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STUDIES ON SOME APPLE VIRUS DISEASES IN NEW HAMPSHIRE

JOSEPH G. BARRAT

Dapple apple symptoms on the fruits of the variety Starking.

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By
Joseph G. Barrat

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Avery E. Rich

Wm. G. Smith

R. A. Kilpatrick

M. C. Richards

L. J. Conklin

A. R. Hodgdon

May 29, 1958
Date

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

SECTION I

INTRODUCTION

Apples (*Malus pumila*[1]) are grown as a basic fruit commodity throughout the temperate world. Their culture varies from country to country depending on the environmental conditions which are present. All of these apples are subject to disease and insect pests which also vary, some being common to all areas while others are restricted to certain locales.

Apples are the most important fruit crop in the United States. The average annual production of marketed apples is approximately 113,800,000 bushels (10). The State of New Hampshire produces one million bushels of these marketed apples. Of course, there are many thousands of bushels of apples which do not reach the market for various reasons, including disease losses. It is estimated that in the United States that approximately 8,263,000 bushels annually do not reach the market because of the apple scab disease alone (80).

Losses from apple diseases are generally attributed to fungi and bacteria. The virus diseases besetting them are considered more or less oddities which do not appreciably affect the quantity and quality of apples produced. The

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1. All plant names are in accordance with Rehder's Manual of Cultivated Trees and Shrubs (108) or Gray's Manual of Botany (39).

writer believes that apples are almost in the same position as potatoes; namely, most apples are infected with virus entities, but they differ from potatoes in that this fact is not yet recognized. The purpose of any investigation of apple virus disorders is to further our recognition of these unapparent disorders and to correct them.

A preliminary review of the literature indicated that no indexing host was available which would detect viruses within the apple tree which do not express themselves by the presence of symptoms. This presence of a virus within a host which does not show symptoms is referred to as a latent virus. Its detection within a plant is demonstrated by the transfer of the virus to another host and the development of symptoms by the second host. This is generally called indexing and is by far the most widely accepted method of determining the presence of latent viruses within a plant. One of the general purposes of this work is to develop an indexing technique which will demonstrate within a particular host the presence or absence of a viral entity in apples. Without this technique, very little progress can be made in the study of apple virus diseases.

The particular purpose of this study of virus disorders of apple trees had its origin in the severe winter of 1933-34 (118, 119). The extremely low temperatures over an extended period of time caused many apple trees to die from freezing injury and caused extensive injury among many of those trees which survived the winter. Most of the injury was located on the trunks of the trees near the soil line and in the crotches of the larger branches near the main trunk. Since the injury was rather restricted it was thought that these areas which lacked low temperature hardiness or exhibited susceptibility to winter injury could be replaced by grafting which would substitute a hardier trunk and scaffold limbs.

Using this as a basis for an experiment, Dr. W. W. Smith, Horticulturist at the New Hampshire Agricultural Experiment Station planted apple trees at his farm in Gilford, New Hampshire. In place of the usual varietal tree trunk the selections Virginia Crab and Florence Crab were used. The apple varieties in which these trunks were grafted were McIntosh, Cortland, Red Spy and Northern Spy. This work was initiated in 1940, and by 1948, disorders which were of unknown cause and which did not permit the proper development of the trees appeared in the substituted trunks (more commonly referred to as body-stocks or interpieces). In addition, symptoms appeared on the fruits of the Cortland variety of a noticeable discoloration which reduced their market value. The disorder occurring in the Virginia Crab bodystock has been called "wood pitting", "stem pitting" or "tristeza" (118, 119). The symptoms on the Cortland fruit have been described and called "dapple apple" (120).

The purpose of these investigations has been to determine the nature of the dapple apple disorder and to increase our knowledge of this and other apple virus diseases.

SECTION II

REVIEW OF LITERATURE

The start of our knowledge of plant virus diseases is usually attributed to Iwanowski, who in 1892 could not remove the infective entity of tobacco mosaic with fine bacterial filters. Beijerinck, a few years later, conceived the possibility that another agent smaller than bacteria could exist and cause the tobacco disorder. Beijerinck in 1896 called the infective sap "contagium vivum fluidum" and, thus, gave official recognition to viruses (17).

