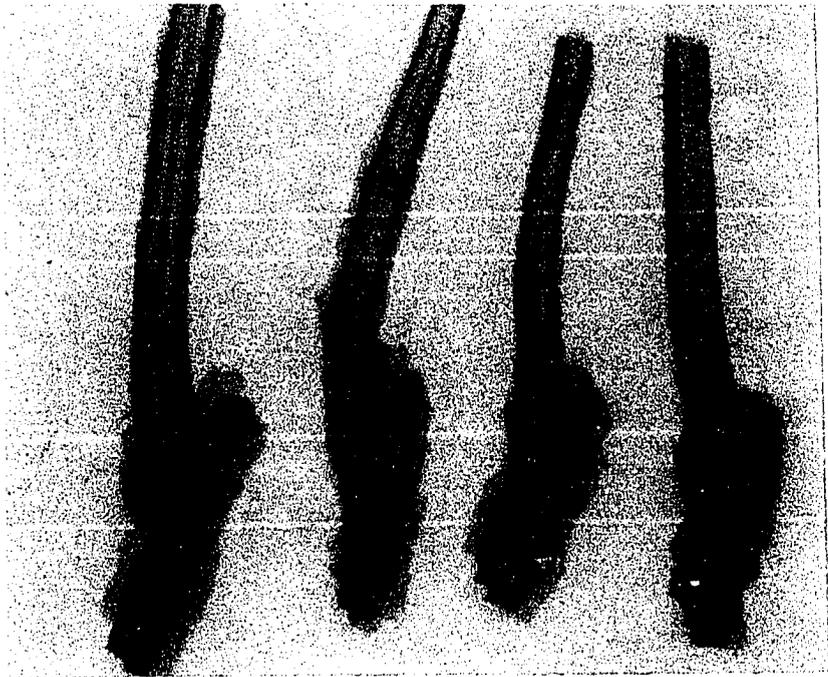
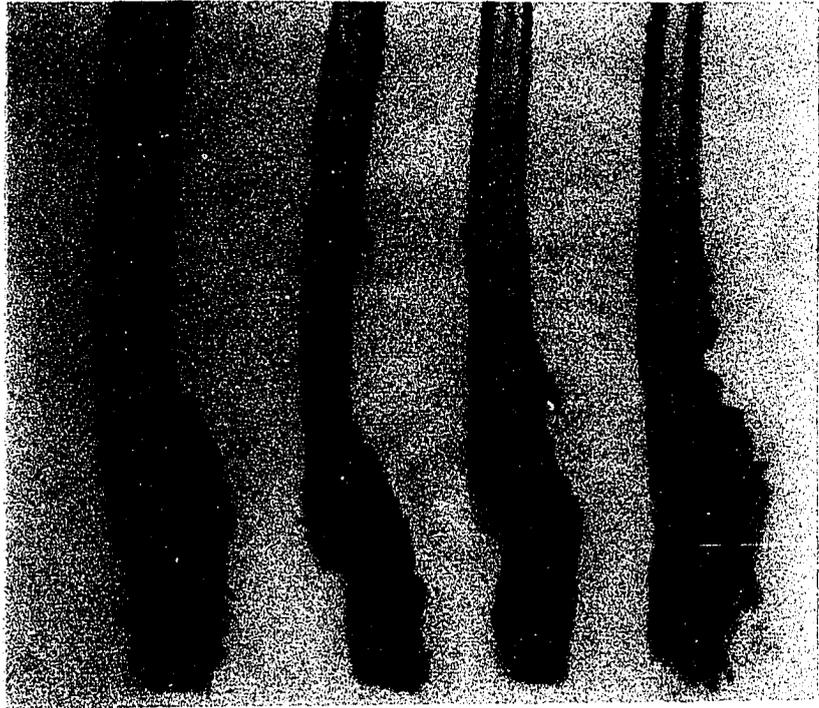


Fig. 6 Top: Exterior appearance of graft unions of "healthy"
Halehaven peach trees propagated on P. tomentosa.

Fig. 6 Lower: Graft unions of diseased Halehaven on
P. tomentosa.

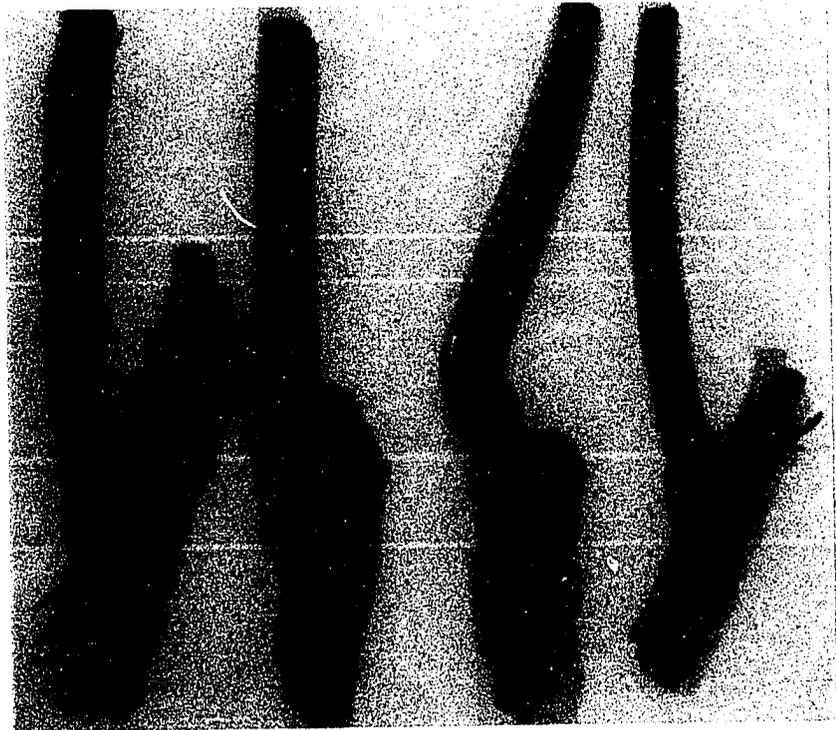


Fig. 7 Top: Longitudinal sections of the graft unions of "healthy" Halehaven peach trees propagated on P. tomentosa (same four trees shown in fig. 6, top).

Fig. 7 Lower: Longitudinal sections of graft unions of diseased Halehaven on P. tomentosa (same four trees shown in fig. 6, lower).

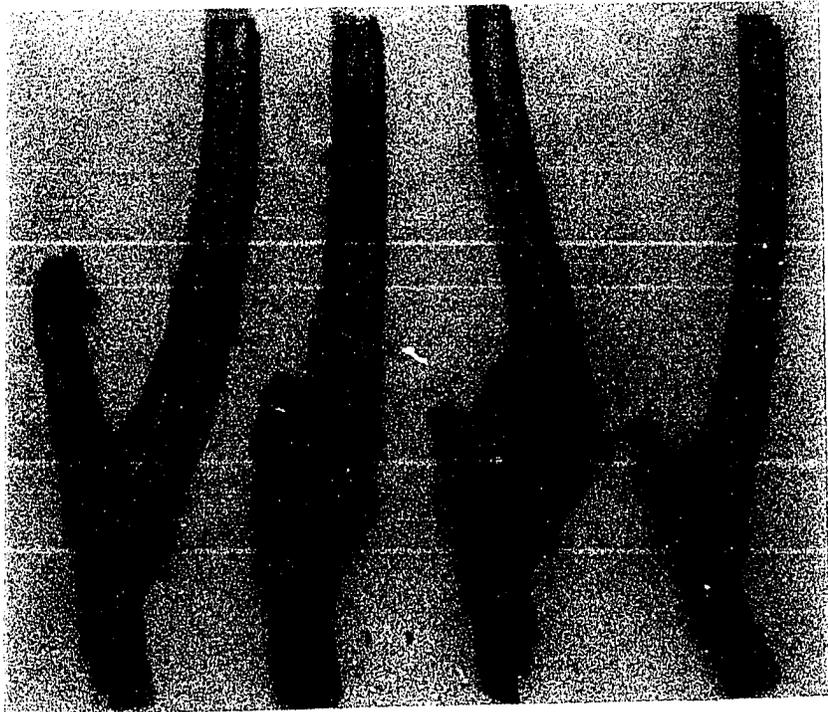
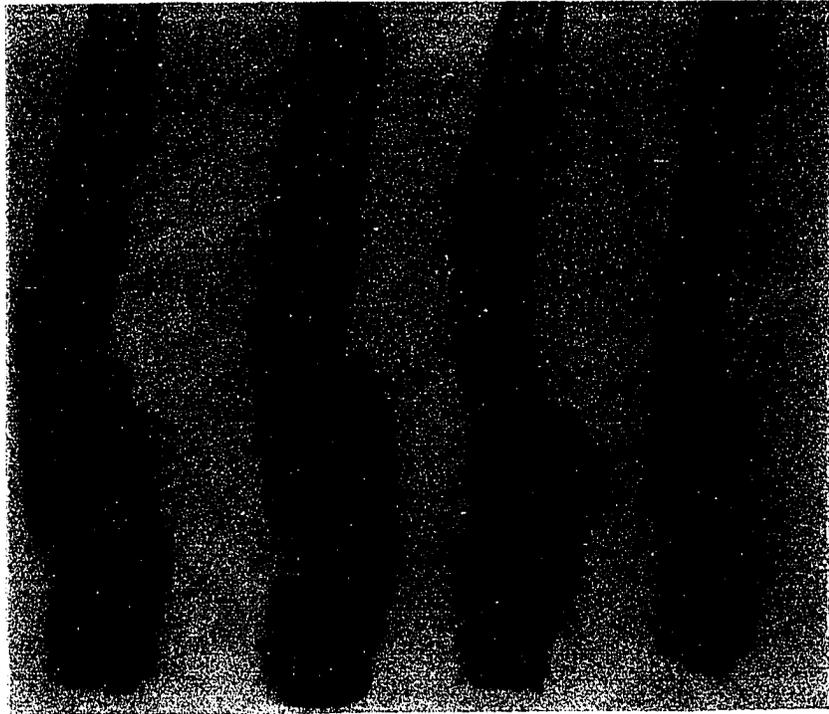


Fig. 8 Top: Exterior appearance of graft unions of "healthy"
Polly peach trees propagated on P. tomentosa.

Fig. 8 Lower: Graft unions of "diseased" Polly on P.
tomentosa.

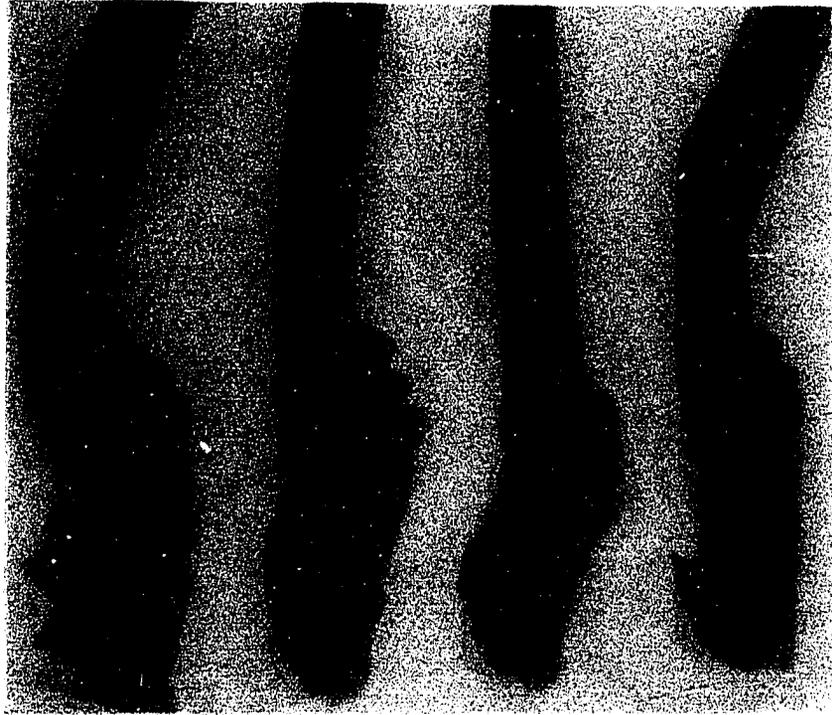


Fig. 9 Top: Longitudinal sections of the graft unions of "healthy" Polly peach trees propagated on P. tomentosa (same 4 trees shown in fig. 8 top).

