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THE HISTOLOGY OF THE BUD GRAFT UNION IN PRUNUS

by

William Ellis Fletcher

A Dissertation Submitted to the
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The Requirements for the Degree of
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INTRODUCTION

The use of selected rootstocks for dwarfing fruit and ornamental plants has been an established practice in Europe for many centuries (Dana, 1952 and Scholz, 1957). It has been only recently however, that the use of dwarf fruit and ornamental plants has gained widespread acceptance in the United States. This has developed primarily because of increased production costs in commercial orchards that have bearing trees of relatively high stature. Contemporary architecture, featuring the single story dwelling, has also created a demand for low-growing trees and shrubs which will maintain the scale of the home-lot landscape complex.

In the period immediately prior to World War I, Hedrick (1914) was convinced that dwarf fruit trees were of little value to the commercial orchardist of that era, but he acknowledged that trees of a smaller stature might have a place in the commercial operation when more information became available on the mechanism of dwarfing and the performance of specific rootstock-scion combinations. Hedrick also suggested that the use of dwarf fruit trees would be limited to filler plantings in bearing orchards and for training as special landscape features.

As a result of continued research and experience, the use of vegetatively propagated dwarf fruit trees has gained
in popularity. Reasons for this trend may be itemized as follows:

a. reduction in operational cost and damage to trees as a part of the necessary cultural operations (Bailey, 1914; Banta, 1955b; Brase and Way, 1959; Mahlstede and Haber, 1957; Sax, 1956a; 1957; Scholz, 1957 and Tukey and Brase, 1939b);

b. the facility of handling a greater number of varieties per unit area (Bailey, 1914; Hedrick, 1915; Sax, 1956a; 1957 and Tukey and Brase, 1939b);

c. reduced injury to developing fruit and trees as a result of severe winds (Hedrick, 1914 and Scholz, 1957);

d. earlier bearing and increased yields in comparison to standard sized trees (Bailey, 1914; Banta, 1955b; Brase and Way, 1959; Day, 1934; Dickson and Samuels, 1956; Fowler, 1955; Graves, 1950; Hartmann and Kester, 1959; Hewetson, 1944; Hobbis, 1944; Lorang, 1960; Mahlstede and Haber, 1957; Overholser et al., 1943; Roberts, 1959; Sax, 1956a; Scholz, 1957; Shaw, 1946b; Tukey and Brase, 1939b; 1940 and Upshall, 1959);

e. higher quality, improved color and better flavored fruits (Bailey, 1914; Bostock and Riley, 1885 and Tukey and Brase, 1940);

f. a change from predominantly biennial bearing to that of annual production (Banta, 1955b); and
g. trees which are more adaptable to mechanization (Brase and Way, 1959; Roberts, 1959 and White, 1961).

Some of the disadvantages of grafted dwarf trees would include such factors as:

a. shorter life of dwarf trees in comparison to standard units (Bailey, 1914; Hobbis, 1944; Mahlstede and Haber, 1957 and Shaw, 1946b);

b. increased need for pruning and maintenance practices as compared to that required for standard trees (Bailey, 1914; Gourley and Howlett, 1946; Hobbis, 1944 and Shaw, 1946b);

c. increased difficulty of maintaining and propagating desirable types (Hedrick, 1915 and White, 1961);

d. higher initial cost of the plant (Bailey, 1914; Hedrick, 1915; Hobbis, 1944 and Scholz, 1957);

e. difficulty of securing dwarf trees which are true to name (Hedrick, 1915);

f. changing of annual bearing habits into that of biennial production with certain combinations (Fowler, 1955 and Roberts, 1959);

g. the need for a larger number of trees per unit area of production (Hedrick, 1915 and Scholz, 1957);

h. the difficulty of finding compatible or winter hardy stocks that are suitable for dwarfing purposes (Hedrick, 1915; Scholz, 1957 and White, 1961);
i. the tendency for many dwarfing stocks to sucker (Hedrick, 1915; Preston, 1954; Sax, 1957; Shaw, 1946a and White, 1961); and

j. the large number of trees that are poorly anchored or have an undesirable root system attributable to the dwarfing stock employed (Banta, 1955b, Brase and Way, 1959; Gourley and Howlett, 1941; Hedrick, 1915; Roberts, 1959; Sax, 1953; 1957; Scholz, 1957; Shaw, 1946a; 1946b and White, 1961).

The fact that there exists a diversity of opinion on the advantages and disadvantages for use of dwarf trees, particularly in the commercial orcharding operation, suggests that much has yet to be learned about methods of producing and growing this type of plant material.

Trees of smaller stature produced by use of either dwarfing rootstocks or interstocks, regardless of the limitations, appear to hold the answer to the continued success and growth of the commercial fruit industry. In fact, they may be the only solution to the economic problem of the fruit industry brought about by the elimination of prime orchard land by increased population, urban expansion and the increased cost of maintenance.

The economical production of dwarf fruit trees is one aspect which will lead to the increased use of this type. Only through a better understanding of the phenomena that are required to produce successful stands of dwarf plant
material in the nursery can the industry hope to reduce production costs, thereby encouraging greater utilization of low growing production units.

One specific problem which has been partially investigated is concerned with the use of the western sand cherry (Prunus besseyi) as a possible dwarfing rootstock for peaches (Agrios, 1960). Although this rootstock has many attributes that make it one of the best of the dwarfing peach stocks, delayed incompatibility resulting in loss of plants in the nursery has created a serious problem for plant propagators.

This study was undertaken to investigate the histological process of bud union development between the peach and the western sand cherry. It was the purpose of this project to follow the normal sequence of events that result in a successful union between component parts, as well as to investigate the possible reasons for delayed incompatibility or graft failure.
LITERATURE REVIEW

History of Grafting

The propagation of plant material by means of grafting is one of the oldest arts of plant-craft (Bailey, 1914) and antedates the earliest recorded history (Buck, 1954). Many authors acknowledge the antiquity of grafting and relate how the practice was discussed by such ancient scholars as Aristotle, Theophrastus, Cato and Pliny (Bailey, 1914; Hort, 1916; White, 1961 and Zielinski, 1955).

One of the first recorded references to peach culture was contained in the literature some 2,000 years before the rise of the Roman empire (Cullinan, 1937). The cultivation of peaches is said to date back to the time of Confucius in the fifth century before the Christian era and in the Ritual in the tenth century before Christ (De Candolle, 1895). Roberts (1949) reported that peach varieties were mentioned in Chinese literature as early as 1560 B.C. This would imply that these people had knowledge of clonal propagation techniques such as layerage or graftage. It would appear logical that some realization of stock-scion incompatibility was known at that time.

As early as 200 B.C. detailed descriptions of the techniques for grafting and budding fruit trees and the recognition of incompatibility were recorded by Cato (White, 1961).
Grafting of the wild and the "good" olive trees was mentioned in the Holy Bible. A discussion of the result of such combinations was recorded in the book of Romans (11: 16-18).

For if the firstfruit be holy, the lump is also holy: and if the root be holy, so are the branches. And if some of the branches be broken off, and thou, being a wild olive tree, wert grafted in among them, and with them partakest of the root and fatness of the olive tree; Boast not against the branches. But if thou boast, thou bearest not the root, but the root thee.

Not only was the practice of grafting firmly established at this time, but specific dwarfing stocks were also in existence and being used. The selection and use of dwarfing stocks was recorded some 2,000 years ago in the older Greek literature (Maney, 1942).

Graftage, involving procedures which are essentially unchanged from those described in oldest writings, remains today as an extremely important propagation technique (Buck, 1954). By 1821, some 119 different methods of grafting had been described in horticultural writings (Bailey, 1914).

Today, this number remains essentially unchanged. Grafting as a method of propagation remains as the primary method for increasing the numbers of fruit trees, despite the development of such techniques as seed stratification, use of automatic misting systems for rooting cuttings and the use of root promoting chemicals.
Graft Incompatibility

There appears in the literature on rootstock-scion relationships, a number of conflicting reports and conclusions that do not agree with the data presented. In many instances facts are based upon casual observations and there is a noticeable lack of standardized terminology. This has been evident for some time according to Tukey (1937) who wrote:

The present renewal of interest in America concerning rootstocks for fruit trees and the relations of stock and scion has resulted in the appearance of publications on these subjects involving terms which have not been standardized and which present a wide variation in form and phraseology, often too lengthy and not infrequently ambiguous.

A review of all literature dealing with stock-scion reactions, although intimately related to compatibility, is not a primary concern in this study. Many excellent reviews have been published on this aspect of graftage (Amos et al., 1936; Brase and Way, 1959; Dana, 1952; Gleisberg, 1957; Graves, 1950; Hartmann and Kester, 1959; Hatton, 1930; Herrero, 1951; Hoblyn, 1951; Katyal, 1949; Roberts, 1949; Rogers and Beckbane, 1957; Scholz, 1957; Tukey and Brase, 1933; Vyvyan and Maggs, 1954; White, 1961 and Zeiger and Tukey, 1960).

A number of studies have been conducted on various aspects of the graft union formation. The greatest majority of these have been concerned with the grafting technique which makes use of several buds on a scion rather than the bud graft
which involves only a single bud. Considerably little has been recorded on the histological healing and growth of the completed bud graft union.

Herrero (1951) wrote:

Apparently very little work has been carried out in order to discover whether there is any relationship between the histological structure of the components of a grafted tree and their compatibility...

Buck (1954) stated:

The histological mechanism of the healing of a graft is known to be based to a large extent upon the activities of the cambium and its derivative tissues. The details have been worked out in the tongue-and-whip graft, but the histology of the bud graft has been virtually ignored.

Later, Scholz (1957) maintained:

There is a need for some good histological study of graft unions of dwarf apple trees to determine their compatibility status.

Much of the research work on graft failure has been performed on unions after the failure became evident. In so doing, it has been extremely difficult, or impossible, to determine if the reasons given for the failure were the primary cause or whether it was an effect of the lack of healing of the union. Although there are many reasons for graft failure or incompatibility, they are difficult to separate from one another. In a number of cases, failure has not been due to lack of affinity between the components, but rather to some other cause. These symptoms may closely resemble conditions that are the same as those caused by pathological, physiological or environmental factors.
There are a number of recognized degrees of incompatibility. These vary with the method of grafting, the type of plants being joined and the growing environment. Incompatibility varies from the complete failure of the graft union to form, to the rapid growth and development of the symbionts for a period of years followed by the breaking apart of the components at the region of union (Argles, 1937; Gourley and Howlett, 1941; Hartmann and Kester, 1959; Herrero, 1951 and Mahlstede and Haber, 1957). This classification would include all of the possible intermediate stages inclusive of poor growth, abnormal unions and all stages of general decline of the components between the extremes. The only criterion for incompatibility is the interruption of cambial and vascular continuity which leads to the breaking apart of the stock and scion at the point of union. This definition was proposed by Mosse (1962) and differs from both the earlier and more recent proposals which utilize vague terminology in describing incompatibility. This is also in agreement with the work conducted by Lapkins (1959) in which the discontinuity of wood and/or bark tissue was found to be the most reliable sign of incompatibility.

The remaining symptoms of incompatibility are extremely difficult to use as methods for diagnosing potential disorders with certainty. Argles (1937) suggested that:
The term incompatibility should be applied only to such combinations as show distinct failure (i.e., failure to unite, failure to grow in a healthy manner, or premature death), and only where such failure can be attributed with a reasonable degree of certainty to differences between stock and scion.