The word "virus" is of Latin origin from "virulentus", which exactly translated means poison or toxin. At one time the word was associated with bacteria, and bacterial and viral phrases were synonymous. With the introduction of filters fine enough to exclude bacteria, it became more common to refer to these agents as filterable viruses. These entities, after all else is removed, are now called viruses (17).

A virus has been defined as "a submicroscopic entity, probably nucleo-protein in nature, capable of reproducing itself only in vivo, and usually producing certain symptoms in the host" (109). A virus is also defined as an exceedingly minute obligate parasite which grows and multiplies within, and at the expense of, living hosts and is too small to be seen with the ordinary light microscope (110).

Although 1892 is the starting point of virus knowledge some pertinent work in fruit trees had been done before that time. E. F. Smith (55) in 1888 transmitted the peach yellows by grafting, thus demonstrating that it was an infective agent not caused by bacteria or fungi. Beach yellows had caused considerable attention and was first recognized in 1791 in this country (55). Apple mosaic is also a disease of long standing, having been noted first about 1827 in France (97).

Apple virus diseases have not received as much attention as other fruit tree virus diseases until recently. Holmes (62), in his 1938 classification, lists _Marmor mali_ (apple mosaic virus) as the only virus causing a disease of apples. Smith (117), in his 1957 book, briefly describes 8 virus diseases of apples and lists 2 as synonymous. Anderson (1) in 1956 described 4 apple virus disorders and makes reference to 3 more. The Review of Applied Mycology (9) in 1957 recorded 12 viruses on apples. Many strains have also been listed; some of which are in error.

The brief summaries presented by Anderson (1) and Smith (117), although recent are inadequate for the separation of virus diseases and establishing the occurrence of a new one. An extensive review of the literature was necessary to establish dapple apple as a new virus disorder and to present a discussion of apple virus diseases.

This review of the literature will attempt to record those apple virus diseases which have been described previous to 1958 and to relate to each other only those which are obvious. It is hoped that this review will aid in understanding the symptoms of apple virus infection and clearly present a needed summary of apple virus disorders.

Virus Symptoms Expressed in Foliage and Stems

Name: Apple Mosaic Virus Khristov 1934. (Apple Infectious Variegation Virus Bradford and Joley 1933; Pyrus virus 2 Smith 1937; Marmor mali Holmes 1939)

Disease Name: Apple Mosaic, Infectious Variegation.

Symptoms: Apple mosaic is a disorder of the leaves in which whitish or cream-colored areas are present. These areas may be either of polygonal shapes which are more or less restricted by the veins, or somewhat of a blotchy condition covering the lamina of the leaf which tends to follow the veins. The amount of discoloration may be as little as a fleck on a single leaf or it may cover the entire leaf. Intermediate forms are present. Necrotic areas appear on the more severely affected leaves, and defoliation of these leaves is common. Strains, based on the severity of symptoms have been described. Reddish-brown streaks on the bark of young shoots and cream-colored patches on the fruit may occur in very sensitive varieties. The virus entity causing apple mosaic has been transmitted (25, 31, 32, 97, 100)[1].

Discussion: Considerable work has been done with the apple mosaic virus. Perhaps the most important is the subdivision of apple mosaic symptoms into 3 strains--severe mosaic, mild vein-banding, and mild mosaic (100).

Severe mosaic is the most intense form causing extensive vein-banding and large chlorotic areas which become necrotic in late summer, followed by much defoliation. Severe

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1. References refer to the entire symptom description.

mosaic, probably on the very susceptible varieties Cox's Orange Pippin and Lord Lambourne, is expressed by cream-colored blotches on the fruit and by reddish-brown streaks on the bark of young shoots.

Mild vein-banding mosaic may cause the full range of symptoms but is predominantly expressed by a mosaic banding of the primary and secondary veins and by a mosaic network in the tertiary veinlets.