Fig. 9 Lower: Longitudinal sections of graft unions of "diseased" Polly on P. tomentosa (same 4 trees shown in fig. 8 lower).

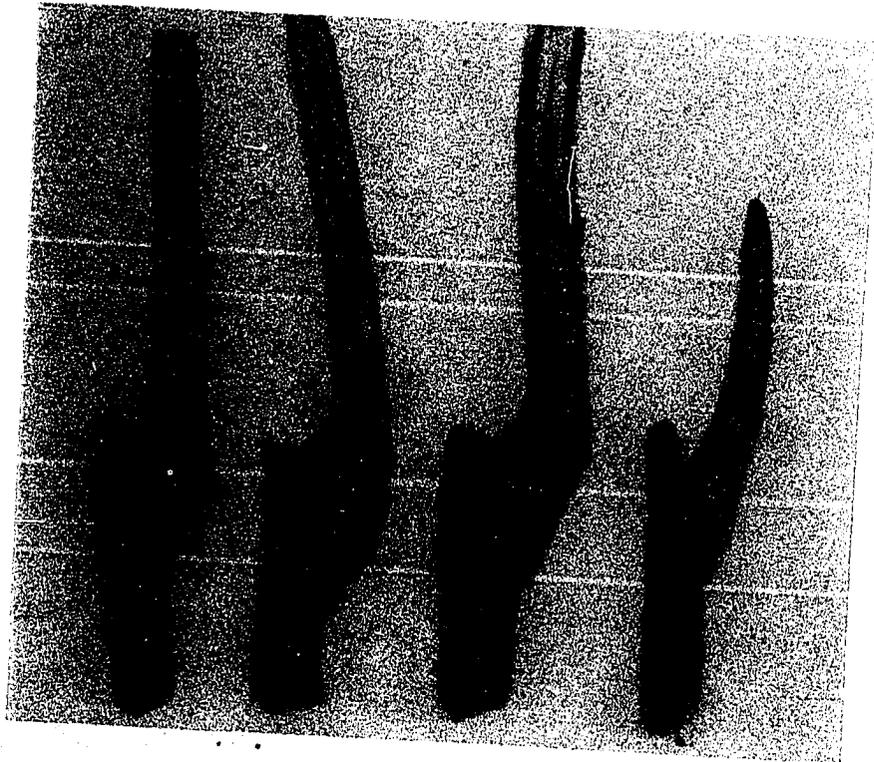


Fig. 10. Graft unions of Elberta peach on peach rootstock.

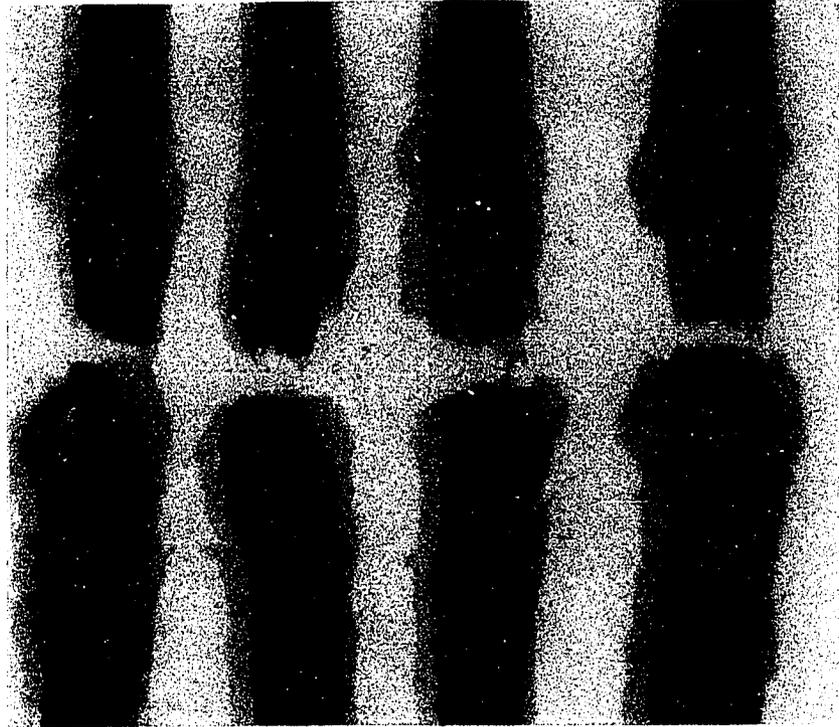


Fig. 11 Top: Graft unions of "healthy" Elberta scions
on P. tomentosa rootstocks.

Fig. 11 Lower: Graft unions of diseased Elberta scions on
P. tomentosa rootstocks.

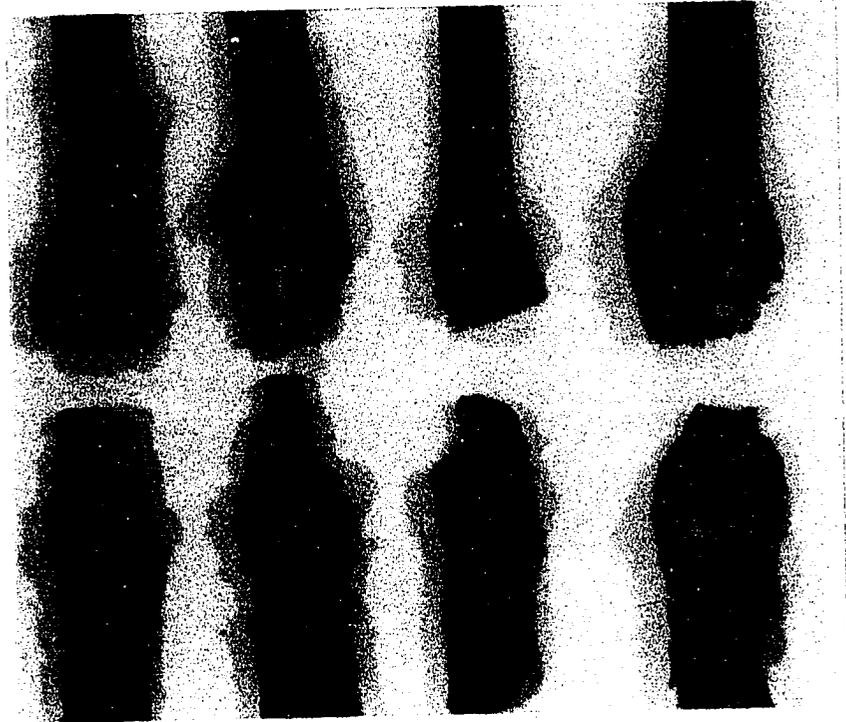


Fig. 12 Top: Graft unions of "healthy" Golden Jubilee scions on P. tomentosa rootstocks.

Fig. 12 Lower: Graft unions of diseased Golden Jubilee scions on P. tomentosa rootstocks.

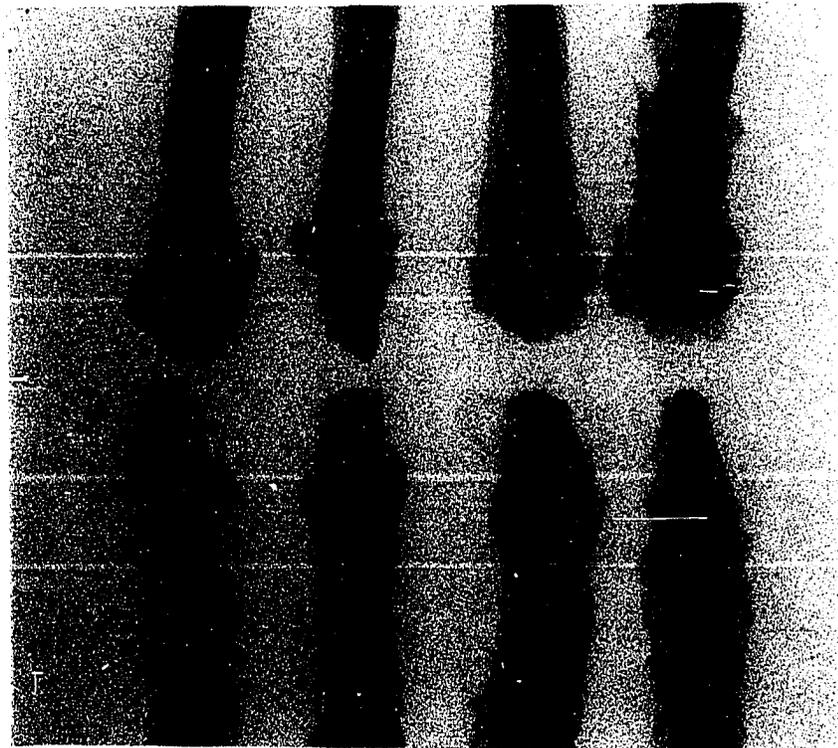
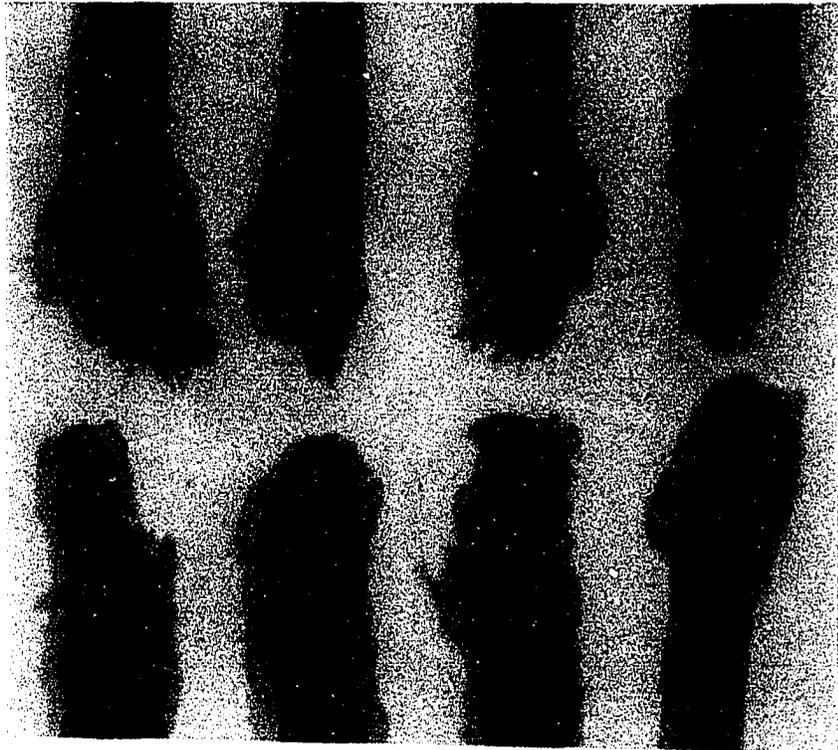


Fig. 13 Top: Graft unions of "healthy" Halehaven scions
on P. tomentosa rootstocks.

Fig. 13 Lower: Graft unions of diseased Halehaven scions
on P. tomentosa rootstocks.