Such systems as proposed by Argles (1937) and Herrero (1951) make use of various outward symptoms, the time of symptom expression, the influence of a virus and similar causes and effects. All of these proposals are of questionable value since the majority begin at the time of graft union healing, even though their ultimate effect is generally not realized for a period of years. A review of the different methods that have been proposed for measuring incompatibility appears in work conducted by Evans and Hilton (1957) and Mosse (1962). These workers (Evans and Hilton, 1957) pointed out the need for proven evaluation measures for assessing rootstock and scion incompatibility has long been recognized, but has only been a project of research during the past fifty years.

Many plants, although closely related, can not be successfully grafted or budded upon one another. In order to explain this failure of the stock and scion to unite or function in a desirable manner, horticulturists have proposed several descriptive terms. Among the terms most commonly appearing in the literature are incompatibility (delayed, physiological, anatomical and inherent), uncongeniality, lack of affinity, graft union disorder, graft union disease and graft union decline. There is no consistency in the specific
use of these terms to describe disorders of graft unions.

While there is considerable information on incompatibility and the failure of a selected rootstock to grow, reports indicate that some material is so compatible that better growth is obtained on a foreign rootstock than if the plant was allowed to remain on its own roots (Crane and Marks, 1952; Hartmann, 1958; Lagasse, 1935; Sax, 1950 and Vaile, 1938).

Close botanical relationship does not afford the propagator an infallible criterion of compatibility. This factor has been borne out by work that has been conducted with successful interfamily and intergeneric grafting. While intergeneric grafts are now commonplace, the grafting of plants belonging to different families are considerably more rare. Jones (1934) recorded that:

On the whole, the more nearly related systematically are scion and stock the more likely union between their tissues occur. Grafts between genera frequently succeed...but when the relationship is more distant than generic, graft unions cannot be brought about.

The majority of the reported cases of interfamily grafts have been performed outside of the United States (Awdejew et al., 1958; Al'benskii, 1960; Derickson, 1930; Moiseeva, 1958; Nazarov, 1956; Nickell, 1948; Sambamurty and Sundararaj, 1954 and Zebrak, 1937).

While the majority of workers stress the importance of the intimate contact between the cambial layers of the grafted
components, it has been shown that horticultural plants without a continuous cambium could be successfully grafted. Muzik and La Rue (1954) stated that:

These results demonstrate that many monocotyledonous plants can be grafted and follow a regular process of regeneration and union, formation of a contact layer, enlargement of cells next to this layer, division of these cells, disappearance of the contact layer between vascular bundles.

Earlier, La Rue and Reissig (1946) reported that cambium is not an absolute requisite for successful grafting. They reported that in leaf grafts, union is made by callus which develops on cut surfaces and especially at the cut ends of the vascular bundles. Other workers have shown that in the setting of grafts, actual contact of cambium layers is not essential but rather the contact of cambium derived callus is important.

Roberts (1949) is only one of the numerous writers who contends that there is no way of determining, in advance, what stocks and scions will be compatible or incompatible or in what degree any of the antagonisms will be expressed. He further states that incompatibility is the major problem in grafting.

There are numerous factors which enter into the successful healing or take of any type of graft union. While most workers have acknowledged the importance of the relationship of plants involved in the graft combination, as well as the
timing of the operation, other aspects to incompatibility have been stressed in the research work to date. Differences in the rate of growth between the stock and scion have been proposed as one reason for the failure of graft components to unite (Argles, 1937; Blodgett et al., 1960; Borzini, 1940; Chandler, 1925; Chang, 1938; Day, 1953; Delamarter, 1922; Gardner et al., 1952; Haas and Halma, 1929; Heppner and McCallum, 1928; Herrero, 1951; Mattoon, 1952; Moreira, 1938; Skinner, 1952; Swingle, 1953; Trunk, 1933; Tukey and Brase, 1935; van der Hoop, 1932; Webber, 1926 and White and Mahlstede, 1960). It is also pointed out by some of these workers that there are those graft combinations which exhibit more pronounced differences in the rate of growth than those considered to be incompatible, yet for all practical purposes are considered to be congenial. There are also those types which exhibit no difference in the rate of growth yet are considered to be uncongenial in combination with each other.

The formation of masses of callus tissue and/or bark inclusions at the point of union has been a common cause of incompatibility (Bailey, 1923; Chang, 1938; Coe, 1924; Dirks, 1925; Evans et al., 1961; Herrero, 1951; Higdon, 1956; Korovin, 1961; Mosse and Herrero, 1951; Proebsting, 1926; 1928; Schneider, 1954; Schuster and Miller, 1933; Sorauer, 1922 and Waugh, 1904).
Failure to form or maintain cambial continuity between the stock and scion has been another reason for the uncongeniality of graft partners (Bradford and Sitton, 1929; Coe, 1924; Eames and Cox, 1945; Herrero, 1951; Higdon, 1956; Katsura, 1960; Lendner, 1940; Mosse, 1955; 1960a; Mosse and Garner, 1954; Mosse and Scaramuzzi, 1956; Roach and Thompson, 1947 and Swingle, 1953).

Abnormalities of the xylem or phloem areas of the joined tissue systems have given rise to many uncongenial graft unions. The failure, nonformation or degeneration of the phloem tissue, even though the immature xylem portions of the union have united, may result in graft failure (Batjer and Schneider, 1960; Bradford and Sitton, 1929; Evans et al., 1961; McClintock, 1948; Mosse, 1955; 1960a; Nauriyal et al., 1958 and Shalla et al., 1961). The reverse sequence, that is the union of phloem but destruction of the xylem, has also been reported (Armstrong and Brison, 1949 and Proebsting, 1928). Imperfect union of the xylem and phloem regions have been found to result in the failure to unite or poor growth (Sax, 1954b and Shaw, 1946a). Failure of the fibers of the stock and scion to interlock has also resulted in the breaking apart of grafted components (Eames and Cox, 1945; Garner, 1944; Mosse, 1962 and Mosse and Scaramuzzi, 1956).

Studies on the effect of the graft union on the transport of water, mineral salts or elaborated food materials produced
by the plant has been a source of research by many workers. In some of these investigations the flow or movement of materials was restricted. In other studies an increased rate of transport was noted. Occasionally the union made no difference in the rate of movement of water, nutrients or elaborated food products. Either the increase or decrease in the normal quantity of water, essential elements or synthesized materials usually resulted in some degree of incompatibility (Bailey, 1923; Berry, 1938; Booth, 1914; Bregger, 1948; Bukovac et al., 1958; Chandler, 1925; Chang, 1938; Chester, 1931; Coe, 1924; Cooper et al., 1952; Dana, 1952; Day, 1934; Dickson and Samuels, 1956; Fillmore, 1951; Gardner et al., 1952; Hayward and Long, 1942; Heinze et al., 1942; Herrero, 1951; 1956; Howard and Heppner, 1929; Kostoff, 1928; Mahlstede, 1961; McClintock, 1948; Mosse, 1960a; Mosse and Garner, 1954; Mosse and Herrero, 1951; Pearse, 1940; Proebsting, 1926; Randhawa and Upshall, 1949; Rao and Berry, 1940; Roach and Thompson, 1947; Roberts, 1934; 1949; Sax, 1953; 1956b; Sax and Dickson, 1956; Scholz, 1957; Schneider, 1954; 1959; Schuster and Miller, 1933; Slyaskiy, 1956; Sorauer, 1922; Swarbrick et al., 1946; Verner, 1955; Vyvyan, 1936 and Warne and Raby, 1939).

The particular chemical or biochemical nature of either the stock or scion may influence the degree of compatibility

The production of so-called toxic substances produced by virus activity in both or either the stock and scion has been cited as another cause for the failure of certain graft combinations (Bitters and Parker; 1953; Calavan et al., 1958; Crane, 1945; Darlington, 1944; Frolich, 1958; Gardner et al., 1946; Grant et al., 1961; Hartmann and Kester, 1959; Herrero, 1951; Higdon, 1956; Janick, 1963; McAlpin, 1948; McAlpin et al., 1948; Mahlstede, 1961; Mahlstede and Haber, 1957; Milbrath and Zeller, 1945; Mosse and Scaramuzzi, 1956; Olson, 1954; Scaramuzzi, 1959; Schneider, 1962; Shalla et al., 1961; Shaw and Southwick, 1944a; 1944b; Smith, 1954; Toxopeus, 1936; Tukey and Brase, 1944; Tukey et al., 1954; Webber, 1943; Weeks, 1948; Williams and Campbell, 1957 and Yerkes and Aldrich, 1946).

The invasion of the wounded surface of a graft union by fungi or the presence of bacteria or fungi on either or both of the graft units may prevent the formation of a normal union or cause rotting (Ahlgren, 1955; Baker and Davis, 1953; Baker and Thomas, 1946; Caroselli, 1957; Glenn, 1947; Gorenz, 1953; Hamond, 1935; Hess, 1954; Hess and Welch, 1954; Hoogendoorn, 1952; Lendner, 1940; Mahlstede, 1958; McDaniel, 1958;

Differences in the genetic make-up, inheritance and chromosome number of the graft components have also been studied as possible causes of incompatibility (Kester, 1961; Mukherjee and Cameron, 1958; Ostendorf, 1933; Posnette and Cropley, 1959; Tukey and Brase, 1934 and Webber, 1920; 1926).

Rather than a direct influence of the graft union, inherent peculiarities attributable to the stock, scion or interstock combination may result in uncongeniality (Batjer and Schneider, 1960; De Stigter, 1956; Garner, 1947; 1953; Hodgson et al., 1938; Hubbell, 1934; Jiménez, 1957; Kormos and Kormos, 1955; Kostoff, 1928; Mosse, 1961; Nauriyal et al. 1958; Posnette and Cropley, 1959; Sax, 1953; 1954b; Scaramuzzi, 1956; 1957; Toxopeus, 1936; Tukey et al., 1962; Wellensiek, 1949 and Williams and Campbell, 1957).

Many of the causes of incompatibility can be traced, not to the lack of relationship of components, but rather to the lack of care in the placement of the scion or to the propagation technique involved (Amos et al., 1936; Argles, 1937; Bange, 1940; Beckett, 1933; Bennett, 1927; Bradford, 1929; Bradford and Sitton, 1929; Delamarter, 1922; Dufour, 1936; Esau, 1960; Evans et al., 1961; Garner, 1935; 1947; 1951; Garner and Hammond, 1938; Hatton et al., 1929; Hayward and Went, 1939; Heppner and McCallum, 1927; Jackson and Zak, 1949; Johansen and Kraus, 1959; Joley, 1960; Kains and McQuesten,
Differences in the gross anatomy of the two components have been found to influence the success of the graft union (Beakbane and Thompson, 1939; 1947; Bradford and Sitton, 1929; Crafts, 1934; Garner, 1947; Mendel, 1936 and Thompson, 1952).

Cultural and environmental factors have been observed to effect the success of grafting. The application of growth substances to the graft union or to the stock and scion prior to joining has been shown to generally result in more rapid healing of the union. Usually the speed at which callus development proceeds and the time required for the actual joining of the tissues determines the failure or success of a given union. The use of these materials to stimulate cell division may materially effect the uniting process, (Deboer, 1947; Evenari and Konis, 1938; Hansen and Hartmann, 1951; Koberidze, 1958; Kordes, 1942; Kruyt, 1947; Maiti et al., 1959; Padfield, 1952; Roelofsen and Coolhaas, 1939; Samish and Gur; 1962; Sitton, 1931 and Swingle, 1940).