Mild mosaic lacks the more intense chlorotic areas as well as any extensive banding of the veins. It is expressed by streaks or flecks which are usually small and obscure and, therefore, may be overlooked entirely.

More recently, three minor strains have been described (100): a vein clearing type, a line pattern type and a ring spot type. The last named is somewhat reminiscent of Cochran's findings in 1950 (35).

Apple mosaic has been known since 1825-7 in France (97), making this disorder one of the oldest recorded virus diseases. It was first noted in the United States in New York in 1910 (19), Connecticut, 1914 (59), Maine, 1916 (89), Massachusetts, 1924 (49), and Michigan, 1933 (25). In 1923 Blodgett (19) successfully transmitted "infectious variegation" by experimentation. In 1934 Christoff (31) in Bulgaria named the disorder "apple mosaic" and furthered its transmission to quince and pear. In 1937 Thomas (123) expanded the host range of apple mosaic to include the following genera and species: Cotoneaster, <u>Eriobotrya japonica</u>, Photinia, Rosa and <u>Sorbus</u>

pallescens. Work in New Zealand added Crataegus to the list (3). Kirkpatrick (69) successfully transmitted the disorder to peach, and Gilmer (47) associated the plum line pattern virus disease with apple mosaic by inoculating apple seedlings with a plum line pattern virus from Shiro plum and by inoculating Shiro plum with apple mosaic from apple. Typical symptoms of plum line pattern were produced from the virus in the apple mosaic buds in Shiro plum and vice versa. Posnette and Ellenberger (102), using some strains of apple mosaic virus and plum line pattern virus, were also able to induce symptoms typical of plum line pattern and apple mosaic by cross inoculation. This work indicates that the plum line pattern disease of plums and also of peaches is the same virus entity responsible for the symptoms of apple mosaic in apples.

Methods of transmission other than by budding or grafting have been reported. Yarwood (132) mechanically transmitted the symptoms of apple mosaic by means of carborundum abrasion from infected apple leaves to tobacco and other plants and was able to return the virus to apple by means of dodder (Cuscuta subinclusa and C. campestris). Yarwood noted that the symptoms of apple mosaic in tobacco were similar to tobacco streak virus. Fulton (44) found differences in the reactions between tobacco streak virus and Yarwood's strain of apple mosaic and concluded that apple mosaic was not a strain of the tobacco streak virus because of the lack of cross protection between the two entities in tobacco. Virus entities of apparently related composition, but yet offering some dissimilarity in symptom expression, are known as strains. When one strain is present in a plant it will to some extent protect the plant from infection by another strain even though the latter is more severe. This type of defense is known as cross-protection.

Posnette and Cropley (100) were unable to transmit apple mosaic mechanically and believed, therefore, that Yarwood had a different virus which caused apple mosaic symptoms in apple. They were also able to show cross-protection between mild mosaic, mild vein banding and severe mosaic symptoms in apple. The milder strains of apple mosaic offer cross-protection to the severe strain (17, 97).

Cochran (35) inoculated apple seedlings with material from peach trees infected with peach ring spot virus and noted rings and mottle patterns similar to apple mosaic in the apple seedlings. Upon returning this selection to peach, ring spot symptoms developed. Other selections of apple mosaic produced no symptoms on peach.

From the above it is evident that there are at least two virus entities which may cause symptoms of apple mosaic in apple. The true apple mosaic consists of at least three strains and is not mechanically transmissible. Another entity causing similar symptoms in apple is associated with peach ring spot. Yarwood's strain may or may not be the same.

Apple mosaic is generally considered to be systemic within the tree. It has been noted many times, however, that buds from infected trees when used as inoculum may fail to transmit the disease (30, 74, 95, 100). Posnette (100), by using buds from vigorously growing shoots, with and without symptoms on the leaves, found that symptomless shoots did not transmit the apple mosaic virus as readily as buds from a shoot with leaf symptoms, thus indicating that there are areas within a plant which are free of the virus, and that the apple mosaic virus does not penetrate all portions of the plant at the same rate. Also, leaves produced in the summer show less infection than leaves produced in the spring.