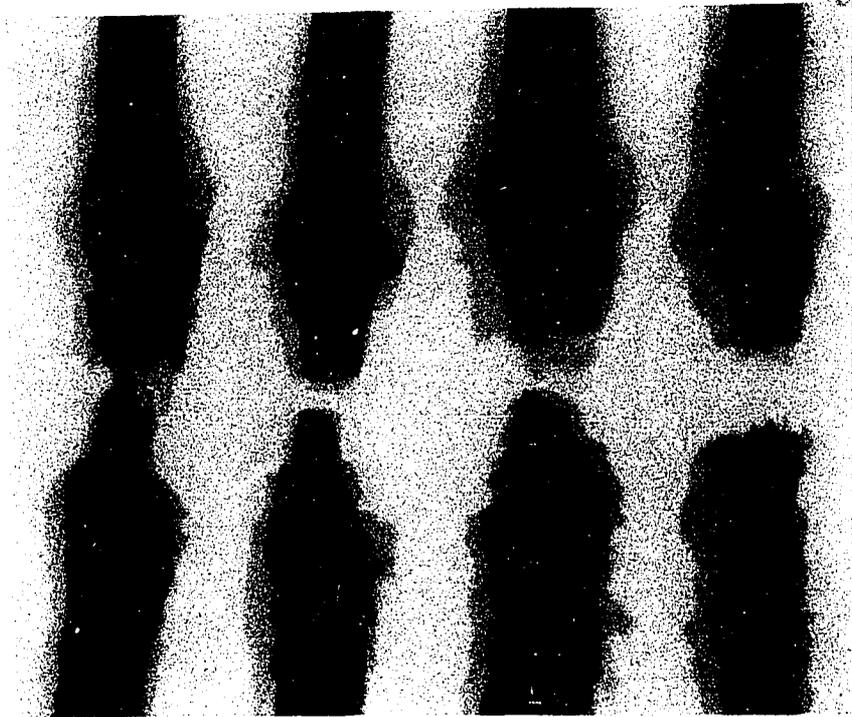
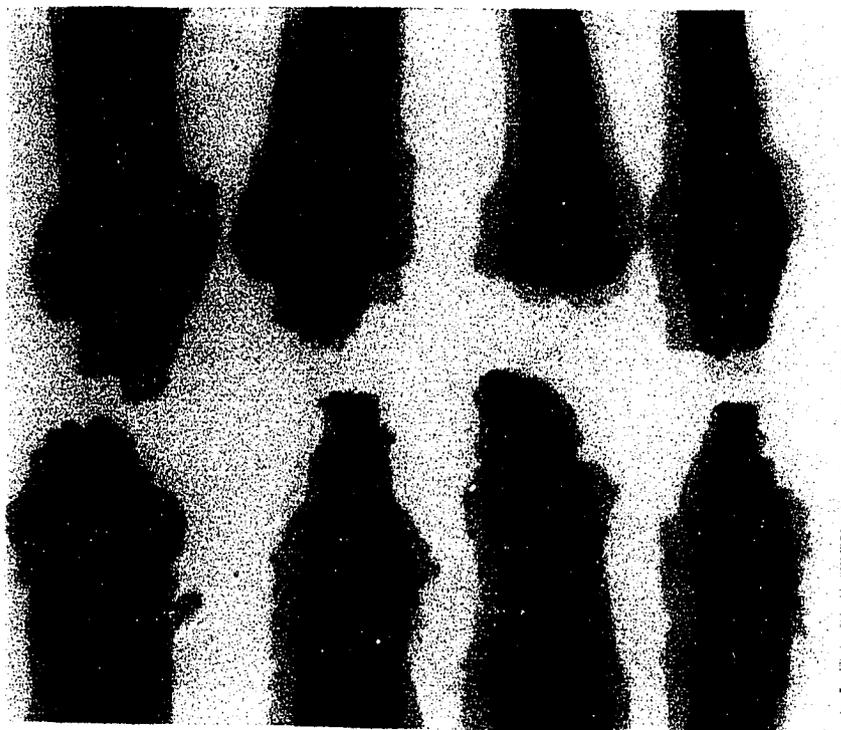


Fig. 14 Top: Graft unions of "healthy" Polly scions on P. tomentosa rootstocks.

Fig. 14 Lower: Graft unions of diseased Polly scions on P. tomentosa rootstocks.



Examination of the exterior appearance of graft unions showed that almost all diseased trees developed a small overgrowth immediately above the graft union (Fig. 2, 4, 6, and 8). There was also a rather sharp constriction at the point of union of scion and stock. In healthy trees this constriction, though nearly always present, tended to be shallow and smooth, not as deep as on diseased trees. In the peach trees grafted on peach there was no scion overgrowth or any constriction (Fig. 1).

Gum secretion was evident at the graft union of some trees. Gum was present on many though not all trees with symptoms, but appeared only at the unions of peach grafted on P. tomentosa, not at the unions of peach on peach.

When peach trees on P. tomentosa were broken at the graft union, the just disjoined surfaces of the scion and stock were far from normal in appearance (Fig. 11-14). They were irregular in shape, ranging from round to ovoid or ellipsoid and frequently with irregular boundaries. The color of such disjoined surfaces ranged from reddish-brown to brownish-black, with the exception of occasional white sections which seemed to be the only normally united and functioning portions of the graft union. On many diseased trees the entire graft union surfaces were discolored. Likewise many were rough, furrowed and with small or large breaches or swellings. In most cases there was a central ridge on the union surface of the scion and opposite it a groove in the stock extending

across the surface lengthwise, while the rest of the area was ridged in radius-like fashion (Fig. 11-14).

Some such graft union constrictions, overgrowths, surface aberrations and discolorations were present even on healthy peach trees with P. tomentosa rootstocks, though on the trees with no symptoms there were usually fairly large white, healthy areas on the union surfaces. In contrast, when peach trees on peach roots were broken at the graft union, the uniting tissues had to be forcibly torn apart, and the graft union surfaces were entirely white and without surface aberrations, and there were few constrictions and no overgrowths (Fig. 10).

In median longitudinal sections through the graft union of peach trees on P. tomentosa rootstocks, a more or less continuous line of brownish discoloration between the wood of stock and scion was clearly evident (Fig. 3, 5, 7 and 9). In some cases this line seemed to extend across the entire extent of the graft union, while in others there were small sections of healthy, non-discolored tissue. In similar sections of peach on peach there was no such line of discoloration (Fig. 1 lower).

The frequency and the degree of sprouting by the rootstocks was rather small, and there appeared to be no striking difference between diseased and healthy trees in this respect except that severely diseased trees failed to develop sprouts. No adventitious roots were found on any peach scions, healthy

or diseased.

Since the propagation of peach on P. besseyi had been discontinued the previous year no observations could be made at that time to establish any differences in the appearance of the union areas of peach on P. tomentosa and on P. besseyi. Subsequent observations, however, of "diseased" peach on P. besseyi in Topeka, Kansas, in the summer of 1959, showed that the graft union areas were uneven and discolored but usually less necrotic than those of peach on P. tomentosa. Similar were the results of observations in southwestern Iowa in 1960, with the difference that the union areas were much less necrotic than on P. tomentosa and in some cases they appeared completely healthy.

EXPERIMENTS ON THE POSSIBLE
VIRUS EFFECT ON STOCK-SCION UNION

Though the virus-like symptoms on dwarf peach, at least on P. tomentosa rootstocks, seem to be associated with poor graft unions and not due to X virus, there is nevertheless a possibility that virus in the scion, rootstock, or both, might accentuate symptom expression, perhaps by adversely affecting stock-scion union.

The experiments described in the following pages were designed to provide information concerning the behavior of compatible and less compatible stock-scion combinations as affected by the presence of the usually latent necrotic ring spot virus in one or both of the propagative units.

Virus Effect on Bud Take

When the Bud or the Rootstock
or Both are Infected with NRS Virus

That the presence of a virus in one or both of the propagative units has a detrimental effect in the successful union of scion and stock and subsequently in the growth of the scion has been shown by Milbrath and Zeller (1945, 1948), Cochran et al (1951), Gilmer et al (1957), Costa et al (1954), and others. Furthermore, it was observed in routine indexing experiments, performed during the winter of 1960 in the greenhouse, that when a necrotic ring spot virus-infected cherry bud was placed on P. tomentosa, the virus would be transmitted

but the bud itself would commonly die. Of 71 necrotic ring spot virus-infected Fruitmorency or Early Richmond sour cherry buds inserted on P. tomentosa, 54 were dead and 17 were living ten weeks after budding. However, all 71 P. tomentosa seedlings were showing ring spot symptoms. On the other hand, among 149 virus-free buds similarly placed on P. tomentosa, only 13 were dead at the end of ten weeks after budding. Although these observations were of results more striking than those reported by other investigators, they were a confirmation of the interference of the virus with bud take.

In order to investigate further this interference an experiment was devised so as to involve budding NRS-infected peach buds on NRS-free rootstocks, NRS-free buds on NRS-infected rootstocks, NRS-infected buds on NRS-infected rootstocks and NRS-free buds on NRS-free rootstocks. Three rootstock species were used: 1. peach, P. persica, California Lovell seedlings, 2. P. besseyi seedlings, and 3. P. tomentosa seedlings. Budding was at three dates: before (July 28), during (August 18), and after (September 8) "peach budding time" in the nursery.

On July 2, 1959, buds from "Special Montmorency" sour cherry trees, known to be infected with necrotic ring spot virus, were inserted in 1400 peach seedlings, 800 P. besseyi seedlings and 900 P. tomentosa seedlings. These were half the seedlings to be used in this experiment; a similar number were left unbudded and were assumed to be NRS-free.

On the same day 60 nursery block Elberta trees were indexed on Shirofugen for NRS virus and immediately afterwards 30 of these were budded with two buds from a "Special Montmorency" sour cherry tree infected with NRS virus. The indexing was for the purpose of determining the presence or absence of NRS virus in the Elberta trees which were to provide buds for the experiment. Budding of 30 Elberta trees with NRS virus-infected "Special Montmorency" buds was for the purpose of insuring a source of NRS virus-infected peach buds.

On July 28, 450 peach, 260 P. besseyi and 300 P. tomentosa seedlings infected with NRS virus were budded, half with Elberta peach buds taken from the 30 trees that had been inoculated with NRS virus and the other half with buds from the 30 non-inoculated trees. Similar buddings of the same numbers of seedlings were done on August 18 and September 8, 1959. By this procedure each fourth of the seedlings of the three rootstocks were budded as follows: One-fourth with necrotic ring spot virus infected sour cherry buds (first) and peach buds from NRS virus inoculated trees; one-fourth with NRS virus infected cherry buds (first) and NRS virus free peach buds; one-fourth with peach buds from NRS virus inoculated peach trees only; one-fourth with NRS virus-free peach buds only.

Preliminary indexing of 25 seedlings of each rootstock which had been budded with NRS virus-infected buds indicated

that that virus transmission was unsuccessful. There is the possibility that the virus in the diseased cherry bud failed to pass into the rootstock. In fact, when 300 such buds on P. tomentosa were examined six weeks after budding, all but 17 were dead. However, as has been pointed out earlier, in greenhouse experiments P. tomentosa seedlings became infected with the virus, even though the buds were found dead upon examination a few weeks after budding. There is also the possibility that other factors slowed down the movement of the virus to the peripheral growth, from where detection of the virus was attempted.

Similar indexing revealed failure of virus passage into the 30 Elberta trees in which two infected buds had been inserted. However, 13 of the trees from which buds were taken for budding were naturally infected with the NRS virus. Thus some seedlings were budded with virus-infected peach buds, though less in number and not in the exact distribution planned for the experiment. Only the budding of September 8 was done with precisely equal numbers of virus-free and virus-infected peach buds and in the prearranged distribution.

Early in the spring of 1960, all seedlings were cut back above the bud and on May 10, all buds on the three rootstocks were examined one by one for signs of growth. At that time the maximum growth of shoots was less than 15-20 cm. A majority of the living buds were growing and consisted of a short, tender stem. Some, however, had produced only a small

leaf, with no other signs of growth. All live, growing buds or shoots were counted. Table V contains the numbers of trees budded, and the successful bud unions, while table VI contains the percentages of bud take in relation to the virus content of the rootstocks and/or scions.

It can be seen from the totals (table VI) that the highest percentage (33.1 per cent) bud take was with virus-free buds inserted on virus-free rootstocks; the next highest percentage (27.8 per cent) was with necrotic ring spot virus-infected buds on necrotic ring spot virus-infected rootstocks. This difference is significant at the 0.05 level. With virus-infected buds inserted on virus-free rootstocks the percentage bud take was 23.2 per cent, while with virus-free buds on infected rootstocks it was 25.9 per cent. The difference between these two percentages is statistically nonsignificant, but the difference of each of the two percentages (23.2, 25.9) and the 33.1 per cent bud take with virus-free buds and rootstocks is highly significant even at the 0.01 level. Only the 23.2 percentage is significantly different at the 0.05 level from the 27.8 percentage obtained when both buds and rootstocks were virus infected. Thus it appears that in general, the budding of virus-free buds on virus-free rootstocks resulted in more bud take than when either or both carried the NRS virus. However, there is indication that bud take was better when both buds and stocks were infected with virus than when either of the two alone carried virus.