The soil temperature and type in which the rootstock is growing may determine whether a given scion will form a suc-
cessful union (Banta, 1955a; Cooper and Olson, 1951; Cooper et al., 1957; Day, 1953; Dorsey, 1919; Embleton et al., 1962a; 1962b; Garner, 1947; Hansen, 1948; Hartmann, 1958; Nauriyal et al., 1958; Nelson and Tukey, 1955; Overholser et al., 1943; Shaw, 1936; Stuart, 1937 and Tukey and Brase, 1939a; 1939b). In addition to the influence of soil temperature, the air temperature adjacent to the developing union will effect the rate and degree of the healing (Burton, 1952; Denisen, 1958; Hansen and Hartmann, 1951; Hess, 1961; Holmes, 1957; Hoogendoorn, 1952; Janick, 1963; Mergen, 1955; Perry, 1955; Ravestein, 1957; Shippy, 1930; Sitton, 1931 and Thompson and Hesse, 1950). The relative humidity in the area immediately surrounding the graft union may also influence the healing sequence and subsequent stand (Denisen, 1958; Hoogendoorn, 1952; Janick, 1963; Mergen, 1955; Ravestein, 1957; Samish and Gur, 1962; Shippy, 1930; Whitehouse, 1954 and Sitton, 1931.

The age of the wood used in the grafting operation is known to influence the rate and degree of healing (Bailey, 1914; Garner, 1951; Greene and Reines, 1958; Hartmann and Kester, 1959; Hess, 1958; Hu, 1956; Mergen, 1954; Roberts, 1931; Samish and Gur, 1962; Sitton, 1931 and Tukey and Brase, 1931). The amount of water applied to the stock as well as stock nutrition often influences the success of any one budding or grafting operation (Bailey, 1914; Duruz, 1955;

Other factors, appearing less frequently in the literature, that have been observed to influence the take of a bud or graft would include: the supply of oxygen to the developing union, the pH of the tissue systems (Dirks, 1925; Ellen-gorn, 1951; Hartmann and Kester, 1959 and Shippy, 1930), the formation of sphaeroblasts in the union (Garner and Nicoll, 1961) and insects (Garner and Hammond, 1939).

Histology of the Graft Union

One of the earliest reviews pertaining to studies of the graft union was presented by Bailey (1923). In this review, it was reported that Herse, in 1908, had given a good review of the work on grafting and a very detailed and complete account of the development of a union by budding. Coe (1924) wrote that of those workers investigating the structure of grafts and the manner of graft union formation, Goppert in 1874, Sorauer in 1875, Waugh in 1904, Schmitthenner in 1907, Ohmann and Herse in 1908, and Bailey in 1923 had significantly contributed to the literature on this subject. The fact was also noted that the present knowledge of graft union formation was based on the work of Goppert and Sorauer.

This review was followed closely by that of Bradford and Sitton (1929) which contained the same basic information on
the development of the graft union. Later, Mendel (1936) presented a more comprehensive review of the theories and research work on the healing processes involved in the union of the scion with the stock. Other workers who have studied the anatomical and physiological processes of the healing graft union include Voechting in 1892, Zimmermann in 1901, Steffen in 1908, Funck in 1929, Silberschmidt in 1932, and Kausche and Kaan-Albest in 1934.

The most complete works of graft union histology are those of Ohmann and Herse (Coe, 1924) and Sorauer and Ohmann (Bradford and Sitton, 1929). Since these publications, the most complete study of graft union histology has been that of Sass (1932). The papers of Mendel (1936) and Buck (1953b) have been the most outstanding contributions to the literature of the histological development of the shield graft.

The fact that the sequence of events leading to the formation of a successful union in both the bud and scion grafts was similar, was first pointed out by Bradford and Sitton (1929). Following the initial union, the subsequent development is essentially the same for both types of grafts.

Although considerable information on the callusing of natural wounds, pruning wounds and the development of callus on cuttings are readily available, less is known about the orderly development of callus during the healing process of
the graft union. The processes are essentially the same as related to derivative tissue. Reviews on wound healing and regeneration of callus by plant parts may be found in the works of Bloch (1941 and 1952), Kostoff (1928) and Swingle (1940).

The histology of the developing union has been found to vary with the type of plant material being grafted, the type of grafting procedure utilized and the existing environmental conditions. In order to determine the derivative tissue involved in the healing of a specific union, as well as the rate of healing, each individual combination would have to be tested under known environmental conditions. In discussing the formation of callus, it should be noted that some workers refer to callus as being any tissue forming on the wound surface, regardless of the stage of development. Other investigators use the term callus to refer only to the young and undifferentiated tissue present on or in the area of the actual wound.

In order to summarize the work which has been reported on the histology of the developing union, the literature has been separated into two categories, (1) derivative tissues responsible for graft union healing and (2) the rate of union development.

**Derivative tissues responsible for graft union healing**

In 1853, Trecul (Dirks, 1925) stated that all parts of the young tissue remaining on the barked wood of the stem took
part in the formation of callus tissue in the graft union. Contrary to the more widely accepted early theories on wound healing, Goeppert (Bradford and Sitton, 1929) realized that tissues, in addition to the cambium, were capable of giving rise to callus tissue. Goppert (Coe, 1924) also maintained that an intermediary cell tissue was primarily responsible for the union of the stock with the scion and that this tissue was primarily produced from the cambium tissues of each graft component.

Coe (1924) reported that Sorauer in 1875 and Schmitthenner in 1907 considered callus to be the result of cells originating from the young sapwood of the stock and the bark of the shield, as well as directly from the cambium tissue. Waugh (1904) emphasized that callus, resulting from divisions of the cambium, was the primary tissue involved in both the healing and establishment of the actual graft union. Ohmann in 1908 (Bradford and Sitton, 1929) stated that in the graft union, the cambium zone was responsible for practically all of the callus involved in the healing operation.

Bailey (1923) maintained that the cambium was the most important tissue system in forming a union between the budded components of apple. Through its action a continuous layer of wood was produced in the completed union. It was noted, however, that wound tissue also developed from medullary rays and from newly formed parenchyma cells. The production of callus tissue was more rapid from the stock than from the scion.
The cambium was also observed by Coe (1924) to be the most active tissue from the standpoint of callus production. He also reported that the stock was responsible for the greatest production of wound parenchyma with the apple. The layer of cells between the cambial region of the stock and scion apparently do not lignify, but rather continue to divide. These cells then gave rise to a meristematic layer which resulted in the production of new wound wood. This, in turn, formed a continuous cambium, connecting the stock and bark flaps with that of the bud shield.

In a study of apple grafts, callus was found to be the result of new cell production by cortical parenchyma, cambium, xylem parenchyma and vascular rays (Fisk, 1927). In agreement with the work of Bailey (1923) and Coe (1924) it was also reported by Kostoff (1928) that the callus tissue in graft unions was produced chiefly by the stock.

With bud grafts of the apple and pear, Bradford and Sitton (1929) reported that the union is established through parenchyma derived from the meristematic xylem of the stock and the cambium of the bud shield. Countryman (1931) observed that the callus tissue found in the graft union of apples could originate from the primary cortex, the region including the endodermis and pericycle, the phloem or the cambium.

Sass (1932) worked on the formation of callus knots on apple grafts as related to the histology of the graft union.
He observed that callus was produced by tissues located outside of the xylem cylinder, and that cambium may contribute very little of the actual callus tissue involved in a union. Any living tissue of the bark, with the exception of the periderm, was capable of proliferating. The unobstructed contact between the respective calli of the stock and scion involved the continued proliferation of a "mixed" callus.

In the ring grafting of apples (Roberts, 1937) new xylem tissue originated from the cambium of the scion rather than from the stock in the developing union. Further experiments with ring grafting were conducted by Yeager (1944) and these supported the findings of Roberts (1937) in that the new xylem tissue in the developing union was of cambium origin derived from the scion portion of the graft rather than the stock.

Mosse and Labern (1960) recorded that callus formation in apples originated almost entirely from rootstock tissue. Callus was produced mainly from the exposed surface of the xylem cylinder, to a lesser extent from the inside of the bark flap and very little was contributed from the sides of the bud shield. In later work, Mosse (1962) concluded that wound callus in the graft is derived from undifferentiated xylem and the internal surface of the bark flap. Callus was found to be formed to a lesser extent from the cambium and cortical tissue of the bud shield.

In the bud graft union of roses the entire inner surface
of the bark shield produced new wound tissue, with the most abundant production being from the cambial zone (Sorauer, 1922). Following the meeting of the wound tissue of the stock and scion, a continuous cambial ring was formed. Essentially there were three areas which were responsible for the production of the tissues uniting the stock and scion, namely, those of the bark of the stock, those of the callus on the exposed wood body and those of the scion. Other work by Sorauer in 1924 (Bradford and Sitton, 1929) demonstrated that callus formed from any tissue could materially assist in the establishment of the graft union. In budding, this tissue was derived from the shield, the wood surface of the stock and from cambium found on the inside of the bark flaps.

With the bud graft of roses, Buck (1954) found that the cambium did not contribute callus during the healing process. The callus involved in the healing of the rose union was derived from immature, recently derived, secondary phloem and secondary xylem in the immediate vicinity of the scion bud. The cambial continuity between the stock and scion was established by a bridging cambium, which was derived from the proliferated callus of the stock and scion. Cambial union between stock and scion was completed in this manner.

Sharples and Gunnery (1933) noted that the cambium contributed nothing to the early formation of wound callus in the grafting of hibiscus. Both the bark and wood callus were
observed to have been formed primarily from the medullary ray elements. There was usually no indication of cambial activity until the callus cushion was completely laid down. Contrary to the majority of reports in relation to the superiority of the stock in the ability to produce callus tissue, equal amounts of callus tissue was observed to be formed by the two graft partners.

In a study of the histology of the bud graft union of citrus, Mendel (1936) observed that the first cell divisions occurred at the injured ends of the medullary rays at the outer periphery of the xylem cylinder. It was further noted that cells of the wood parenchyma and secondary bark take part in callus formation only later in the process of union development. Those portions that were observed incapable of cell division or callus development were those of the older wood and pith. New callus development was formed most rapidly by the stock and slowest by the bud shield.

Callus was observed to have originated from either stock or scion or both components in the bud graft of mango (Soule, 1951). The tissues found to be taking part in the formation of the graft union were any of the meristematic cells in the pith, wood rays of the most recently formed secondary xylem, the cambium, phloem and the cortex.

Cells originating from medullary rays, phloem rays and
cortex were observed by Mergen (1954) to have been active in producing the tissues which formed the bridge between the stock and scion in pine grafts. The fact was also emphasized that wound callus was not only produced by meristems already present at the time of grafting, but that parenchymatous cells of pith, medullary rays, phloem and cortex can assume meristic functions. Although both the stock and scion was observed to produce callus, the greatest development was from the stock portion.

Went (1938) found that the union between grafted peas occurred as a result of the regeneration of the vascular bundles and that the stock did not begin growth until after the actual union of stock and scion had taken place.