The spread of apple mosaic throughout the world can be attributed directly to man and his grafting techniques. One hundred per cent infections of apple varieties are known, in which the result of infection can be directly traced to the use of infected scionwood (60, 94). Woolly aphid resistant rootstocks such as that of the Northern Spy in which the apple mosaic virus is symptomless (100, 122) have also been important means of spreading the virus in Australia.

Although no insect vector has yet been claimed for any of the apple mosaic virus diseases, there have been reports of the spread of apple mosaic in orchards (2, 20, 122). Blodgett found that spreading took place in older trees and down rows where the possibility of natural root grafts was highest. He noticed an increase from 31 to 138 infected trees out of a total of 914 over a 10 year period. Several workers have pointed out that infection by the apple mosaic virus is potentially and actually detrimental to crop production (6, 37, 77, 100). Mallach (77) contends that over a 5 year period increase in tree growth was 40 per cent less, yield was 55 per cent less, and that in young trees girth was decreased 46 per cent. Dyer (37), comparing yields of infected and non-infected trees in South Africa, reports a yield of 294 pounds of fruit from healthy trees and 195 pounds from mosaic infected trees. Posnette and Cropley (100) indicated in their studies that infection with the severe strain suppresses tree growth 30 per cent, and reduces yield as much as 30 to 40 per cent in some varieties. Harvey (54) considers present tree infections to be of importance. He cites the loss of green photosynthetic area as reducing the tree's efficiency. Also, the pre-harvest leaf fall exposes the fruits to direct sunlight resulting in sun scald injury.

Posnette and Cropley noted in some trees of several varieties a peculiar condition in which a strain of the apple mosaic virus is latent. This condition was discovered when trees were indexed with Cox's Orange Pippin. These varieties are: Crawley Beauty, Edward VIII, Egremont Russet, Ellison's Orange, Laxton's Fortune, Grenadier, Jonathan, Laxton's Supberb, Miller's Seedling and Orleans Reinette. Heat inactivation of the severe strain of apple mosaic has been reported by Posnette and Cropley (100). A treatment of 37° C. for 27 days in an incubation chamber seemed to be most satisfactory. A comprehensive coverage of the apple mosaic disease may be found by referring to the papers by Bradford and Joley, 1933; Posnette and Cropley 1956, and Posnette and Ellenberger 1957.

<u>Geographical Distribution</u>: Argentina (40), Australia

(13), Belgium (112), Bulgaria (31), Canada (60), England (74), France (112), Germany (77), Holland (66), Italy (33), Kenya (4), New Zealand (30), Norway (104), Nova Scotia (60), Switzerland (21), Tasmania, Union of South Africa (4), Union of Soviet Socialist Republic (7), United States (25) and Yugoslavia (64), and is probably present in every apple growing district of the world.

Varieties: Many, including Jonathan, Golden Delicious, Starking Delicious, McIntosh, Baldwin, Northern Spy, Cortland, Cox's Orange Pippin, Bough Sweet, Lady Sudeley, Duchess of Oldenburg, Gravenstein, Lord Lambourne, Allington Pippin, Lane's Prince Albert, Bramley's Seedling, Beauty of Boskoop, Golden Pearmain, Ballarat, Astrachan.

Name: Apple Rosette Virus van Katwijk 1953
(Pyrus virus 6 (Smith) van Katwijk)

Disease Name: Apple rosette.

Symptoms: Apple trees infected with the apple rosette virus bear little if any fruit. This disorder may affect a single limb or the whole tree. The virus entity in this disorder affects the leaves of the tree so that they are misshapen, smaller than normal, and a cluster of them gives a rosette appearance. The base of an affected leaf is somewhat wedge-shaped, while the serrations along the margin of the blade are longer and sharper than normal. The lower half of the leaf blade is often less serrated than the upper half. Some leaves appear to be less misshapen, but the blade is somewhat curled. The virus entity causing the disorder has been transmitted (65).