Table V. Numbers of seedlings budded and of buds, budlings and trees counted according to

Rootstock sp.		Peach on Peach				Peach on <u>P. besseyi</u>			
Budding date		BR ^a	BG ^b	GB ^c	GT ^d	BR ^a	BG ^b	GB ^c	GT ^d
NRSE ^e /NRSR ^f	July 28	118	98	64	32	-	-	-	-
	August 18	-	-	-	-	128	69	40	38
	September 8	147	21	15	14	131	3	1	1
	Total	265	119	79	46	259	72	41	39
NRSE ^e /VFR ^g	July 28	96	59	31	29	42	24	14	12
	August 18	-	-	-	-	137	80	45	44
	September 8	150	17	9	9	92	4	2	2
	Total	246	76	40	38	271	108	61	58
VFB ^h /NRSR ^f	July 28	156	95	50	45	350	113	53	53
	August 18	288	138	79	65	126	40	16	12
	September 8	150	22	12	12	87	0	0	0
	Total	594	255	141	122	563	153	69	65
VFB ^h /VFR ^g	July 28	212	161	45	41	127	79	50	50
	August 18	306	127	87	54	119	26	14	11
	September 8	150	53	38	28	129	4	3	2
	Total	668	341	170	123	375	109	67	63
TOTAL		1773	791	430	329	1468	442	238	225

^aBR = Buds budded to rootstocks (summer of 1959).

^bBG = Beginning to grow (counted on May 10, 1960).

^cGB = Grown to budlings (counted on June 22, 1960).

^dGT = Grown to trees (counted on September 22, 1960).

^eNRS

^fNRS

^gVFR

^hVFB

according to the rootstock species, date of budding and virus treatment.

<u>aseyi</u>		Peach on <u>P. tomentosa</u>				TOTAL			
^c	GT ^d	BR ^a	BG ^b	GB ^c	GT ^d	BR ^a	BG ^b	GB ^c	GT ^d
-	-	-	-	-	-	118	98	64	32
10	38	49	9	4	4	177	78	44	42
1	1	150	1	1	0	428	25	17	15
11	39	199	10	5	4	723	201	125	89
14	12	213	15	11	10	351	98	56	51
15	44	67	15	8	8	204	95	53	52
2	2	157	8	3	3	399	29	14	14
51	58	437	38	22	21	954	222	123	117
53	53	295	39	25	20	801	247	128	118
16	12	248	32	16	16	662	210	111	93
0	0	157	2	1	1	394	24	13	13
59	65	700	73	42	37	1857	481	252	224
50	50	118	21	21	19	457	261	116	110
14	11	264	50	33	25	689	203	134	90
3	2	190	14	13	4	469	71	54	34
57	63	572	85	67	48	1615	535	304	234
38	225	1908	206	136	110	5149	1439	804	664

^eNRSB = Necrotic ring spot virus-infected buds.

^fNRSR = Necrotic ring spot virus-infected rootstocks.

^gVFR = Virus-free rootstock.

^hVFB = Virus-free buds.

Table VI. Percentages of growing buds, budling stands and grown trees in rel

		Peach on Peach			on <u>P. besseyi</u>		
Budding date		Grow- ing buds	Bud- lings	Trees	Grow- ing buds	Bud- lings	Trees
NRSB/NRSR	July 28	83	54.2	27.1	-	-	-
	August 18	-	-	-	53.9	31.2	29.0
	September 8	14.2	10.2	9.5	2.2	0.7	0.0
	Aver.	44.9	29.8	17.3	27.7	15.8	15.0
NRSB/VFR	July 28	61.4	32.2	30.2	57.1	33.3	28.0
	August 18	-	-	-	58.3	32.8	32.0
	September 8	11.3	6.0	6.0	4.3	2.2	2.0
	Aver.	30.9	16.3	15.4	39.8	22.5	21.0
VFB/NRSR	July 28	60.8	32.0	28.8	32.2	15.1	15.0
	August 18	47.9	27.4	22.5	31.7	12.7	9.0
	September 8	14.6	8.0	8.0	0.0	0.0	0.0
	Aver.	42.9	23.7	20.5	27.1	12.2	11.0
VFB/VFR	July 28	75.9	21.2	19.3	62.2	39.3	39.0
	August 18	41.5	28.4	17.6	21.8	11.7	9.0
	September 8	35.3	25.3	18.6	3.1	2.3	1.0
	Aver.	51.0	25.4	18.4	29.0	17.8	16.0
TOTAL		44.6	27.0	18.5	30.1	16.2	15.0

own trees in relation to the rootstocks budded (recorded in table V).

on <u>P. besseyi</u>		on <u>P. tomentosa</u>			TOTAL		
Bud- lings	Trees	Grow- ing buds	Bud- lings	Trees	Grow- ing buds	Bud- lings	Trees
-	-	-	-	-	83.0	54.2	27.1
31.2	29.6	18.3	8.1	8.1	44.0	24.8	23.7
0.7	0.7	0.6	0.6	0.0	5.8	3.9	3.5
15.8	15.0	5.0	2.5	2.0	27.8	17.2	12.3
33.3	28.5	7.0	5.1	4.6	27.9	15.9	14.5
32.8	32.1	22.3	11.9	11.9	46.5	26.0	25.4
2.2	2.1	5.0	1.9	1.9	7.2	3.6	3.5
22.5	21.4	8.6	5.2	4.8	23.2	13.9	12.2
15.1	15.1	13.2	8.4	6.7	30.8	15.9	14.7
12.7	9.5	12.9	6.4	6.4	31.7	16.8	14.0
0.0	0.0	1.2	0.6	0.6	6.0	3.3	3.2
12.2	11.5	10.4	6.0	5.2	25.9	13.5	12.0
39.3	39.3	17.7	17.7	16.1	57.1	25.3	24.0
11.7	9.2	18.9	12.5	9.4	29.4	19.4	13.0
2.3	1.5	7.3	6.8	2.1	15.1	11.5	7.2
17.8	16.8	14.8	11.7	8.3	33.1	18.8	14.4
16.2	15.3	10.7	7.1	5.7	27.9	15.6	12.8

On peach rootstocks the results were in conformity with the general pattern. Highest percentages were with virus-free buds and virus-free rootstocks (51.0) and with both components infected (44.9). Lowest percentage was with buds only infected (30.9), while with rootstocks only infected the percentage bud take was 42.9. On Prunus besseyi and P. tomentosa rootstocks, bud take percentages were low (especially on P. tomentosa) and somewhat erratic. On all three rootstocks, however, bud take was relatively good when both rootstock and bud were virus-free.

On June 22, 1960, another count was made of the same trees, but at this time only budlings grown enough to be tied to the common nursery iron rod were counted. These were the budlings that could be expected to grow into normal, salable trees. The numbers of budlings on the initially budded rootstocks in relation to the virus content of the respective buds and rootstocks are presented in Table V, while the percentages of budlings in relation to the rootstocks budded are presented in Table VI.

In the June 22 count there was a significantly greater budling stand when both scions and stocks were virus-free (18.8 per cent) or both were virus-infected (17.2 per cent) than when the buds only (13.9 per cent) or the rootstocks only (13.5 per cent) carried the virus. The differences between the 18.8 per cent stand (VFB or VFR) and the 13.9 per cent stand (NRSB on VFR) and the 13.5 per cent stand (VFB on NRSR)

are statistically significant at the 0.01 level, while the differences between the latter two and the 17.2 per cent (NRSB on NRSR) are statistically significant at the 0.05 level. However, there is no significant difference between the budling stand (18.8 per cent) from virus-free stocks and scions and that (17.2 per cent) from virus-infected stocks and scions, nor is there a statistically significant difference between the budling stand (13.9 per cent) from virus-infected buds only and that (13.5 per cent) from virus infected rootstocks only.

By comparing the corresponding sections of table VI it can be seen that there is an almost proportional reduction in the percentages of the budlings (17.2 compared to 27.8 per cent) when both buds and stocks were virus infected. Similarly the budlings were 60.0 per cent of the bud take when only the buds were carrying the virus, 52.1 per cent of the bud take when the rootstocks only carried the virus, and 56.7 per cent of the bud take when both buds and stocks were virus-free.

On September 22, 1960, a final count was made of the trees which developed from the budlings counted on June 22. It can be seen in tables V and VI that there were slightly fewer trees on September 22 than there were budlings on June 22. A few trees had been broken by the cultivator, so that not all failures of budlings to develop into trees could be attributed to relatively poor bud union. The reduction in the number of trees in relation to the number of budlings was independent of the presence or absence of NRS virus in the scion and/or the

rootstock. Fig. 15 presents in a diagrammatic form the percentages of peach buds growing on the three rootstocks in relation to virus content of scions and rootstocks.

A summary of the data on bud take when virus-infected or virus-free buds are used on virus-free and virus-infected rootstocks is presented in tables VII and VIII. In table VII we can see that the bud take (25.3 per cent) with necrotic ring spot virus-infected buds is less than the bud take (29.2 per cent) with virus-free buds, regardless of the presence or absence of virus in the rootstock. This difference is statistically significant at the 0.01 level. It is worth noting, however, on P. besseyi rootstocks, there was better bud take (33.9 per cent) with virus-infected than with virus-free buds (26.8 per cent).

Considering the performance of virus-infected and virus-free rootstocks regardless of buds placed on them, it can be seen that on virus-free rootstocks there was a higher bud take percentage (29.2) than on virus-infected rootstocks (26.4 per cent); the difference between the two percentages is statistically significant at the 0.01 level. All three rootstocks followed this pattern.

In table VIII are recorded the budling stands counted on June 22. The stand (16.0 per cent) obtained with virus-free buds is greater than that (14.8 per cent) obtained with necrotic ring spot virus infected buds, but the difference is not statistically significant. If the stands on each rootstock

Fig. 15 Percentages of peach buds growing on three rootstocks in relation to virus content of scions and rootstocks.

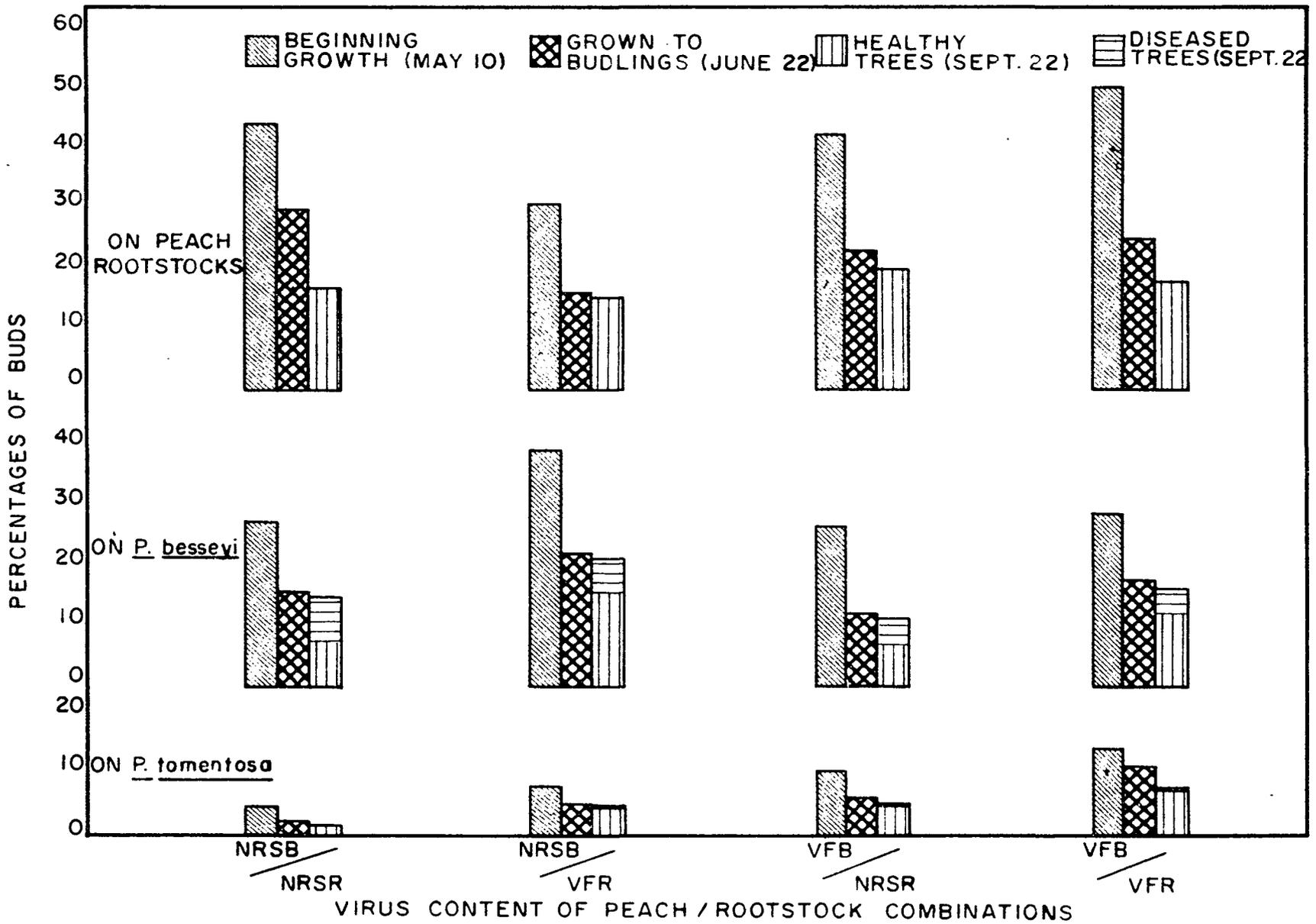


Table VII. Summary of bud take results on virus-free and virus-infected buds and rootstocks. Count of May 10, 1960.