Juliano (1941) observed that in grafts of Nothopanax, before union between the stock and scion is effected, callus cushions are first formed by the activity of the parenchyma of both bark and the pith as well as from the ray cells of both symbionts. From this callus a cambial bridge was produced which joined the cambial ends of the stock and scion. Although callus development began with the stock, the contribution of callus by both graft components was nearly the same. The original cambium layer had no part in the production of callus tissue. It was in the callus cells adjacent to the pre-existing cambium that new elements first appeared.

Working with tobacco grafts, Crafts (1934) reported that
phloem and xylem elements formed directly from callus which also gave rise to a true cambium layer between these vascular elements. This cambium layer continued development and rapidly formed a continuous layer with the cambia of the stock and scion.

With grafts in general, Barnes and MacDaniels (1947) regarded the successful union of grafted components as the ability of the cambia of both stock and scion to produce callus and to unite, thus forming a continuous cambium layer over the union of stock and scion which gave rise to the normal complement of conducting tissue.

Whitehouse (1954) noted that the union between grafted components of woody stems was accomplished as a result of callus formation by both the stock and scion, and that upon meeting, these tissue systems fuse. Mahlstede and Haber (1957) maintain that after the bud shield has been inserted in the stock, healing in the form of callusing, progresses from the immature secondary phloem and xylem in the vicinity of the bud.

In describing the steps involved in the healing of a graft union, Hartmann and Kester (1959) conclude that after the cambial region of the stock and scion have been placed in intimate contact with each other, the outer layers of cells in the cambial regions of both components produce parenchyma
cells which intermingle. This newly formed callus tissue in line with the original cambium layers differentiate into new cambium cells. The new cambium cells give rise to the new vascular tissue, xylem and phloem, and thus establish a vascular connection between the stock and scion. The union is accomplished entirely by cells which are developed after the actual graft has been made.

The rate of union development

Callus production following the grafting operation was found by Du Hamel in 1758 (Coe, 1924) to occupy the entire space between the stock and scion after only three weeks of growth. Goeppert in 1874 (Bradford and Sitton, 1929) noted that only during the second year was the graft union completed through cambium continuity. Schmitthenner in 1907 (Coe, 1924) also reported that a considerable time may elapse before the cambial regions of the components unite.

Herse in 1908 (Sass, 1932) conducted a detailed study of the histology of healing in the apple graft. He was able to show that the respective calli of stock and scion coalesce and that in well matched grafts a new cambium appears, bridging the callus between the members in about ten weeks.

The uniting callus tissue that closed the wound in bud grafts of apple was observed to be completed in ten to fourteen days after budding (Bailey, 1923). During this relatively short period of time the entire space underneath the bud
shield was filled with callus. The cambia of the stock and scion was joined in twenty-one to twenty-eight days and had by this time produced a new continuous wood layer. While the sides and the lower end of the bud shield area was completely filled with callus by the sixth day, the top of the bud shield was not filled until after some eight days.

Working with apples, Fisk (1927) records that the cambium is differentiated in the callus tissue of the graft union some twenty-one to twenty-eight days after the graft was made. Callus wounds were reported to have healed in only a period of five to ten days following the placement of the graft (Kostoff, 1928). In other work with apples, (Countryman, 1931) callus was found to develop from all living tissues of the bark, with the exception of the periderm, and this activity may begin two days after grafting.

Sass (1932) reported that if the stock and scion of apple grafts are well matched, the gap between components may be filled by callus in less than two weeks. The period of callus bridging is variable, but many instances of successful cambial union were noted in grafts that were three weeks old. The gap between the xylem cylinder of the stock and scion does not become filled with callus during the first season and there is evidence that the gap does not become filled during subsequent years.

Mosse and Labern (1960) record that callus begins to de-
velop two days after budding and that in two to three weeks all of the internal cavities of the bud union of apple are completely filled by this callus. Mosse (1962) found that in twenty-four hours meristematic activity may be observed, and at this time wound callus begins to form. It is also reported that after two to three weeks following budding, tracheids begin to differentiate in the callus region.

In a study of the bud graft union of citrus, Mendel (1936) observed that cell divisions begin almost simultaneously in all tissues adjacent to the wounded area and may be found to be occurring within twenty-four hours after the insertion of the bud shield. The first callus bridges were observed to be present within five days, and differentiation of the callus in the bark flaps was noted to be present in ten days. Callus tissues produced by the bud shield were noted to be in the process of differentiation fifteen days after budding. The first occurrence of tracheids was observed in the callus of the bark flaps in fifteen days while a period of twenty days was required for the appearance of tracheids in the callus of the bud shield. The first appearance of meristematic layers in the callus between the shield and bark flaps was observed fifteen days after budding. Lignification of the callus was found to be completed in the bark flaps within twenty-five to thirty days, while that portion located under the shield required some thirty to forty-five days.
The histological development of two types of bud shields used in the propagation of citrus, was studied by Singh and Singh (1947). One type had the wood removed from the face of the bud plate and the other was left with wood inside of the shield. For the bud in which the wood was removed, only a slight amount of callus developed. The bud shield was connected to the stock, only in the middle region of the shield, after seven days. Two weeks later, most of the region between the stock and scion was filled with callus tissue. The entire surface under the shield was noted to have united within a period of three weeks. After four weeks, vascularization had taken place, connecting the stock and scion. After two months there was complete intermingling of tissues and the vascular development in the bud shield had been completed. At this period the union was considered complete. The union between the stock and shield containing the small section of wood took longer to unite. Seven days following bud insertion there was only a light amount of callus formed around the edges of the shield. By the end of two months vascular differentiation was just beginning.

In other work with citrus, Khan and Salem (1959) found that the bud union was not complete after a period of one year. Katsura (1960) reported that after a period of two months following bud placement a solitary ring of cambium had been formed. Randhawa and Bajwa (1958) found the union to be
complete in six weeks.

In the budding of mango, Soule (1951) reported that there was no proliferation of callus parenchyma in the graft union. However, in observations made eight days following budding, the necrotic plates covering the face of the injured tissue had been ruptured by wound callus, and the scion was firmly attached to the stock by means of this wound parenchyma. Twelve days after budding, the union was found to be essentially complete at one or more points and the cambial tissues of the stock and scion had united in these areas. The process of union formation was found to be complete in twelve days, although proliferation of callus did not begin until some time between the fourth and eighth day after budding. Union had proceeded far enough two to three weeks after budding to ensure a successful take. After twenty-four days, the union was surrounded by several cylinders of new tissue which was continuous between stock and scion. If conditions were not favorable for union, the stock and scion would proliferate parenchymatous callus for many weeks with no indication of cambial bridge formation.

With grafts of pine, Mergen (1954) found that callus development was quite pronounced and that there were direct connections between the stock and scion after seven days. Most of the portion in the union was completely filled with callus tissue after three weeks. After a period of five weeks, the cavity between the components had been completely filled and
differentiation had begun. After six weeks a complete and continuous bridge between the respective graft partners was clearly apparent.

Burchardt (1935) reported that in a period of two to three weeks the union between components had occurred and was at this time complete with cacao.

The bud union of roses and the time involved in the healing operation was studied by Buck (1954). Three days after the buds were inserted into the stock, cell division was observed in the uninjured cells bordering the necrotic plates formed over the cut surface of the stock and scion. This division resulted in the formation of callus which ruptured the necrotic plate. Two to four days after budding, the terminal cells of the xylem rays and cambial derivatives began active division. Callus cells adjacent to the uninjured stock cambium began division in three to five days. The calli of stock and scion were found to have merged within a period of four to six days. After five days, a layer of cork cambium was observed to have developed behind the necrotic cell plate and at this time contact between the stock and scion calli was completed. After ten days, an uninterrupted band of cambiform tissue was noted to extend across the face of the stock. The union of cambial elements of the stock and scion was complete in a period ranging from ten to fourteen days. The arc of cork cambium cutting off the edge of the scion also had developed within
this period of time. Between twelve and fourteen days, the stock-scion interface became filled with callus and lignification had begun. Between fourteen and twenty-one days, cambiform tissue formed arcs of secondary xylem in the stock and secondary phloem in the scion portion of the bud graft.

The first day after grafting *Nothopanax*, Juliano (1941) noted that definite activity could be observed in parenchymatous cells, first in the cortex and then in the pith. After the sixth day, a distinct meristematic strip responsible for the regeneration of callus cells had already formed in the bark. By the time the graft was thirteen days old, there were connections throughout the area of the stock and scion. Some fifty-four days after grafting, when the callus was fully formed between symbionts, there was still an absence of cambial cells in the callus cushion.

Vascular strands developing from callus parenchyma were found to connect the stock and scion as quickly as five days after grafting with tobacco (Crafts, 1934).

Included in the work of Mendel (1936) are observations on the time required for the formation of callus in a graft union, as well as the time required for cambium to become continuous between the graft components. Sorauer in 1883, found that distinct changes at the wounded surface of budded roses can be detected within twelve hours, and that in ash a callus deposit
some sixteen cells in depth can be observed after two days. Callus formation began no later than two days following the grafting of beets in work performed by Voechting in 1892. In 1901, Zimmermann reported that in grafts of coffee, callus development in the wounded area was clearly evident within two days. The cambium of the stock and scion was partially completed after some twenty-four days. Ohmann in 1908 found that callus development proceeds in about ten days for grafts, and almost immediately in budding. In the same year, Steffen reported that in the flap cavity of budded roses the callus from the union had completely closed the wounded area in a period of fourteen days. Kausche in 1934 observed that wound callus formation started within twenty-four to thirty-six hours after grafting, and that cambial connections arising from this wound callus was completed in seventeen days for budlings of the rubber tree.

The process of wound healing in grafts has been reported for plant materials that are not woody in nature. Nickell (1948) in describing the graft union between sweet clover and sunflower noted that vascular connections between stock and scion formed in three weeks as a result of the differentiation of xylem and phloem strands in the pith parenchyma. After eleven weeks there was extensive development of vascular connections in the union. The anatomical relations between the
graft union of guayule and sunflower have been discussed by Artschwager (1951). The anatomical changes that accompanied the union in this plant material involved the elimination of the contact layer between graft components, and the formation of transfusion windows which became evident seven days after grafting. The establishment of vascular connections was noted shortly thereafter.

Muzik (1958) found that parenchyma bridges were formed between the stock and scion portion in two months with grafts of the orchid. No evidence that vascular tissue had formed across the graft union after a period of two years was found. It was further noted that vascular connections were evident in six to eight weeks following the grafting of grass.

Grafting of the Peach

At the turn of the century, peaches were generally propagated by seeds, although a few selected varieties were budded (Blake, 1914). Budding and grafting was not as popular because dwarfing and various stock disorders were encountered as a result of these operations. Budding was preferred to scion grafting as a method of vegetative propagation of peaches since a better graft union was obtained (Cullinan, 1937; Hartmann and Kester, 1959; Herrero, 1951; Mahlstede and Haber, 1957 and McClintock, 1948). Failures resulting from scion grafting were attributed to the production and deposition of wound gum by
the injured tissue systems of the stock and scion used in the grafting operation. Many plants produce wound gum as the result of injury to the xylem or wood portion of the stem. As a result these types can only be propagated, with success, by bud grafting, since this permits the insertion of the bud exterior to the central core of xylem. Other forms of grafting necessarily injure the xylem tissue and the wound gum produced severely retards or completely eliminates the development of a satisfactory union. The production of wound gum or injury to the xylem region is not restricted to the stone fruits. In budding citrus, Mendel (1936) found that cutting into the xylem area of the stock prevented the formation of callus along this area. Since the production of callus is considered to be essential for a successful union, grafting techniques which sever the xylem tissues are generally unsatisfactory.