Discussion: The disorder caused by the apple rosette virus occurs in Holland in 2 areas. Single trees of the variety Beauty of Boskoop were found to be unfruitful and to have an upright appearance. From the photographs it appears that only the terminal buds produce leaves which accounts for the impression that the disorder looks like a strong upright rattle (term used by van Katwijk). It is readily detected as being of virus-like nature rather than of some other type of disease-causing agent. The average size of affected leaves measured 6 x 4½ cm as compared to normal leaves of 10 x 8½ cm. The Jonathan variety showed some distortion of the leaves after inoculation but not so clearly as Beauty of Boskoop. Little mention was made of the fruit; apparently it is not formed.

Geographical Distribution: Holland (65, 128).

Varieties: Beauty of Boskoop, Jonathan, Golden Pippin, Glory of Holland.

Name: Apple Proliferation Virus Mulder 1949 (Pyrus virus 5 (Smith)) Apple Witch's Broom Virus Rui 1950

Disease Names: Apple Proliferation, Apple Witch's Broom, Wildness, Wild Apple, Scopazzi del melo.

Symptoms: At first an apple infected with true proliferation virus gives the impression that many witches' brooms are growing in the tree. The virus mainly affects the rapidly growing water shoots. After a limited amount of growth the water shoots and some terminal shoots start to die back while the axillary buds send forth new shoots which are smaller than normal, giving a proliferated or witch's broom appearance to the branch. Apparently a cluster of shoots is thus located on the branch and, therefore, resembles a faggot broom. The side shoots arise from buds which should be fruit buds and thus are instrumental in reducing the yield of the tree. The stipules on the leaves of both shoots of the proliferated tree are abnormally large and somewhat distorted. From the photograph, the true leaf blade is more rounded than normal and slightly distorted. The leaves on affected trees are pale. The apple proliferation virus has been transmitted (90).

Discussion: There is some confusion concerning the proper name of this disorder--whether it should be called apple proliferation or witch's broom. This disease, although known for many years in Italian nurseries, was first recognized and reported as a virus disease by Mulder in 1949 in Holland (90). In 1950 Rui (113) in Italy published on the disorder, calling it "witch's broom". Also in Italy Fogliani in 1952 (43) gave some evidence of its virus nature, but Refatti and Ciferri (107) in 1954 claimed not to have conclusive proof of it. Ciferri in 1956 (33) reported transmission of this virus entity to pear as well as apple. Smith (117) in his textbook uses the term "Apple Witch's Broom Virus" and refers to "Apple Proliferation Virus" as a synonym. However, both terms are used in present literature, and it is still a matter of choice.

The disease in Italy assumes somewhat the same general symptoms in mature trees and has been reported to affect as much as 10 per cent of the Jonathan variety in the nursery (107).

In Italy an early development of dormant axillary buds was observed on 1-year old apple nursery stock. The vigor of affected plants is reduced with 7 to 30 secondary branches being formed. The leaves are mostly small, narrow and rather pale green, but the stipules show the same characteristic enlargement. Apple witch's broom has been noticed to spread from tree to tree in some orchards, apparently by natural root grafts. Evidence that it may be spread by pruning operations is offered by Fogliani (43).

While visiting Europe Dr. L. C. Cochran and G. Strout observed apple witch's broom on nursery seedlings and suggested it belongs to the "yellows" rather than the "mosaic" group (34).

The apple proliferation virus causes symptoms distinct from those of apple rosette virus.

<u>Geographical Distribution</u>: Austria (42), Germany (42), Holland (90), Iraq (129), Italy (43), Switzerland (42).

<u>Varieties</u>: Golden Delicious, Jonathan, Canada Pippin, Champagne Pippin, Morgenduft, Beauty of Boskoop, Berlepsch, Golden Pearmain, Annurca, Abbondanza, Landsberger Rienette, Laxton's Superb, Signe Tillisch, Cox's Orange Pippin, Blenheim, Golden Pippin.