Buds	BUDS						ROOTSTOCKS					
	NRSV-infected			Virus-free			NRSV-infected			Virus-free		
	BR ^a	BG ^b	BT ^c	BR ^a	BG ^b	BT ^c	BR ^a	BG ^b	BT ^c	BR ^a	BG ^b	BT ^c
<u>Rootstock sp.</u>												
Peach	511	195	38.1	1262	596	47.2	859	374	43.5	914	417	45.6
<u>P. besseyi</u>	530	180	33.9	938	262	26.8	822	225	27.2	646	218	33.7
<u>P. tomentosa</u>	636	48	7.5	1272	158	12.2	899	83	9.2	1009	123	12.2
TOTAL	1677	423	25.2	3472	1016	29.2	2580	682	26.4	2569	758	29.6

^aBR = Budded to rootstocks.

^bBG = Beginning to grow.

^cBT = Per cent bud take.

Table VIII. Summary of bud take results on virus-free and virus-infected buds and rootstocks. Count of June 22, 1960.

Buds	BUDS						ROOTSTOCKS					
	NRSV-infected			Virus-free			NRSV-infected			Virus-free		
	BR ^a	GB ^b	St ^c	BR ^a	GB ^b	St ^c	BR ^a	GB ^b	St ^c	BR ^a	GB ^b	St ^c
<u>Rootstock sp.</u>												
Peach	511	119	23.2	1262	311	24.6	859	220	25.6	914	210	23.0
<u>P. besseyi</u>	530	102	19.2	938	136	14.4	822	110	13.3	646	128	19.8
<u>P. tomentosa</u>	636	27	4.2	1272	109	8.5	899	47	5.2	1009	89	8.8
TOTAL	1677	248	14.8	3472	556	16.0	2580	377	14.6	2569	427	16.6

^aBR = Budded to rootstocks.

^bGB = Grown to budlings.

^cSt = Per cent stand.

are compared separately we see that on peach there is no significant difference between the 23.2 per cent stand obtained with virus-infected buds and the 24.6 per cent with virus-free buds. On P. besseyi the stand (14.4 per cent) with virus-free buds is smaller than that (19.2 per cent) obtained with virus-infected buds and the difference is statistically significant at the 0.01 level. On the contrary, on P. tomentosa the stand (8.5 per cent) with virus-free buds is higher than that (4.2 per cent) attained with virus-infected buds and the difference is statistically significant at the 0.01 level.

In comparing virus-free with necrotic ring spot virus-infected rootstocks, it can be seen that the virus-free rootstocks produced a better stand (16.6 per cent) than the virus-infected rootstocks (14.6 per cent), and the difference between the two is statistically significant at the 0.05 level. Examining the performance of each rootstock it is evident that virus-free P. besseyi and P. tomentosa rootstocks gave a better stand than their virus-infected counterparts; both differences are significant at the 0.01 level. On peach, the small difference (25.6 vs 23.0) in favor of the virus-infected rootstocks was not statistically significant.

In general, there were higher percentages of bud take and budling stands from virus-free buds than from NRS virus-infected buds and similar higher percentages from virus-free than from NRS virus-infected rootstocks.

Date-of-budding Effect on
Bud Take When the Bud or
the Rootstock or Both Carry the Virus

If, as has been reported (Cochran et al 1951), necrotic ring spot virus induces a shock effect at the point of bud insertion, with some subsequent recovery and satisfactory healing of the union, it is conceivable that time of budding might not allow sufficient time before dormancy for adequate recovery and healing.

The budding of peaches in southwest Iowa nurseries is done in the period, July to early September. The experiment described previously included early, intermediate and late budding dates. Equal numbers of peach seedlings were budded on July 28, August 18 and September 8, 1959. Similarly, equal numbers of P. besseyi and P. tomentosa were budded on the same dates. The buds and the seedlings were either virus-free or they were infected with NRS virus and the budding was done so that all the combinations were represented, i.e., healthy buds on virus-infected seedlings, virus-infected buds on healthy seedlings and virus-infected buds on virus-infected seedlings. In table IX, the percentage data from table VI have been rearranged and presented so as to compare bud take and subsequent stands from buddings at the three dates.

The over-all percentages of bud take for the three budding dates were: July 28, 40.7; August 18, 33.8; September 8,

Table IX. Percentages of peach buds beginning to grow, grown to budlings and grown to t

Budding date	Rootstock sp.	NRSB/NRSR ^b			NRSB/VFR ^c			BG ^e
		BG ^e	Buds GB ^f	GT ^g	BG ^e	Buds GB ^f	GT ^g	
July 28	Peach	83	54.2	27.1	61.4	32.2	30.2	60.8
	<u>P. besseyi</u>	-	-	-	57.1	33.3	28.5	32.2
	<u>F. tomentosa</u>	-	-	-	7.0	5.1	4.6	13.2
	Aver.	83	54.2	27.1	27.9	15.9	14.5	30.8
Aug. 18	Peach	-	-	-	-	-	-	47.9
	<u>P. besseyi</u>	53.9	31.2	29.6	58.3	32.8	32.1	31.7
	<u>F. tomentosa</u>	18.3	8.1	8.1	22.3	11.9	11.9	12.9
	Aver.	44.0	24.8	23.7	46.5	26.0	25.4	31.7
Sept. 8	Peach	14.2	10.2	9.5	11.3	6.0	6.0	14.6
	<u>P. besseyi</u>	2.2	0.7	0.7	4.3	2.2	2.1	0.0
	<u>F. tomentosa</u>	0.6	0.6	0.0	5.0	1.9	1.9	1.2
	Aver.	5.8	3.9	3.5	7.2	3.6	3.5	6.0
	Mean Total	27.8	17.2	12.3	23.2	13.9	12.2	25.9

^aNRSB = Necrotic ring spot virus-infected buds.

^eBG =

^bNRSR = Necrotic ring spot virus-infected rootstocks.

^fGB =

^cVFR = Virus-free rootstocks.

^gGT =

^dVFB = Virus-free buds.

and grown to trees with budding done at three dates on three rootstocks.

HT ^g	VFB/NRSR ^b			VFB/VFR ^c			Average		
	Buds			Buds			BG ^e	GB ^f	GT ^g
	BG ^e	GB ^f	GT ^g	BG ^e	GB ^f	GT ^g			
30.2	60.8	32.0	28.8	75.9	21.2	19.3	70.9	41.2	25.2
28.5	32.2	15.1	15.1	62.2	39.3	39.3	41.6	22.5	22.1
4.6	13.2	8.4	6.7	17.7	17.7	16.1	12.0	9.1	7.8
44.5	30.8	15.9	14.7	57.1	25.3	24.0	40.7	23.9	18.0
-	47.9	27.4	22.5	41.5	28.4	17.6	44.6	27.9	20.0
32.1	31.7	12.7	9.5	21.8	11.7	9.2	42.1	22.5	20.5
1.9	12.9	6.4	6.4	18.9	12.5	9.4	16.8	9.7	8.4
15.4	31.7	16.8	14.0	29.4	19.4	13.0	33.8	19.7	15.9
6.0	14.6	8.0	8.0	35.3	25.3	18.6	18.1	12.3	10.6
1.1	0.0	0.0	0.0	3.1	2.3	1.5	2.5	1.3	1.14
1.9	1.2	0.6	0.6	7.3	6.8	2.1	3.8	2.7	1.22
3.5	6.0	3.3	3.2	15.1	11.5	7.2	8.8	5.8	4.4
2.2	25.9	13.5	3.2	33.1	18.8	14.4	27.9	15.6	12.8

^eBG = Buds beginning to grow.

^fGB = Grown to budling.

^gGT = Grown to trees.

8.8 per cent. In comparing the average bud take percentages for the three rootstocks with the several virus-free or virus-infected stock-scion combinations at three dates of budding, the 15.1 per cent bud take with virus-free buds on virus-free rootstocks is noticeable and significantly better than the other three percentages (5.8, 7.2, 6.0) for this date. Bud take on all three rootstocks was poor with the late budding; in fact, bud take on P. tomentosa was poor for all three budding dates.

In the second column of each heading (table IX) are recorded the percentages of buds that grew to budlings by the June 22 count. The general mean for all rootstocks and virus-content combinations for the budding of July 28 is 23.9 per cent (which is 41.4 per cent less than the bud take measured on May 10), for the budding of August is 19.7 per cent (41.8 per cent less) and for the budding of September 8 is 5.8 per cent (34.1 per cent less).

The percentages of buds grown to trees are shown in the third column of each heading of the table IX. The numbers of budlings from the July 28 budding that developed into trees were approximately proportional to those of the August 18 and the September 8 buddings. Thus 18.0 per cent of the buds budded in July 28, 15.9 per cent of those budded in August 18 and 4.4 per cent of those budded in September 8 developed into trees. These tree percentages correspond to 23.9, 19.7, and 5.8 per cent of budlings developed from the July 28,

August 18, and September 8 buddings.

These data suggest that the date of budding can markedly affect the percentage of trees that will develop from peach buds budded on the three rootstocks. This effect, however, seems to be limited to the period of development of successful union between bud and stock soon after budding or possibly in the very early stages of bud growth. Once the bud has started to grow, it seems to be of no importance whether the budding had been done early or late in the previous season.

Whether there is any interaction between effect of presence of NRS virus in the stock and/or the scion at time of budding and effect of time of budding can not be determined from these data.

Bud Take of Healthy and Necrotic Ring Spot

Virus-infected Peach Buds on

Peach and Two Dwarfing Rootstocks

The poor bud take of peach on P. tomentosa was noticed as soon as 1957, when the first peach trees showing the virus-like symptoms were observed. This poor bud take and the resemblance of these symptoms to those accompanying certain viruses, plus the possible natural occurrence of viruses like the necrotic ring spot virus in the P. tomentosa seedlings and in the peach buds made it desirable to know what is the effect of necrotic ring spot on bud take of peach on P.

tomentosa and subsequent symptom development. Since P. besseyi was the other dwarfing rootstock, it seemed likewise desirable to have the same information about this stock. The peach rootstock was included in the experiment for comparative purposes and also for the actual study of its behavior as a rootstock with the known presence of a virus. The budding at different dates was included in the experiment to find the most suitable period of the growing season when each rootstock should be budded.

Brase and Way (1959) reported budling stands of peach on P. tomentosa varying from 64 to 86 per cent and on P. besseyi from 66 to 77 per cent, depending on the peach variety. Actually these are percentages of salable one-year-old trees. They also noted that P. besseyi rootstocks must be budded in late July and must be virus-free if a few peach budlings are not to develop a pale green foliage and tend to roll upward toward the midrib in midsummer. These peach budlings also defoliated prematurely and the bud union showed abnormal growth.