The use of dwarfing understocks has become an established commercial procedure for the production of the majority of dwarf fruit trees. Iowa nurseries have been propagating peach varieties by this technique in recent years. The most common understocks for this purpose have been seedlings of the western sand cherry, *Prunus besseyi*, and the Nanking cherry, *Prunus tomentosa* (Agrios, 1960). Commercial varieties of peaches used as scions are budded onto seedling peach rootstocks by means of the "T" or "shield" budding technique. This type of bud grafting is widely practiced and the procedures are adequately
described (Bailey, 1914; Brase, 1956; Hartmann and Kester, 1959; Kains and McQuesten, 1955; Mahlstede and Haber, 1957 and Whitehouse, 1954).

The western sand cherry has several attributes which inherently should make it a good dwarfing stock for peaches (Brase, 1953 and Brase and Way, 1959). These characteristics would include the fact that it is a dwarf plant naturally, that it is very hardy and that it can easily be grown from seed. The propagation of clonal selections would be quite easy, since these plants sucker readily in the field.

Sax (1956a) also advocated the use of these two understocks for the production of dwarf peaches. Brase (1956) maintained that seedlings of *P. besseyi* and *P. tomentosa* had a limited use as dwarfing rootstocks for peach varieties. Overholser et al. (1943) however maintained that peaches, as a rule, were not satisfactorily grown as dwarfs.

In the early 1950's, peach varieties grafted onto *P. besseyi* and *P. tomentosa* in Iowa showed disorders that closely resembled symptoms expressed by the X virus. In addition, nurserymen observed that bud take and the subsequent growth and development in the field were not satisfactory during the first year of growth, when these plants were used as rootstocks. As a result, nurserymen will either have to discontinue the production of peaches propagated on these rootstocks or will have to develop other dwarfing stocks to reduce tree stature if
a solution is found to the existing problem. Extensive studies have indicated that the incompatibility between peach varieties and these rootstocks is not disease induced, as previously postulated, but is attributable to a poor graft union (Agrios, 1960).

Brase and Way (1959) reported a similar problem in the state of New York. Trials began in 1944 involving the use of *P. besseyi* as a dwarfing stock for peaches were observed to have disorders similar to those found in Iowa. Peach budlings developing the first growing season were noted to have pale green leaves that tended to roll upward toward the midrib during midsummer. The budlings expressing this symptom, defoliated prematurely. In addition, bud unions developed abnormally, primarily because of the injury of the phloem tissue of the understock seedlings. In transplanting tests, the budlings that grew normally without bud union disorder developed into typical dwarf trees. Those trees that defoliated prematurely either failed to grow or made poor growth. Death usually followed within two years following transplanting. It was assumed that some of the seedlings of *P. besseyi* used as the rootstock carried a virus that was without symptom in the stock but which, upon transfer to the peach budlings, interfered with normal growth and development. This assumption by Brase and Way (1959) was based upon observations made on *P. tomentosa*, which is a good indicator for the necrotic ring
spot virus.

Delayed incompatibility resulting in the ultimate death of the entire graft complex has been reported earlier in peaches (Howard and Heppner, 1929). Peaches that were grafted onto plum stocks united and produced normal growth until the latter part of the second growing season. At this time the peach variety usually died, followed by the death of the stock. Brase (1953) reported that peaches budded onto P. besseyi generally formed an excellent bud union, but thirty-five to forty per cent of the budlings failed to survive. The author observed that failure was associated with the development of irregular bud unions. The use of P. besseyi as a possible dwarfing stock for peaches has been considered for some time. Hedrick (1914) found that the western sand cherry would unite with peach varieties, but could not conclude if unions would be sufficiently permanent to have commercial applications.

The present report presents the findings of a two-year study designed to ascertain the sequence of events that lead to the establishment of a successful union between the peach, variety Polly, budded on the western sand cherry, an understock generally used for purposes of dwarfing this type of plant material. In addition, it was a purpose of this investigation to determine if these two graft components were generally compatible under the conditions in which they are commercially
propagated. If the graft was incompatible, it was the further objective to determine the earliest symptoms of incompatibility and possible causes for its occurrence.
MATERIALS AND METHODS

Budding and Sampling Procedure

The graft components used in these studies consisted of the peach variety Polly which provided the bud, and the western sand cherry, Prunus besseyi, which served as the rootstock. Buds of the peach were inserted into the stocks, using the standard "T" or "shield" budding technique. This is the procedure used in the commercial production of most stone fruits.

Budsticks, obtained from Mount Arbor Nurseries, Shenendoah, Iowa, were used for all budding performed. These were collected from indexed, scion block trees and were of current season's growth. Immediately following collection of the budstick, leaf blades were removed to reduce moisture loss. The budsticks were then wrapped in moist burlap and stored at 35 degrees F. until used the following day. A portion of the leaf petiole was allowed to remain attached to the stem for ease of bud insertion as well as to serve as an early indication of possible union. Leaf petioles of successful unions usually absciss relatively early, while those not uniting generally remain attached for some time following the placement of the bud.

Only mature buds located near the center of the budstick were selected for use in this experiment. At the time of insertion, the leaves were stripped from the stock plant in the
immediate vicinity of the T incision, in order to facilitate insertion of the buds and the tieing operation.

All budding was performed by the same experienced budder during the two years of the trial in order to reduce mechanical variability. The buds utilized in these studies contained a very thin piece of wood on the interior of the shield. Although the technique involved would be classified as "budding with wood in", it more nearly approaches the procedure of "de-wooding" buds. In commercial practice, either or both procedures would be employed, depending upon the condition of the bark of the budstick at the time of placement. All bud tieing was performed by one individual in order to approach uniformity in the tieing operation throughout the course of the experiment. Commercial rubber budding strips were employed for wrapping, since they tend to insure firm contact between the stock and scion, prevent desiccation of the injured portions and expand during the course of the development of the components. Usually this type of bud-tie does not have to be manually removed from the area of union, since deterioration by sunlight causes the bands to fall after union has been established.

Rootstocks used in these experiments were from established seedlings growing on the Iowa State University Horticulture farm. More than one bud was placed on each selected seedling in order to minimize variation between seedlings and to reduce differences
that might have been present in the experimental area. Only a single bud is usually placed on a given seedling in the commercial operation. When a seedling contained more than one bud, all buds were removed to eliminate possible advantages or disadvantages that might have been imposed on the remaining bud at the time of sampling.

All buds were placed the same day in order to reduce variability which would have been imposed by staggered budding dates. The normal time for budding peaches in Iowa ranges from July until early September. Budding was performed on July 14, 1961 and August 4, 1962. Placement of the buds during the early part of the budding season is considered to be a standard practice when *P. besseyi* is used as a rootstock.

Buds were obtained from stock plants that were certified virus-free by the Plant Pathology Department at Iowa State University. For buds used that were not certified to be virus-free, budsticks were collected from nonindexed trees growing in the nursery. Nonindexed trees were the type that did not meet the necessary standards required for indexing. Trees that are known to be infected with a virus are not used for commercial sources of budwood. In addition, disease relationships between stock and scion have already been determined for the peach-western sand cherry combination by Agrios (1960). The same method of handling was employed for buds that were not indexed as for those that were certified to be virus-free.
From the total number of buds placed, a total of 400 indexed and nonindexed buds were selected for this experiment in each of the two years of study. One-half of the buds were inserted on current season's growth or on branches having a small diameter. The remainder were placed on more mature wood. Precautions were taken during the budding operation to ensure that possible disease transmission could not occur as a result of the use of nonindexed bud sources and the budding technique. All indexed material was budded prior to the utilization of the nonindexed source.

Following the placement of buds, samples were collected periodically for study. Ten buds from each of the indexed and nonindexed material were collected on each date of sampling. Five of these buds were collected from the young and five from the more mature stem regions. Later in the collection schedule bud unions which were not normal in their development, or which appeared to be declining or dying, were also collected. Buds were collected 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, and 60 days following placement upon the understocks. Samples were cut one inch above and one inch below the budding strips in order to ensure that no damage would occur to the tissue systems in and adjacent to the actual union.

Processing of Bud Unions

Following collection of samples, buds were kept moist and cool until their killing and fixation in the laboratory. In the
laboratory, samples were trimmed of excess wood and placed in a formalin-acetic acid-alcohol (FAA) killing and fixing solution (Sass, 1958). The bud grafts were then aspirated in this solution for a period of fifteen minutes to facilitate penetration of the FAA into all parts of the tissue. All buds collected during the early sequence of sampling were processed with the intact budding strips. Sections obtained after twenty-five days did not include the rubber budding strips. Following a period of twenty-two to twenty-four days in the field, many bud strips were observed to break and unwind. For uniformity, all bud-ties were removed at this time.

Fifteen days following the collection of bud grafts, all remaining buds were labeled and used for a study to determine the per cent bud take that was obtained. At this time, one could readily determine whether or not the bud was in the active process of uniting with the stock or whether a failure was imminent.

Following the initial aspiration of material submerged in the FAA, all material was again aspirated at the end of two weeks. All samples remained in the killing and preserving solution until they were processed at a later date.

Samples were dehydrated in a series of alcohol solutions beginning with a fifty per cent concentration. Following dehydration, material was then transferred to a xylene-alcohol
series of solutions preparatory to infiltration. The paraffin technique was chosen in preference to other techniques since it would allow accurate study of the developmental sequence of healing throughout the entire union by the use of serial sections. In addition, larger numbers of processed material could be observed in a reasonable period of time using the paraffin technique. Without a doubt, a more solid matrix such as celloidin would have rendered superior sections, particularly for those unions collected during the very early or the more advanced stages of development.

In order to soften the tissue prior to sectioning, the specimens, affixed to plastic blocks, were placed in containers of water and maintained at a temperature of 40 degrees C. for a thirty-six hour period. Sections were cut on a rotary microtome. The optimum section thickness was determined to be 19 microns.

Considerable difficulty was encountered in affixing the paraffin sections to the glass slides. A variety of adhesives and processes, including the celloidin bath technique proposed by Buck (1954) for woody tissue mounting, were tried without success. The adhesive that was finally selected was Adhesive III, consisting of a mixture of one volume of Solution A to four volumes of Solution B (Sass, 1958). Even with this adhesive, there were many instances when only the developing bud region remained in intimate contact with the slide, leaving the woody portion free. This was conducive to tearing or over-
lapping of the wood region in many of the slides.

The safranin-fast green combination was found to be the most satisfactory for staining of sections.
RESULTS

Field and General Laboratory Studies

Following budding, all grafts were inspected at regular intervals throughout the period of development. As with most woody plant material that can be grafted with success, those unions uniting usually respond differently than with scions that do not unite. The first visible indication of possible success or failure of the bud graft was evident between seven and ten days after budding. At this time the leaf petioles on budshields which were uniting became yellow and were easily brushed off. Shortly thereafter, the petiole abscissed. The petiole on poorly knit or dying shields became black, shriveled and remained firmly attached to the shield.

After field collection and placement of the bud unions in the killing and fixing fluid (FAA), areas of the shield that were dead or in the process of decline could easily be detected. Dead portions of the bud shield were dull brown in color, whereas the regions which were active retained a yellowish-green color. Although this color differential was more pronounced in later sampling periods, these characteristic symptoms were apparent in material that had been budded four days prior to sampling. On later sampling dates, buds in various stages of death blackened and the shield became shriveled and discolored.