In the experiments in southwestern Iowa, Elberta peach buds were used throughout on the three rootstocks. The data are summarized on tables V and VI.

Considering the bud stands (table VI) on each of the rootstocks in all three budding dates, the bud take of peach on peach was the highest (44.6 per cent), the bud take on P. besseyi next (30.1 per cent), and the stand on P. tomentosa

the lowest (10.7 per cent). Comparing the bud take of peach buds on each of the three rootstocks within one budding date, it is evident that when budded on July 28, peach on peach gave 70.9 per cent stand, peach on P. besseyi gave 41.6 per cent stand and peach on P. tomentosa gave 12.0 per cent stand. In August 18 budding, peach on peach gave 44.6 per cent stand, peach on P. besseyi almost the same (42.1 per cent) and peach on P. tomentosa produced 16.8 per cent stand. In September 8 budding, the bud take on all three rootstocks was low: 18.1 per cent of peach on peach, 2.5 per cent of peach on P. besseyi, and 3.8 per cent on P. tomentosa.

Within each rootstock and for all the budding dates there was some variation in the mean bud take percentage depending on whether the buds or rootstocks or both were NRSV-infected. In the case of peach on peach, virus-free buds and rootstocks produced the highest bud take (51.0 per cent, or 59.1 per cent) when the bud takes of only July and September are considered. With both buds and rootstocks carrying virus, the bud take (44.9 per cent) was lower than that with virus-free buds and rootstocks, but higher than the bud take (30.9 per cent) with necrotic ring spot virus-infected buds on virus-free rootstocks and the bud take (42.9 per cent) with virus-free buds on necrotic ring spot virus-infected rootstocks.

The results on P. besseyi rootstock are somewhat erratic. With the peach budded on P. besseyi the highest bud take was attained, surprisingly, with necrotic ring spot

virus-infected buds budded on virus-free rootstocks (39.8 per cent). With virus-free buds the bud take was essentially the same on necrotic ring spot virus-infected rootstocks (27.1 per cent) and on virus-free rootstocks (29.0 per cent) and the same as the bud take (27.7 per cent) of virus-infected buds on virus-infected rootstocks. However, when only the August and September buddings are considered in all three cases, the bud take (27.7 per cent) of necrotic ring spot virus-infected buds and stocks is significantly greater than that of virus-free buds on necrotic ring spot virus-infected rootstocks (18.7 per cent) and on virus-free rootstocks (12.1 per cent).

In the budding of peach on P. tomentosa, virus-free buds and stocks yielded the best bud take (14.8 per cent), while virus-infected buds and stocks yielded the poorest bud take (5.0 per cent). With buds or the rootstocks only virus-infected, the bud take was similar and intermediate (8.6 per cent and 10.4 per cent, respectively).

On June 22, the growing budlings were counted and the data, grouped according to rootstock, date of budding and virus content, are also summarized in tables V and VI. The stand from May 10 to June 22 was reduced by 39.5 per cent on peach, by 46.2 per cent on P. besseyi and by 33.7 per cent on P. tomentosa. The reduction in each item listed in the table was approximately proportional to the reduction of the means, with no outstanding differences. The same is true when the

numbers of buds grown to trees are compared with those of budlings and of growing buds (tables V and VI). Fig. 16 presents in diagrammatic form the bud take and growth of peach buds on three rootstocks during the first growing season.

Growth of Peach Buds on
Three Rootstocks in the
Presence of Necrotic Ring Spot Virus

On July 19, 1960, the height of the peach budlings growing on the virus-free or virus-infected peach, P. besseyi and P. tomentosa rootstocks was measured. The height was taken as the distance from the point of the stock-scion union to the apical or highest other branch tip. The data are summarized in table X.

The diameter of the same budlings was measured on August 15, 1960. Trunk diameter was measured 5 cm above the stock-scion union. Table X presents the summarized data.

The budlings on peach attained an average height of 81.9 cm while those on P. besseyi averaged 62.2 cm. The budlings on P. tomentosa were the shortest with an average of 48.5 cm. The presence of virus in the stock or scion did not produce any significant differences within groups of the same rootstock, although virus-infected buds on virus-free peach and P. tomentosa produced the shortest budlings (76.5 cm and 44.7 cm, respectively).

Fig. 16 Bud take and growth of peach buds on three rootstocks during the first growing season.

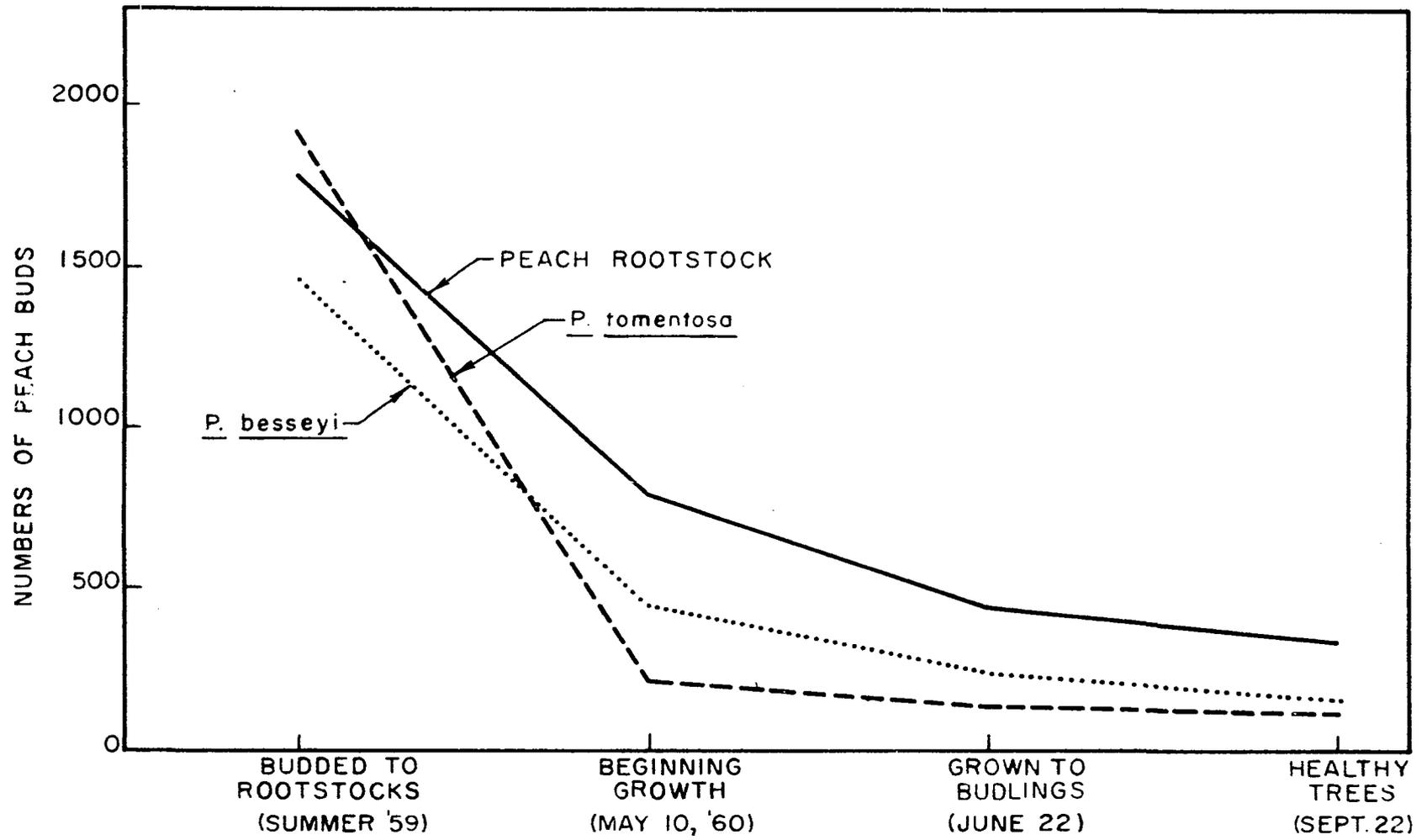


Table X. Height, diameter and condition of peach budlings on peach and on two dwarfing stocks.

	Understock	NRSB/NRSR		NRSB/VFR		VFB/NRSR		VFB/VFR		Total
		Trees	cm (aver.)	Trees	cm (aver.)	Trees	cm (aver.)	Trees	cm (aver.)	
Height ¹	Peach	84	82.4	41	76.5	62	86.0	60	81.1	81.9
	<u>P. besseyi</u>	41	54.2	58	69.7	68	57.8	65	64.8	62.2
	<u>P. tomentosa</u>	4	53.5	21	44.7	37	45.9	49	51.7	48.5
Dia- meter ²	Peach	78	1.04	41	1.10	59	1.27	57	1.04	1.11
	<u>P. besseyi</u>	39	0.70	58	0.98	65	0.80	63	0.85	0.84
	<u>P. tomentosa</u>	4	1.08	21	0.94	37	1.03	48	1.07	1.03
			Per cent		Per cent		Per cent		Per cent	Per cent
Dis- eased ³ trees	Peach	0	0	0	0	1	1.6	0	0	0.4
	<u>P. besseyi</u>	19	48.7	15	25.8	22	33.8	16	25.4	32.0
	<u>P. tomentosa</u>	0	0	1	4.7	1	2.7	1	2.1	2.7

¹Height measured on July 19, 1960, from bud-union to highest branch tip.

²Diameter of trunk measured on August 15, 1960, five cm above graft union.

³Diseased trees counted on August 15, 1960.

Peach on peach also produced trees with greater diameter (1.11 cm) than on the other rootstocks. This diameter though, is not significantly greater than that of peach on P. tomentosa (1.03 cm). Budlings on P. besseyi were the smallest in diameter (0.84 cm), although they were considerably taller than those on P. tomentosa. Again, there were no significant differences in caliber associated with presence or absence of necrotic ring spot virus in the buds and/or in the rootstocks.

There was only one diseased tree in the block of peach on peach. The frequency of diseased trees on P. tomentosa rootstocks was surprisingly low; only 3 trees were showing symptoms. The incidence of diseased trees (32 per cent) in the P. besseyi block was lower than that observed in previous years, but much higher than that observed on P. tomentosa (table X). On the P. besseyi rootstocks there were considerably more diseased trees (48.7 per cent) when both stocks and scions were infected with the necrotic ring spot virus, than when the buds only (25.8 per cent), or the stocks only (33.8 per cent) or neither (25.4 per cent) were infected with the virus.

The symptoms on the trees were exactly like those described for the diseased trees observed in previous years. The stock-scion union of peach on P. tomentosa could easily be broken apart and the union surfaces were showing the brown, dead areas described in previous pages. The stock-scion

union of peach on P. besseyi could not usually be broken at exactly the union surfaces, but whenever this was feasible the scion and stock surfaces were similar to those of P. tomentosa, only less brown and less necrotic.

On September 22, the 225 peach trees on P. besseyi were tested for strength of the stock-scion union. Each tree was bent so that a point approximately 35 cm above the union would touch the ground. In this test 25 trees broke, of which 18 broke at the union and 7 at points other than the union. Nine of the 25 trees that broke were conspicuously diseased and one-fourth to two-thirds of the union surfaces were necrotic. The union surfaces of the other trees were little or not at all necrotic.

On the same date (September 22), 40 peach trees on peach were subjected to the same test described above. Each group of 10 trees represented one combination between virus-free or virus-infected scion and virus-free or virus-infected stock. Eight of the 40 trees broke at the stock-scion union, four of which had completely healthy, white union surfaces, while the other four exhibited discoloration of the upper sections of the union surfaces. In all the last four cases the understock was cut back at or very little above the level of the bud and the bud surface was almost in entirety in contact with dead understock tissue. There were no diseased trees in this block.

Survival of Peach Buds on

P. besseyi and P. tomentosa 20 Days after Budding
in the Presence of NRS Virus in Buds and Rootstocks

In February 1960, 500 P. besseyi and 550 P. tomentosa seedlings in storage were inoculated with necrotic ring spot virus-carrying sour cherry buds in the laboratory. At the same time an equal number of P. besseyi and P. tomentosa seedlings were budded with virus-free buds. The budding was done with the bark shield method described under "Methods". All seedlings were returned to storage and kept there until April 12, 1960, when the weather and soil conditions were favorable for planting outdoors on the premises of Mount Arbor nurseries in Shenandoah, Iowa. On August 3, there were 986 P. besseyi and 759 P. tomentosa seedlings growing in the field, and they were budded with peach buds, half of which were carrying the necrotic ring spot virus and half were virus-free. Table XI shows the numbers of virus-inoculated seedlings of each species that were budded with virus-free and virus-infected buds and also the virus-free seedlings budded with virus-free or virus-infected peach buds.

The numbers and percentages of living buds recorded in table XI are much higher than those reported in previous tables for both rootstocks. This count includes all buds still living on the rootstocks only 20 days after budding. It even considers as living the buds in which the part of bark above the bud was dead.

Table XI. Living peach buds on August 23 on virus-free and virus-infected P. besseyi and P. tomentosa stocks budded with virus-infected and virus-free buds on August 3.

Buds	NRSB ^a /NRSR ^b			NRSB ^a /VFR ^c			VFB ^d /NRSR ^b			VFB ^d /VFR ^c			TOTAL		
	ER ^e	Lv ^f	Pc ^g	ER ^e	Lv ^f	Pc ^g	ER ^e	Lv ^f	Pc ^g	ER ^e	Lv ^f	Pc ^g	ER ^e	Lv ^f	Pc ^g
Rootstock sp.															
<u>P. besseyi</u>	247	157	63.5	247	162	65.5	246	151	61.3	246	179	72.7	986	649	65.8
<u>P. tomentosa</u>	184	69	37.4	202	84	41.5	176	63	35.8	197	81	41.1	759	297	39.1

^aNRSB = Necrotic ring spot virus-infected buds.

^bNRSR = Necrotic ring spot virus-infected rootstocks.

^cVFR = Virus-free rootstocks.

^dVFB = Virus-free buds.

^eER = Budded to rootstocks.

^fLv = Living.

^gPc = Per cent.

The bud take on P. besseyi (65.8 per cent) was much higher than that on P. tomentosa (39.1 per cent). The highest bud take on P. besseyi was attained with virus-free peach buds on virus-free stocks (72.7 per cent). The difference between this and the bud take (63.5 per cent) of virus-infected buds on virus-infected stocks or the bud take (65.5 per cent) of virus-infected buds on virus-free stocks is statistically significant at the 0.05 level, while the difference between 72.7 per cent and the bud take (61.3 per cent) of virus-free buds on virus-infected rootstocks is statistically significant at the 0.01 level.

The bud take on P. tomentosa at this date was apparently not significantly altered by the presence of necrotic ring spot virus in the buds and/or in the rootstocks. None of the differences between bud take percentages is statistically significant at the 0.05 level.

It is worth noting that on both rootstock species bud take was higher when the rootstocks were virus free (72.7 and 65.5 per cent on P. besseyi, 41.1 and 41.5 per cent on P. tomentosa) than when the rootstocks were infected with the virus (in which case the bud take was 61.3 and 63.5 per cent on P. besseyi and 35.8 and 37.4 per cent on P. tomentosa), although not all these differences are significant.

Subsequent growth and development of these buds and symptom expression by budlings from them remain to be observed.

DISCUSSION

The nature of the symptoms on dwarf peach trees on Prunus besseyi and P. tomentosa rootstocks strongly suggested that they might be caused by X virus. The fact that, beginning in midsummer, symptoms became progressively more striking on an increasing number of trees supported this possibility.

That the malady, whatever the cause, was associated with the rootstocks was indicated by the lack of symptoms on regular peach trees propagated from the same budwood on peach roots, and by the scattered distribution of individual trees with symptoms in the dwarf peach blocks. (Propagation procedure involves budding understocks consecutively in the row with buds from the bud stick in hand, then likewise with buds from the next bud stick, etc.).

Considerable evidence is indicative that the symptoms were not virus induced. In all attempts at transmission, involving altogether 31⁴ trees, there was no symptom expression as a result of inoculation. Thirty-eight of 50 trees which showed symptoms in 1957 produced foliage after they were transplanted in the greenhouse (20) or in the field (30). Foliage in all cases was symptomless. There were symptomless sprouts on the P. tomentosa rootstocks of peach trees with symptoms in the field in 1958.

Cochran et al (1951) reported that necrotic ring spot

complex can cause disturbance at the graft union. In one transmission experiment with P. tomentosa necrotic ring spot was detected in only 2 of 40 peach trees, 20 of which previously had shown disease symptoms.

That a disturbance at the base of the tree might be involved was first evident when peach seedlings girdled at the base by label wires showed top symptoms suggestive of the dwarf peach disease. The P. tomentosa rootstocks of diseased dwarf peach trees were healthy, but the poor graft union with the peach scion was clearly evident as reported here in detail.

Whatever the malfunction resulting from the poor graft union, it apparently was accentuated in the excessively wet soil areas in the nursery block under observation in 1958. This association with excessive soil moisture, plus the extensive reddening of leaves and twigs of trees with the poorest graft unions, suggests the failure of translocation of mineral nutrients from roots to tops and/or excessive accumulation of carbohydrates in the tops of diseased trees.

In the 1959 and 1960 experiments, presence of virus in the stock and/or scion did seem to result in somewhat lower percentage bud take. In general, when either the buds or the stocks were infected with necrotic ring spot virus, there was a marked interference with stock-scion union and significantly lower bud take and budling stand percentages. However, when both buds and rootstocks were virus-infected, the effect

on stock-scion union, although apparently unfavorable, was not great enough for the percentage bud take and budding stands to be significantly lower than those from virus free stocks and scion. The above observations are in agreement with those reported by Cochran et al (1951) in relation to ring spot virus and peach varieties propagated on peach.

Hildebrand (1953) budded Montmorency on ring spot virus-infected mahaleb rootstocks and on healthy or diseased mazzard rootstocks with fair success, but he got no bud take upon budding diseased Montmorency buds on healthy mahaleb. In the experiments reported herein the virus effect when peach was budded on peach or on P. tomentosa was similar in most respects. Thus on both rootstocks the limitation of bud take was about the same whether the virus was in the bud or in the rootstock only. On P. besseyi, however, the percentage bud take was significantly less when the rootstocks only were virus-infected than when virus-infected buds were placed on virus-free rootstocks.

Although budding peach in mid-August on P. besseyi and on P. tomentosa gave as good a bud take as budding in July, the low bud take observed soon after the August budding makes budding of peach on any of the three rootstocks in late July seem preferable to budding on either of the later dates. Brase and Way (1959) have already suggested that P. besseyi rootstocks must be budded in late July. Budding in early September was unsuccessful on all three rootstocks in this

experiment.

Concerning the value of P. besseyi and P. tomentosa as dwarfing rootstocks for peach, the data presented show that both sustained a very low bud take percentage. P. besseyi gave a higher initial bud take and budling stand than P. tomentosa and seemed to be affected less by the presence of necrotic ring spot virus in the buds or stocks at the time of budding. However, the much greater percentage of diseased trees at the end of the first growing season on P. besseyi than on P. tomentosa, more or less nullified the better bud take on P. besseyi. The low bud take of peach on P. tomentosa and the high incidence of trees with late summer stock-scion incompatibility symptoms in peach on P. besseyi casts some doubt on the suitability of either of these two rootstocks for dwarf peach tree propagation, at least under the conditions prevalent in southwest Iowa nurseries.

SUMMARY AND CONCLUSIONS

In late summer of 1957 one-year-old trees of several peach varieties propagated on P. tomentosa and P. besseyi showed a diseased condition characterized by yellowing and rolling of the leaves, reddening of leaves and twigs, inhibition of growth and, in extreme cases, death of the trees. The similarity of the symptoms with those described for X-disease of peach along with the fact that no other pathogen was detected in any part of the diseased plant, led to the speculation that the cause of the disease could be the X virus or perhaps some other virus.

Attempts were made to transmit virus with dormant buds in the greenhouse and with buds from growing trees in the greenhouse and in the field. The attempted transmissions were to P. tomentosa seedlings, first year Elberta peach trees and California Lovell peach seedlings. A total of 314 trees were budded; in no case were any symptoms expressed.

Peach trees with and without symptoms in 1957, when transplanted in the greenhouse and in the field, did not manifest symptoms in the subsequent growth, although a few of the trees failed to grow at all.

The evidence indicates that the disease is caused by scion-stock incompatibility rather than by a virus. Observations of diseased and healthy peach trees of four varieties grafted on P. tomentosa rootstocks during the summer of 1958,

directed attention to the following points:

1. The more diseased was a tree the weaker and more discolored was its graft union. Longitudinal sections of the trees at the graft union area revealed a characteristic line of discolored or dead tissue extending across the entire union area or across the major part of it.
2. Peach seedlings with stems damaged by wire girdling showed symptoms similar to those of diseased trees.
3. Healthy peach trees on peach roots always had sound, white graft unions which never separated when pulled, the scion or the stock breaking instead.
4. Many peach trees grafted on P. tomentosa roots were exuding gum at the graft union.
5. Percentage of diseased trees on P. besseyi rootstocks was by far larger (45 per cent) than on P. tomentosa (6 per cent).
6. On the same rootstock, P. tomentosa, different peach varieties showed different percentages of diseased trees (Halehaven 13.9 per cent, Golden Jubilee 22.1 per cent, Elberta 22.9 per cent, Polly 25.2 per cent).
7. The effect of poor graft union was more pronounced in locations with too much soil moisture, where the percentage of diseased trees was from 38.5 per cent (Golden Jubilee) to 73.7 per cent (Polly), as compared to 17 per cent and 8.6 per cent diseased trees of the same varieties, respectively, in well drained locations.

In experiments conducted in the summers of 1959 and 1960, approximately equal numbers of virus-free or necrotic ring spot virus-infected seedlings of peach, P. besseyi and P. tomentosa were budded with virus-free or necrotic ring spot virus-infected Elberta peach buds in late July, middle August and early September. The bud take and budding stand were counted in the spring and summer of 1960.

The data presented suggest that the stock-scion union of peach on peach, P. besseyi and P. tomentosa was interfered with by the presence of necrotic ring spot virus in the buds or in the rootstocks at the time of budding. The interference of the virus was expressed as reduction in the bud take and budding stand percentages, and was most evident when only the buds or only the rootstocks carry the virus. Bud take on P. besseyi was reduced when only the rootstocks were virus-infected but was not affected when only the buds contained the virus.

When both buds and rootstocks were infected with virus, their union was not impaired significantly and the resulting bud take was almost as high as that with virus-free buds and stocks. Possible exception was P. besseyi rootstock, results with which were somewhat erratic.

The earlier the budding of peach on peach the higher was the bud take percentage (July 28, 70.9 per cent, August 18, 41.6 per cent, September 8, 12.0 per cent). Budding on P. besseyi and P. tomentosa in late July resulted in no better

bud take (41.6 per cent, 12.0 per cent) than budding in mid-August, (42.1 per cent, 16.8 per cent) but the bud take dropped sharply (2.5 per cent, 3.8 per cent) when these two rootstocks were budded in early September.

Bud take percentage on P. besseyi was generally much higher than that on P. tomentosa. Peach budlings grew tallest on peach (81.9 cm) and they were also taller on P. besseyi (62.2 cm) than on P. tomentosa (48.5 cm), but they were of smaller trunk diameter (0.84 cm) on P. besseyi than on P. tomentosa (1.03 cm) in the measurement of August 15. By the end of the first growing season there was a much higher incidence of peach trees showing stock-scion incompatibility symptoms on P. besseyi (32 per cent) than on P. tomentosa (4 per cent). Although the foliage symptoms of diseased trees were the same on both rootstocks, appearance of the stock-scion union area was less necrotic on P. besseyi than on P. tomentosa. There were more diseased trees (48.7 per cent) on P. besseyi with both stocks and scions virus-infected than when only one of the two (25.8 and 33.8 per cent) or none (25.4 per cent) carried the virus at time of budding.