At regular intervals following budding, attempts were made
to dislodge the inserted bud and shield. As early as six days after budding, it was difficult to remove the shield from the stock in healthy unions. After eight and ten days, the shield portion could not be separated from the stock without tearing the entire surface. Upon separation, the majority of the callus adhered to the stock interface, suggesting that most of this tissue had been produced by the stock. This observation was later confirmed by the microscopic examination of stained, sectioned material.

Two weeks after budding, the first shoot was observed to begin development. Although many buds had greatly enlarged their original size by this time, this was the first instance of actual bud break. By the end of the growing season for plants budded in July, 1961, budlings ranged from four to twenty-four inches in length. For plants budded in August, 1962, three buds had developed by the fifteenth day. The same range in length was observed at the termination of the growing season.

Throughout the field trials there were bud shields that remained dormant, even though they were green and contained swollen buds. Following overwintering in the field, only two of these buds were observed to break dormancy and start growing. Both were included in the trial performed in 1962. The remaining buds of this type did not appear to be alive. Ques-
tionable buds declined and were dead by the time that the budlings in the field had developed new leaves.

Bud shields of unions that appeared to be in a state of visual decline were generally completely desiccated. However, bud shields that had dried out at either end and still remained green in the area adjacent to the bud were occasionally observed (Figure 1).

Fungal development was noted on some bud grafts during the 1961 field trials. The fungal hyphae appeared between the fourteen and twenty day sampling period and was the only time that fungus activity was observed. Since bud unions were still covered by the bud ties at this time, the extent or influence of fungus development was difficult to determine. Buds on which fungi developed remained dormant until their removal from the growing area. After bud tie removal, no fungal activity was noted. Fungi were observed only on unions that appeared to be normal.

No attempt was made to statistically compare the final stands of buds placed in the two sizes of stocks used or between the two years included in this study because a great number of the developing bud unions were removed for microscopic examination. Records were maintained on the number of buds that remained dormant and green, on those that started into growth and on those that were either dead or in various stages of decline.
Figure 1. Random samples of normal bud unions taken at different periods during the observation schedule. The sections A to E were collected 60, 40, 30, 20 and 10 days after budding, respectively. Note the various patterns of shield decline and rapidity of development following formation of a continuous cambium. In sections A to C, all buds had started growth and the developing shoots were removed.
The information recorded in the following table includes both budlings that remained in the field and those that were removed during the process of examination. It is assumed that those which had developed during the course of the growing season would have produced functional budlings.

Table 1. Influence of age of understock and condition of bud on the field stands of Polly peach on *Prunus besseyi*

<table>
<thead>
<tr>
<th>Budding date</th>
<th>Scionwood source</th>
<th>Buds dead</th>
<th>Buds green</th>
<th>Budlings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Budding Scionwood</td>
<td>YW</td>
<td>MM</td>
<td>YW</td>
</tr>
<tr>
<td>July 14 Indexed</td>
<td>YW</td>
<td>10%</td>
<td>11%</td>
<td>32%</td>
</tr>
<tr>
<td>1961 Nonindexed</td>
<td>MM</td>
<td>12%</td>
<td>15%</td>
<td>21%</td>
</tr>
<tr>
<td>August 4 Indexed</td>
<td>MM</td>
<td>16%</td>
<td>19%</td>
<td>31%</td>
</tr>
<tr>
<td>1962 Nonindexed</td>
<td>MM</td>
<td>22%</td>
<td>16%</td>
<td>31%</td>
</tr>
</tbody>
</table>

* Young wood; current season or small in diameter

* More mature wood; not current season or larger in diameter

During growth and development of budlings in the nursery, various degrees of overgrowth of the scion occurred at the point of union (Figures 2 and 3). In no instance was the scion smaller in diameter than the stock. No consistent morphological pattern in the union could be correlated with overgrowth of the scion. In general, these were characterized by larger areas devoid of tissue or those lacking continuity between the stock and scion, in comparison to those unions which were more
Figure 2. Exterior appearance of peach scions budded onto *Prunus besseyi* rootstocks. All unions are two-year old, with the exception of the larger specimen. This is from a three-year old bud graft.

Figure 3. Longitudinal sections of the bud unions contained in Figure 2, showing the internal structure of bud grafts.
nearly equal in size. Areas included in the stock-scion interface of these unions were either composed of nonliving tissue or were completely devoid of connective tissue regions.

The presence of wound gum on the exterior of the stock, immediately adjacent to the inserted bud shield, was observed infrequently and could not be considered as extensive.

After three years, budlings that were allowed to remain in the field ranged from 3-1/2 to 6 feet in height. Two-year old budlings ranged from 2-1/2 to 3-1/2 feet in height. With the exception of three trees, all budlings developed normally without any symptoms of disease. Two of these three "diseased" trees were propagated using indexed scionwood.

Laboratory Studies

The technique involved in making the bud graft resulted in injury to tissues of both stock and scion. The removal of the bud from the budstick resulted in various degrees of injury, ranging from partial to complete destruction of cells immediately adjacent to the interior portion of the shield. Injury was also observed in the cambial region of the stock when the bark flaps were lifted prior to bud insertion. Microscopic examinations of cross sections made through the stock incision revealed that portions of the cambium, as well as immature xylem tissue were separated from the more mature, woody tissue on the interior portion of the bark flaps. No distinct or completely
intact layer of cambium was detected on either the exposed tissues of the stock or on the bark flap. Since the bud shield contained a small portion of the xylem, a small arc of intact cambium was present.

An initial study was made to determine the morphology of the stem of the understock, *Prunus besseyi* (Figures 4, 5 and 7).

**Normal bud union histology**

In order to form a basis for the interpretation of the processes leading to the union of grafted components, sections through the medial portion of the bud shield were selected for presentation (Figure 6).

In sections of unions collected as early as one day after budding, the outermost layer of wounded cells was evident as a thin, necrotic, brown layer over the interface of the two components. This layer, hereafter referred to as the necrotic plate or necrotic cell region, was believed to be the result of the death of two or more layers of cells which had been injured in the budding sequence.

Although organized cell activity was not detected in bud grafts two days old, areas of sporadic cell division were evident. Four days after budding, many cells in the process of division were evident in all of the uninjured cell regions adjacent to the cut or torn surfaces of the incision (Figure 8). The uninjured cells located at the terminal end of the xylem
Figure 4. Gross section of *Prunus besseyi* stem, the rootstock used for peach dwarfing in these studies. 30x.

Figure 5. Orientation drawing for Figure 7. 20x. The darkened area represents the portion of tissue included, from Figure 4, in the diagramatic presentation.

Figure 6. Orientation drawing for Figures 8 to 23. 14x. All photographs are from sections of bud unions included in the darkened area.
Figure 7. Diagramatic drawing of *Prunus besseyi* stem. 340x. Included are the major tissue systems referred to in this study. Other areas that appear in the photographs of the cross sections presented are also recorded.

A-epidermis with cuticle, B-periderm, C-cortex, D-protophloem fibers, E-phloem ray, F-cambium, G-secondary xylem and H-xylem ray.
Figure 8. Cross section through the bud union area four days after budding. 36x. Events are considered to be normal.

A-Scion, B-Wound gum ribbon, C-Callus development from xylem ray and secondary xylem and D-Stock.

(Note: in both photographs, the darkened area on the stock-scion interface is due to both the necrotic plate and the overlapping of tissues from the stock and scion in the immediate area.)

Figure 9. Cross section through bud union at the lateral extremities of bud-stock juncture four days after budding. 36x. Events are considered to be normal.

A-Scion, B-Bark flap, C-Wound gum ribbon, D-Callus development from xylem ray and secondary xylem, E-Cell division in phloem of bark flap and F-stock.
rays of the stock were observed to enlarge and divide tangentially. These new cells were large, thin walled and the zone varied from one to four cells in width. The area of greatest cell division was located in the stock, in an area adjacent to the two lateral extremities of the inserted bud (Figure 9). Only later in the developmental sequence was active cell division noted in the scion and the stock portion immediately below the bud shield. Even though cell division was observed in all areas of the union at approximately the same time, the rapidity of new cell development was proceeding at different rates.

Small bands of brightly stained gumlike material were observed in cross sections of all unions in the process of healing. These deposits varied in length, but were of uniform width. While some unions had only a small amount of this material, others contained an amount equal in width to some thirty to thirty-five callus cells. In no case did this band of wound gum extend across the entire stock-scion interface. These deposits were restricted to unions in which both components were actively producing callus tissue.

While an occasional callus strand was observed to have penetrated the necrotic cell region by the fourth day, considerably more had penetrated the area by the sixth day following bud insertion. In the bark flaps, rapid tangential cell divisions were present after four days in the two lateral ex-
tremities of the inserted bud. These callus cells quickly divided and began to fill the cavity located at this point. This development generally occurred parallel to the torn surface of the bark flap and stock region. The same pattern was detected for the callus strands produced from the ends of the broken xylem rays of the stock and for the terminal cells of broken phloem rays and immature secondary phloem in close contact with the injured area of the scion. Extensive development of callus strands proceeded mainly through the formation and enlargement of wedge shaped cell groups located at the terminal ends of these structures.

Following penetration of the necrotic plate by the callus strands, continued radial and tangential cell division resulted in the filling of the cavities between stock and scion. The immature xylem area adjacent to the cut surface enlarged and divided, forming layers of cells which usually did not penetrate the necrotic plate.

On the sixth day, direct contact between the calli of stock and scion was first observed. At this time, the necrotic plate had been ruptured in several places and had become scattered throughout the areas of the stock-scion juncture. Only occasionally did necrotic tissue form any type of barrier that would prevent the union of tissues produced by the two components at this sampling period. In these cases, wound gum was usually present in the immediate vicinity of the necrotic
tissue, which indicated that wounding was more severe than in the majority of sections observed. At this time, bridges of calli were observed between all areas of the bud shield and stock. The lateral peripheral areas adjacent to the inserted shield became filled with callus tissue rapidly. Since divisions were both tangential and radial, callus strands clearly defined in sections sampled earlier in the budding sequence could no longer be detected, making the subsequent identification of derivative tissue impossible.

At the end of eight days, inspection of serial sections throughout the vertical length of the T incision showed that the lower one-half of the bud shield was completely connected to the stock by means of contact bridges of callus (Figures 10 and 11). At this time, contact of the calli of stock and scion may be considered as complete for all areas of the stock and bud shield, with the exception of the uppermost portion of the bud shield.

By the tenth day, complete union was established in the distal portion of the bud shield, although some areas had not been completely filled with callus. It should be noted that in the upper region of the shield, intimate contact of tissue systems may never occur. In this event, components are distinctly separated by either an area void of tissue or one that contains a thin layer of nonliving cells.

Between the eighth and tenth day following budding, the
Figure 10. Cross section through the bud union area eight days after budding. 36x. Events are considered to be normal.

A-Bark flap, B-Scion, C-Wound gum ribbon, D-Merged calli of stock and scion, E-Necrotic plate fragments and F-Stock.

Figure 11. Cross section through bud union at the lateral extremities of bud-stock juncture eight days after budding. 36x. Events are considered to be normal.