LITERATURE CITED

1. Argles, G. K. 1937. A review of the literature on stock-scion incompatibility in fruit trees, with particular reference to pome and stone fruits. Commonwealth Bur. Hort. and Plantation Crops, East Malling, Eng. Tech. Commun. 9:5-15.
2. Armstrong, W. D. and F. R. Brison. 1949. Delayed incompatibility of a live oak-post graft union. Proc. Amer. Soc. Hort. Sci. 53:543-545.
3. Bailey, J. S. 1923. A microscopic study of apple graft unions. Unpublished M. S. Thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
4. Blodgett, E. C. 1937. Fruit diseases in Idaho. Plant Dis. Rptr. 21:89-95.
5. ———. 1939. Some obscure plant diseases in Idaho. Plant Dis. Rptr. 23:216-218.
6. Bodine, E. W. and L. W. Durrell. 1941. Virus diseases of peach in Western Colorado. Plant Dis. Rptr. 25:474-475.
7. Bradford, F. C. and B. G. Sitton. 1929. Defective graft unions in the apple and the pear. Mich. Agr. Expt. Sta. Tech. Bul. 99.
8. Brase, K. D. and R. D. Way. 1959. Rootstocks and methods used for dwarfing fruit trees. N. Y., Geneva, Agr. Expt. Sta. Bul. 783.
9. Cation, D. 1949. Transmission of cherry yellows virus complex through seeds. Phytopathology 39:37-40.
10. Chang, W. T. 1938. Studies in incompatibility between stock and scion with special reference to certain deciduous fruit trees. Jour. Pomol. and Hort. Sci. 15:267-325.
11. Chester, K. S. 1931. Graft-blight: A disease of lilac related to the employment of certain understocks in propagation. Jour. Arnold Arb. 12:79-146.
12. Cochran, L. C. 1946. Passage of the ring spot virus through mazzard cherry seeds. Science 104:269-270.

13. _____ . 1950. Passage of the ring spot virus through peach seeds. (Abstract) *Phytopathology* 40:964.
14. _____ , L. M. Hutchins, J. A. Milbrath, G. L. Stout, and S. M. Zeller. 1951. Ring spot. In "Virus diseases and other disorders with virus-like symptoms of stone fruits in North America." U.S. Dept. Agr. Agr. Handbook. No. 10:71-80.
15. Costa, A. S., T. J. Grand, and S. Moreira. 1954. Behavior of various citrus rootstock-scion combinations following inoculation with mild and severe strains of tristeza virus. *Proc. Florida State Hort. Soc.* 67:26-30.
16. Countryman, Mary C. 1931. The histology of overgrowths on apple graft unions. Unpublished M. S. Thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
17. Davidson, T. R. and J. A. George. 1957. Symptoms of sour cherry yellows and necrotic ring spot in relation to time of infection. (Abstract) *Canad. Phytopath. Soc. Proc.* (25):13.
18. Dirks, C. O. 1925. The relation of hydrogen-ion concentration to the formation of graft unions. Unpublished M. S. Thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
19. Fink, H. C. 1950. Detection of necrotic ring spot virus and its occurrence and spread in sour cherry nurseries. Unpublished Ph. D. Thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
20. Fisk, V. C. 1927. Anatomical studies of non-pathogenic and pathogenic overgrowths on grafted apple trees. Unpublished M. S. Thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
21. Frindlund, P. R. 1959. Time and temperature requirements for the transmission of the necrotic ring spot virus of *Prunus*. *Plant Dis. Rptr.* 43:993-995.

22. Garner, R. J. and D. H. Hammond. 1938. Studies in incompatibility of stock and scion. II. The relation between time of budding and stock-scion incompatibility. Commonwealth Bur. Hort. and Plantation Crops, East Malling, Eng. East Malling Res. Sta. Ann. Rep. for 1937:154-157.
23. Gilmer, R. M. 1955. Imported mahaleb seed as carriers of necrotic ring spot virus. Plant Dis. Rptr. 39:727-728.
24. _____ and K. D. Brase. 1960. Relative infection potentials of rootstock and scion in increasing virus incidence in the deciduous tree fruit nursery. (Abstract) Phytopathology 50:240.
25. _____, K. D. Brase, and K. G. Parker. 1957. Control of virus diseases of stone fruit nursery trees in New York. N. Y., Geneva, Agr. Expt. Sta. Bul. 779.
26. Helton, A. W. 1956. Latent viruses in stone fruit trees. Idaho Agr. Expt. Sta. Bul. 260.
27. Heppner, M. J. and R. D. McCallum. 1927. Grafting affinities with special reference to plums. Calif., Berkeley, Agr. Expt. Sta. Bul. 438.
28. Herrero, J. 1951. Studies of compatibility graft combinations with special reference to hardy fruit trees. Jour. Hort. Sci. 26:186-237.
29. Hildebrand, E. M. 1941. Rapid transmission of yellow-red virosis in peach. Contr. Boyce Thompson Inst. 11:485-496.
30. _____. 1953. Fruit virus diseases in New York in retrospect. Plant Dis. Rptr. Suppl. 222:185-223.
31. Hobart, O. F., Jr. 1954. Introduction and spread of necrotic ring spot virus in sour cherry nursery trees. Unpublished Ph.D. Thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
32. _____. 1956. Passage of cherry virus through Prunus root grafts. Iowa State College Jour. of Sci. 31:49-54.

33. _____, H. C. Fink, and W. F. Buchholtz, 1955. Virus spread in nursery blocks of sour cherries in southwest Iowa. Iowa State College Jour. Sci. 30:249-253.
34. Holmes, K. D. 1957. Propagation of some of the stone fruit trees. Pl. Propag. Soc. Proc. 7:164:167.
35. Kunkel, L. D. 1938. Contact periods in graft transmission of peach viruses. Phytopathology 28:491-497.
36. Laubert, R. 1914. Über die Blattrollkrankheit der Syringen und die dabei auftretende abnorme Starkeanhaftung in dem Blättern der kranken Pflanzen. Gartenflora 63:9.
37. Mason, S. C. and W. L. Hall. 1897. Miscellaneous fruit notes. Kan. Agr. Expt. Sta. Bul. 75.
38. Milbrath, J. A. 1950. Latent ring-spot virus of cherries reduces growth of nursery trees. Plant Dis. Rptr. 34:374-375.
39. _____ and S. M. Zeller. 1945. Latent viruses in stone fruits. Science 101:114-115.
40. _____ and _____ 1948. Indexing fruit trees for virus. Amer. Nurseryman 88:7-8.
41. Millikan, D. F., Jr. 1955a. Stone fruit virus investigations. I. Inoculation studies of the ring spot virus complex in sweet cherry. Missouri Agr. Expt. Sta. Research Bul. 582.
42. _____ 1955b. The influence of infection by ring spot virus upon the growth of one-year-old Montmorency nursery trees. Phytopathology 45:565-566.
43. _____ 1959. The incidence of the ring spot virus in peach nursery and orchard trees. Plant Dis. Rptr. 43:82-84.
44. Mosse, Barbara. 1955. Symptoms of incompatibility induced in peach by ring grafting with an incompatible rootstock variety. Commonwealth Bur. Hort. and Plantation Crops, East Malling, Eng. East Malling Res. Sta. Ann. Rep. for 1954:76-77.

45. Olson, E. O. 1958. Responses of lime and sour orange seedlings and four scion-rootstock combinations to infection by strains of the tristeza virus. *Phytopathology* 48:454-459.
46. Overholser, E. L., F. L. Overley, J. H. Schultz, and D. F. Allmendinger. 1943. Nursery fruit trees, dwarf and standard understocks, their handling and planting. *Wash. Agr. Expt. Sta. Pop. Bul.* 170.
47. Overholzer, P. C. J. 1947. The Bitter Seville rootstock problem. *Farming in So. Afr.* 22, 255:489-495.
48. Peterson, G. W. 1958. Transmission and latency of cherry necrotic ring spot virus in Prunus tomentosa. Unpublished Ph.D. Thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
49. Proebsting, E. L. and C. J. Hansen. 1943. Leaf scorch and die back of apricots. *Proc. Amer. Soc. Hort. Sci.* 42:270-274.
50. Rawlins, T. E. and K. G. Parker. 1934. Influence of rootstocks on the susceptibility of sweet cherry to the Buckskin disease. *Phytopathology* 24:1029-1031.
51. Reeves, E. L., E. C. Blodgett, T. B. Lott, J. A. Milbrath, B. L. Richards, and S. M. Zeller, 1951. Western X-disease. In the "Virus diseases and other disorders with virus-like symptoms of stone fruits in North America." U.S. Dept. Agr. *Agric. Handbook No.* 10:43-52.
52. Rhoads, A. S. 1930. Devitalization of trees on lemon rootstocks. *Plant Dis. Rptr.* 14:169.
53. Richards, B. L. and L. M. Hutchins. 1941. A new virosis of peach in Utah, resembling X-disease (Yellow-red virosis). (Abstract) *Phytopathology* 31:19.
54. _____ and B. N. Wadley, 1950. Status of our knowledge of the Western X-virus in Utah. (Abstract) *Phytopathology* 40:969.
55. Roberts, R. H. 1929. Some stock and scion observations on apple trees. *Wisc. Agr. Expt. Sta. Res. Bul.* 97.

56. _____ 1949. Theoretical aspects of graftage. Bot. Rev. 15:423-463.
57. Sax, Karl. 1956a. What's new in plant propagation? National Hort. Mag. 35:116-118.
58. _____ 1956b. The story behind dwarf fruits. Hortic. 34:203-233.
59. _____ 1957. Dwarf ornamental and fruit trees. Pl. Propag. Soc. Proc. 7:146-155.
60. _____ and A. Q. Dickson. 1956. Phloem polarity in bark regeneration. Arnold Arbor. Jour. 37:173-179.
61. Schneider, H. 1945. Anatomy of Buckskin-diseased peach and cherry. Phytopathology 35:610-635.
62. _____ 1957. Chronic decline, a tristeza-like bud-union disorder of orange trees. Phytopathology 47:279-284.
63. Schuster, A. L. and P. W. Miller. 1933. A disorder of Persian (English) walnuts grafted on black-walnut stocks, resulting in girdling. Phytopathology 23:408-409.
64. Swingle, R. U. 1953. Some facts and theories concerning compatibility in relation to plant propagation. Pl. Propag. Soc. Proc. 3:23-35.
65. Thomas, H. E. and T. E. Rawlins. 1941. Buckskin disease of stone fruits. (Abstract) Phytopathology 31:864.
66. _____, _____ and K. G. Parker. 1940. A transmissible leaf casting yellows of peach. Phytopathology 30:322-328.
67. Wagnon, H. K., J. R. Breece, and A. Schollocker. 1958. Stubby twig, a new virus disease of peach and nectarine in California. Phytopathology 48:465-468.
68. _____, J. H. Traylor, H. E. Williams, and J. H. Weinberger. 1960. Observations on the passage of peach necrotic leaf spot and peach ring spot viruses through peach and nectarine seeds and their effects on the resulting seedlings. Plant Dis. Rptr. 44:117-119.

69. Warne, L. G. G. and Jane Raby. 1939. Water conductivity of the graft union in apple trees with special reference to malling rootstock No. IX. Jour. Pomol. 16:389-399.
70. Waugh, F. A. 1904. The graft union. Mass. Agr. Expt. Sta. Tech. Bul. 2.
71. Way, R. D. and R. M. Gilmer. 1958. Pollen transmission of necrotic ring spot virus in cherry. Plant. Dis. Rptr. 42:1222-1224.
72. Weeks, W. D. 1948. Further scion and stock combinations with Spy 227. Proc. Am. Soc. Hort. Sci. 52:137-140.
73. Willison, R. S., G. H. Berkeley, and G. C. Chamberlain. 1948. Yellows and necrotic ring spot of sour cherries in Ontario. Distribution and Spread. Phytopathology 38:776-792.
74. Zeller, S. M. and A. W. Evans. 1941. Transmission of Western X-disease and marginal leaf spot of peach in Oregon. Plant Dis. Rptr. 25:452-453.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. W. F. Buchholtz for his kindly advice and constructive criticism during the course of these investigations and in the preparation of this thesis. The author is indebted to Mount Arbor Nurseries for the materials and facilities necessary for the completion of this problem.