A-Bark flap, B-Scion, C-Wound gum ribbon, D-Merged calli of stock and scion, E-Necrotic plate fragments, F-Stock and G-Mechanical tearing.
formation of new cells occurred between the bark flaps and the scion in such profusion that the flaps were being pushed outward from the shield. Cambium regeneration in the region of the bark flaps and under the bud shield was noted during this period.

Callus that was formed in the area of the bark flaps began to differentiate ten to twelve days after budding (Figure 12). In this same period, activity of cambium in the areas adjacent to the point of bud insertion was also observed. Only later in the normal sequence of union was the same phenomenon noted for the intact cambium areas of the bud plate, and in the stock region. During the time between bud insertion and twelve days, the ends of the bark flaps were observed to be in varying stages of plasmolysis. After twelve days, most of the dead bark flaps became separated from the living portion by a cork cambium layer ranging from three to four cells in thickness (Figure 13).

No radical changes were observed in sections of unions collected twelve and fourteen days after budding. There was increased differentiation in the calli and the presence of additional cambiform arcs across the stock-scion interface.

In callus formed underneath the shield, the thickening of cell walls was observed between the fourteenth and sixteenth day (Figure 14). As was noted for the cambium region included in the bark flaps, activity of the cambium in the bud shield
Figure 12. Cross section through the bud union area twelve days after budding. 36x. Events are considered to be normal.

A-Scion, B-Merged calli of stock and scion and C-Stock.

Figure 13. Cross section through bud union at the lateral extremities of bud-stock juncture twelve days after budding. 36x. Events are considered to be normal.

A-Scion, B-Bark flap, C-Cork cambium layer, D-Merged calli of stock and scion and E-Stock.
region was detected as soon as callus differentiation commenced. Cambiform tissue produced secondary xylem, and secondary phloem, as well as a small amount of xylem tissue, in the scion.

The development of what appeared to be the initiation of tracheid formation was observed during the sixteenth and eighteenth day periods in the region interior to the bark flaps (Figure 15). The cambiform arcs were developing in a more orderly pattern and had either progressed into the area between the bark and shield or were closely approaching this region. It was at this time that the first continuous chain of cambial cells between stock and scion was observed.

The healing process for all bud unions studied was nearly the same for the period from one to fourteen days. After this period, distinct differences in both the speed of wound healing and the completeness of the union were observed between samples. Rapid development of new wood was evident by eighteen to twenty days for those unions in which the cambia could be considered united. The extent of development appeared to be related to the completeness of the union.

For all sections of unions examined from the eighteenth to twenty-fifth days, no distinct sequence of events could be established. Lignification was considerably more advanced in callus cells near the bark flap areas than in those directly underneath the bud. Connecting arcs of cambiform tissue were
Figure 14. Cross section through the bud union area sixteen days after budding. 36x. Events are considered to be normal.

A-Scion, B-Merged calli of stock and scion, C-Necrotic cell area, D-Mechanical tearing and E-Stock.

Figure 15. Cross section through bud union at the lateral extremities of bud-stock juncture sixteen days after budding. 36x. Events are considered to be normal.

A-Scion, B-Bark flap, C-Cork cambium layer, D-New wood production, E-Wound gum ribbon, F-Necrotic cell area and G-Stock.
either in intimate association or entirely complete at this time for many of the sections observed (Figures 16 and 17). Other sections were less organized at this same period. For unions in which disorganization was noted, some cells were still in the process of division, while those in nearby areas showed pronounced lignification.

In sections cut from bud unions collected three weeks after budding, it was difficult to establish an orderly sequence of union maturation. Some bud unions exhibited symptoms of visible decline, arrested development, or the rapid increase in size and number of all tissue systems.

After three weeks, all bud unions were found to be in various stages of discontinuity. This ranged from isolated areas of necrotic tissue located in the scion, on the stock-scion interface, or in both stock and scion, as well as the presence of large areas of dead tissue or areas filled with wound gum.

One may conclude that if incompatibility occurs, symptoms would be expressed by the third week following insertion of the bud. All bud grafts included in the foregoing discussion were taken from plants which appeared to have a normal bud union and which showed no outward appearances of abnormality. Buds sampled after twenty days were either swelling or had broken and started to elongate.

Bud unions included in the subsequent discussion were those known to be either dead or which were obviously not normal.
Figure 16. Cross section through the bud union area twenty days after budding. 36x. Events are considered to be normal.

A-Scion and B-Necrotic area.

Figure 17. Cross section through bud union at the lateral extremities of bud-stock juncture twenty days after budding. 36x. Events are considered to be normal.

A-Scion, B-Bark flap, C-Cork cambium layer, D-Stock and E-Wound gum ribbon.
Abnormal bud union histology

Fourteen days after budding, indications of some disorder were observed in many bud unions. These disorders were noted to be present in the scion, the stock or in the interface between the stock and scion. The most common area of decline was associated with the callus between the two components. These areas containing dead or dying cells occurred as early as the fourteenth day or were delayed until a much later period. Occasionally, sections were observed during the latter portion of the sampling period, that is, fifty and sixty days, to have only small areas of necrotic tissue or small inclusions of gum-like deposits. This indicated that these differences from the normal healing process were delayed, or that further deterioration did not occur. Unfortunately, it was impossible to predict whether these unions would have died.

In the order of frequency in which the various disorders were encountered, the following was observed: (1) inclusion of necrotic tissue or wound gum in the calli joining the two components, (2) necrotic areas produced over much of the interface of the stock-scion area, (3) formation of wound gum in the stock-scion interface which extended into the connective tissue and (4) failure of either stock or scion to produce wound parenchyma on a portion of the injured parts.

The frequent presence of necrotic cells, or cell fragments,
and ribbons of wound gum in the newly formed callus regions
were the most common abnormalities observed. The dead tissues
were closely compressed by the rapid development of callus
parenchyma. These inclusions were never of such concentration
as to form a definite barrier or continuous zone between the
components. The normal sequence of bud union formation was
assumed to be delayed as the result of the presence of these
inclusions.

In abnormal unions, necrotic cell regions located adjacent
to the stock-scion interface were seldom ruptured by developing
callus strands. Although these necrotic plates were not uni-
formly located in any type of union, they were more common in
the areas subtending the lateral extremities of the bud shield.
No union that outwardly appeared normal developed a continuous
band of necrotic tissue across the complete stock-scion inter-
face. Development of a continuous plate was limited to unions
having buds which quickly became desiccated following placement,
or to those which declined before bud break.

Masses of wound gum had accumulated in both the stock-scion
interface and in the newly formed adjacent callus areas of ab-
normal unions. In general, the location of these gum masses
was restricted to areas on either side of the central bud shield
axis. The gum deposits were occasionally found to occupy as
much as one-half the entire stock-scion interface area. The
wound gum deposit does not always assume the shape of a sphere.
or globule, but rather may contain one or more finger-like protuberances (Figure 18). None of the bud unions examined were found to have these gumlike deposits extending completely across the stock-scion interface.

In cross sections of abnormal unions, there were localized areas in the stock or scion which produced no wound parenchyma. In the bud shield, this area was generally located along the outer edges. Occasionally, small areas directly beneath the bud trace were also devoid of proliferating tissue (Figure 19). Most commonly, the area that did not produce new tissue, or which became necrotic, was the xylem area of the stock injured at the time the vertical incision was made (Figures 20 and 21).

A similar developmental sequence of wound healing occurred in all bud unions in which the bud and the included shield had failed (Figures 22 and 23). There was an absence of callus tissue in the area exposed by the separation of the bark flap from the stock. The formation of a thick layer of necrotic cells over this injured area prevented callus penetration, if such occurred. A series of rapid cell divisions in the areas subtending the bark flaps produced callus tissue which rapidly pushed toward the central area of the stock. As a result of the pressure exerted by this developing wound tissue, the shield containing the bud was either crushed or pushed out of place. Although wound tissue developing from the bark flap regions was observed to have completely covered the injured stock area within three weeks, this usually required additional time.
Figure 18. Cross section through the bud union area sixteen days after budding. 36x. Events are considered to be abnormal.

A-Scion, B-Necrotic tissue inclusion, C-Necrotic cell area, D-Wound gum mass, E-Stock and F-Mechanical tearing.

Figure 19. Cross section through the bud union area sixteen days after budding. 36x. Events are considered to be abnormal.

A-Scion, B-Cork cambium layer, C-Wound gum ribbon, D-Stock, E-Necrotic cell area and F-Necrotic cell inclusion.
Figure 20. Cross section through the bud union area twenty days after budding. 36x. Events are considered to be abnormal. The injury to the xylem area is a result of propagation technique.

A-Scion, B-Wound gum ribbon and C-Necrotic area.

Figure 21. Cross section through the bud union area twenty-five days after budding. 36x. Events are considered to be abnormal. The injury to the xylem area is a result of propagation technique. Note the arrested development of the scion.

A-Scion, B-Necrotic cell area, C-Wound gum ribbon and D-Stock.
Figure 22. Cross section through the bud union area twenty-five days after budding. 36x. Events are considered to be abnormal. The lateral extension of the developing calli is shown developing over the stock area, following death of the scion.

A-Cork cambium layer, B-Lateral extension of wound calli, C-Desiccated scion and D-Stock.

Figure 23. Cross section through the bud union area thirty days after budding. 36x. Events are considered to be abnormal. The scion has become displaced and forced out of position with the nearly completed wound tissue.

A-Desiccated scion, B-Cork cambium layer and C-Lateral extension of wound calli.
DISCUSSION

The development of a functional graft union between the peach and western sand cherry follows a similar pattern of histological development as has been reported for plants closely related to this genus. Successful union is considered to be dependent upon the simultaneous differentiation of cambiform tissue produced from the calli in the stock-scion interface and the subsequent development of a continuous cambium between the stock and scion. Bud unions that do not follow this normal sequence may decline or die.

In the budding operation, there were slight differences in the ease of opening the bark flaps of the T incision on the understock. Those tissues toward the proximal end of the shoot were more mature than those at the distal end and consequently were more difficult to separate at the desired point. Commercial propagators recognize that ease of separation, or bark slippage is influenced, not only by the age or maturity of the tissues, but also by the variety or species of plant and the season of the year.

In general, the rate of union healing and the region from which new cells and tissue systems were formed agrees with the observations made by other investigators. Slight differences which occurred could be attributed to one or more of the following factors: (1) the method of scion removal from the bud-
stick, (2) the method of bud insertion, (3) the time of observation, (4) the wrapping technique employed, (5) differences in climatic conditions and (5) the difference in the type of plant material involved in the graft combination.

Severe wounding at the time of budding is considered to be the cause for the development of large necrotic areas in certain bud unions. The extent of wounding may be attributed to the propagation technique and/or to the inherent nature of the understock. Not infrequently the individual performing the budding operation will, of necessity, force a bud into contact with the exposed xylem cylinder if the bark does not "slip" readily. This is done in preference to choosing another location on the stock or to impose undue stress on the understock by making a second wound.

In the commercial nursery, a budding crew is generally made up of at least two workers. The duty of the "budder" is to remove selected buds from the budstick, make the necessary incision into the stock and insert the bud and included shield. In this procedure the budder commonly removes the entire shield, with the exception of a small portion of bark above the actual bud. This procedure is used for all buds contained on the budstick. Drying of the cut areas undoubtedly occurs prior to placement of the bud shield in the stock incision. As the budder proceeds down the nursery row, it is the duty of the "tier" to wrap the inserted buds in place. Since it is impossible to
keep up with an experienced budder, additional desiccation of the bud shield and the stock occurs.

In the xylem area immediately below the vertical incision made in the stock, an area is often found which becomes necrotic or fails to become active (Figures 20 and 21). This is the result of cutting too deep into the xylem on the part of the budder. There is no reason to doubt that this is a frequent occurrence in the nursery, especially with the first few stocks budded following sharpening of the budding knife, which is used for both bud removal and preparation of the T incision.

In the normal sequence of healing, the portion of the living tissues of the bud shield and the stock are connected in about one week. Shortly thereafter, the entire cavity area underneath the bud shield becomes filled with wound callus. In the early stages of union development, the supply of water available to the scion portion is perhaps the most important factor in determining the success or failure of the operation. A shortage of water available to living plant cells favors cell differentiation at the expense of cell division and enlargement. If cells on the wounded area of the stock or scion dry, the rupturing of the thicker cells by the developing callus is either retarded or eliminated. If intimate contact is not made between developing callus cells within a relatively short period of time, drying of the bud shield usually results soon after budding. This fact gives basis to the practice of in-
interrupting commercial budding operations when temperatures reach 90 degrees F. This also gives basis to the practice of wrapping the union of certain plants immediately after bud insertion. All of these factors influence the success of the bud union in its early development.

Although cambium is usually considered to be the most active tissue system giving rise to callus cells following grafting of many plants, this was not observed with the plant materials used in these studies. Only a small region of the bud shield contained intact cambium cells and these were slow to form new tissues. However, once there was union between the cambial regions, active division was observed to take place from this continuous cambium layer of stock and scion.

In general, the stock portion is considered to be the major region producing callus tissue which connects the components. The bud shield seldom produces much callus during the initial stages of union formation. There were instances however, when the rate of callus formation was quite similar for both the stock and scion portions. This variation may have been the result of the method of propagation, differences in root-stock maturity, or the maturity of the buds removed from a given budstick. The normal stimulus resulting from wounding of the intact stock, as well as the availability of adequate supplies of water and nutrients, may be additional factors effecting the greater initial activity of the understock.
At the present time, a reliable technique is not available that would enable one to distinguish between cells produced by the two components. The use of fluorescence microscopy may be of assistance in this regard. With the aid of fluorochromes, which are selectively absorbed by certain woods, chemical-fluorescent identification could be made. Certain wood types are autofluorescent and can be distinguished without the aid of fluorochromes when viewed under ultra-violet light.

With other woody plant material, differences between wood formed during a specific period of the same growing season may be demonstrated visually. This distinction may be accomplished by the aid of a phase-contrast microscope, although standard staining procedures reveal no differences. Any modification of technique that would allow for visual detection of differences between cells of the graft components would provide for a more complete understanding of graft union formation.

The major portion of the raised bark flaps contribute little to the bud healing sequence. Most plant propagators agree that approximately three-fourths of the raised bark flap dies and does not enter into the success or failure of the developing bud union. The only proposed functions of the bark flaps are that they serve as additional mechanical support for the bud shield, prevent excess desiccation of the scion, retard entry of possible foreign materials in the immediate vicinity
of healing and prevent excessive crushing of the young tissues as a result of the tying operation.

If incompatibility occurs, it is usually expressed between the second and third week following budding. In this type of union, buds often fail to break into active growth. Even if the buds break, the extent of growth is directly related to the amount of connective tissue produced by the grafted components. The extent of discontinuity in the developing union may be such that the union can support scion growth for a limited period of time. Sudden death or decline of a developing budling may occur once a "limiting factor" develops. Most failures normally occur during the latter portion of the growing season when temperatures are most apt to be high and when rainfall is at a minimum.

Discontinuity between graft components does imply that undesirable growth or incompatibility would be expressed at some time during the growth of the plant. This condition may also be observed in graft unions that are considered to be highly compatible or that are functioning normally. Graft unions may be discontinuous in one or more areas at the point of insertion and not influence the functioning of the union or the performance of the scion variety. It is the extent of discontinuity in a given union that determines whether or not the developing scion variety will perform suitably.

The formation of wound gum in the stock-scion interface
region may be attributed to the presence and/or activity of the shield and included bud. In unions in which no scion development was evident, no wound gum could be detected on the wounded xylem of the stock. This wound gum was apparently produced by the small portion of xylem tissue that is included on the bud shield.

In *P. besseyi*, the bark begins slipping relatively early in the growing season. With most commercial peach varieties, bark slips at a later time in the season. In order to use peach scions at the time when bark is slipping on the rootstock, a portion of recently derived xylem tissue must be included on the bud shield. For most peach varieties this would involve performing cultural operations that would hasten growth and possibly cause earlier bark slippage. The most logical approach to this would be regular irrigation, if needed, during the early part of the growing season and fertilization of those plants included in the stock plant block.

Some bud union sections were observed to have a very disorganized pattern of development throughout the entire uniting area and extending up into the shield portion. Sections of this type were usually those which had masses of wound gum or necrotic tissue. Proliferation of callus cells was observed in the areas not obviously in the process of differentiation into connective or vascular elements. During the forty and fifty day sampling period, buds that had not started into
growth were often found to have no distinct connection between the cambium of the stock and scion.

A distinction was not made between the process of bud union development for indexed and nonindexed buds. The external appearance of bud unions and field response of the developing budlings were no different. Observation of stained sections in the laboratory revealed no differences between the union formed by buds classified as virus-free or those that were not indexed. It may be concluded that for the particular nonindexed stock plants chosen as a source of budwood that no differences in rate or extent of union formation was realized.

No comparison in the sequence of bud healing has been made between buds placed on young wood or more mature wood. Examinations of bud unions for the two ages of stock showed no discernable differences for speed of healing or rate of callus formation. Undoubtedly, differences would have appeared if budding had been performed when bark was not readily slipping on these two ages of stock.

The relatively poor stand of acceptable trees obtained during the course of these experiments would make the commercial use of the western sand cherry as a dwarfing rootstock for peach questionable. In order to be considered economical, a higher number of saleable budlings would have to be produced. Modifying the accepted propagation procedures could possibly increase the number of desirable budlings any given season.
Differences in the per cent bud take in the two years of study agrees with the general range obtained by commercial propagators. The reduced bud take for budding performed relatively late in the second growing season is also in agreement with the reduced stand obtained when peach varieties are budded onto *Prunus besseyi*.

Any procedure that would hasten the process of callus development and differentiation in the early stages of bud union development would improve bud take. It must be recognized that the inherent factors responsible for failure are difficult to eliminate. However, some of the factors which result in failure could be purely physical in nature. Such factors as gauging the optimum time for collecting budsticks, optimum period of bark slippage, increasing the speed of new cell division and similar procedures could be controlled to a great extent by the plant propagator.

Suggested techniques would include: the application of a suitable growth regulating chemical to either the stock or scion in order to speed up the uniting processes; and/or the controlled variation of cultural techniques to speed up, check or delay the rate of maturity of either stock or scion. Other possible approaches could involve the determination of the optimum method of tying or waxing the bud union and determining the budling response by use of scion wood obtained from different areas of the stock plant.
SUMMARY AND CONCLUSIONS

A study was conducted on the histology of the healing process in the peach-western sand cherry bud union. Indications are that unsatisfactory response in the nursery may be due to incompatibility between the two graft components. This study was undertaken to contribute to a better understanding of the sequence of events leading to the establishment of a graft union and the possible reasons for incompatibility between grafted plant material.

During a two-year study, buds of the peach, variety Polly, were placed onto a dwarfing rootstock, *P. besseyi*. Budwood used in these investigations were collected from stock blocks certified to be virus-free as well as from trees which had not been indexed. Buds of these two types were placed onto both young and more mature growth of the dwarfing rootstock by means of the "T" budding technique.

A regular collection schedule was followed in which developing unions, as well as those not growing in a satisfactory manner, were periodically removed for laboratory examination. Samples of unions were collected from one day to a point sixty days after budding. Field observations on symptoms used to detect early union, per cent bud take, per cent budling development and growth after two and three years in the nursery are included.
The developmental sequence is presented for unions considered to be normal as well as for bud grafts that either died or did not develop normally.

The major results of this study are as follows:

1. The mechanical operations of removing the bud and included shield from the bud stick and preparation of the T incision on the understock injures or destroys all of the tissues which have either been cut or torn. These cells form a necrotic plate over the interface of the graft components.

2. The development of callus strands from the terminal, uninjured cells of the immature xylem of the stock occurred rapidly and ruptured the necrotic plate of the stock in the normal sequence of bud healing by the fourth day. Shortly thereafter, the necrotic plate contained on the bud shield was also ruptured by callus growth, mainly the result of divisions in the secondary phloem. Delayed penetration of the necrotic plate by callus tissue results in the death of the bud brought about by the absence of connective tissue and resultant desiccation.

3. Active cell division was observed in some bud unions collected two days after budding. By the fourth day, considerable callus had been produced by dividing cells of the stock. In sequence of meristematic cell activity, the intact cells of the immature xylem, adjacent to the lateral flaps were the first to activate, followed by the extremities of the bud
shield and finally the area immediately below the central axis of the inserted bud.

4. Six days after bud insertion, contact between the calli produced by the stock and scion had been established. By eight days, the lower one-half of the bud shield was connected to the stock by means of callus bridges. The area of the bud shield adjacent to the horizontal stock incision may never fill with callus.

5. The first continuous cambium connecting component parts of the bud graft system occurred shortly after fourteen days. Subsequent development of xylem and phloem tissues by this layer resulted in forcing of the bud away from the stock. Although the intact cambium layers of both the stock and scion contribute little to the early development of a successful union, cambium continuity was established between graft components.

6. Distinct differences in the rapidity of cell division and completeness of healing were observed between unions collected after fourteen days. After three weeks, distinct areas devoid of cells were apparent in all bud unions. Although these areas may persist, they are not believed to influence the effective functioning of the union.

7. Symptoms of incompatibility were expressed as early as the third week and could be discerned anatomically in the stock-scion interface, or in the area of the stock adjacent to
the T incision. In the order of occurrence, disorders between the stock and scion were observed in the following sequence: (1) necrotic tissue inclusions and the presence of wound gum ribbons in the anastomosing calli, (2) necrotic cell areas present on the stock-scion juncture, (3) large deposits of wound gum in the connective tissue region and (4) failure of callus formation on an injured portion of the stock or scion.

8. No discernable differences were observed in the rate of healing, in the morphology of the bud union and in the gross appearance of the budlings when current season's growth or shoots in their second year of development were used as the rootstock. Under the conditions of this experiment, no differences in the morphology of the bud graft union or plant performance was found between seedlings budded with indexed and nonindexed scions.

9. The general processes of development for the peach-western sand cherry bud graft have been found to be basically the same as for other plant material propagated in this manner. These events were as follows: (1) formation of necrotic cell areas over cut or torn tissue regions, (2) formation of callus from the uninjured cells adjacent to the wounded portion, (3) formation of callus in the stock-scion interface, (4) formation of a continuous cambium between scion and stock from resultant callus differentiation and (5) resumption of cambial activity, lignification and connection of vascular tissue.
10. Bud failure between the peach-western sand cherry graft combination may be the direct result of propagation technique, environmental conditions, inherent differences between graft components or the failure of either symbiont to function in a normal manner. Under the conditions prevalent in Iowa nurseries, incompatibility of peach on Prunus besseyi casts doubt on the suitability of this rootstock for dwarf peach tree propagation.


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