<u>Name</u>: Apple Rubbery Wood Virus Prentice 1950
(Pyrus virus 3 (van Katwijk 1953))

<u>Disease Name</u>: Rubbery wood

<u>Symptoms</u>: The rubbery wood virus affects only the branches and stems of the apple tree. The virus is expressed

by the excessive flexibility of these parts which bend readily under the pressure of their own weight and the weight of the fruit upon them. When hand pressure is applied to the branches they bend easily, and to some extent the degree of infection may be determined by the ease with which the bending occurs. Mature trees, under the weight of the branches and fruit, assume a "weeping" habit, the whole tree or a single branch being affected. The fruit produced by infected trees is apparently normal. When compared to non-infected trees, affected trees are stunted. The virus agent causing rubbery wood has been transmitted (66, 77, 103).

Discussion: Symptoms of rubbery wood virus infection are usually expressed by the typical "rubbery" feel of branches in older trees. However, in budded stock it is not uncommon to find a vigorously growing side branch originating from a point a few inches above the ground exceeding the growth of the main leader. In a few varieties the weight of the branches even in nursery stock is adequate to cause the trees to lose their normal upright habit and lie prostrate. During pruning operations the softness of the wood is noticeable and has been described as being of rather "cheesy" texture. Rubbery wood infected trees produce normal fruits, but the yield is less than normal (103).

The cause of the rubbery condition has been attributed to incomplete lignification of certain cells (18). Microscopic examination shows that the walls of the xylem vessels and tracheids are not lignified in the normal manner but are thickened instead with cellulose. These cells in sectioned and stained portions of the stem appear as islands of unlignified tissue surrounded by normal cells. In some branches lignification may be almost lacking. Although not all cells are without lignin there is apparently enough of this structural material lacking to prevent normal rigidity in the limbs.

The occurrence of rubbery wood is not considered to be as important and as wide-spread as apple mosaic; however, its known geographic distribution is being continually expanded. It was first reported in England as an unusual disorder in 1934, and work was furthered again in 1945 (18) and 1949 (103). Posnette and Cropley in 1952 (98), noting increased occurrence of the disorder on the Malling series of rootstocks used in the orchards, undertook an examination of various rootstock stool propagation beds in England. They found that propagation stools of M.I and M.IX[1] rootstocks at the East Malling Research Station were infected with the rubbery wood virus. In further work it was found that some of the commercial stool beds of additional selections of the Malling series were infected. Rubbery wood virus infected clones have been found in most of the old stools of the Malling series except M.II and M.XII. The Malling series of rootstocks have been sent throughout the world as superior understock material. No infections of the rubbery wood virus have been found in the newer Malling-Merton

1. The series of clonally propagated apple rootstocks from the East Malling Research Station are designated as M. for Malling. The Roman numeral indicates the clone.

series (51, 74). The virus was first found to be present in some of the propagation wood of the Lord Lambourne variety. This variety has become quite popular during relatively recent time throughout England and other European apple growing areas. Consequently many trees have been worked over to Lord Lambourne. By this means the rubbery wood virus disease has occurred in many places due to the use of infected propagation material. The rubbery wood virus disease has been reported recently as occurring on Golden Delicious and Stayman apple varieties in the State of Missouri (96). The writer has seen experimental plantings of English apple varieties in Oregon which are typically infected with the rubbery wood disorder. No doubt, in the future we can expect rubbery wood to appear wherever the Malling series of rootstocks has been used.

Detection of the virus is based upon the rubbery "feel" of the limbs. The symptoms of the disease have been separated into three classifications based upon the amount of bending which an infected limb will do under hand pressure. Since this is a qualitative measurement, some experience with known infections would be necessary to determine new infections.

Posnette and Cropley (99) developed an indexing method for the detection of the rubbery wood virus by using the very sensitive variety Lord Lambourne. Usually the clonal rootstock M.II has been used because it is free of the rubbery wood virus. A bud of the tree to be indexed is placed on a 1-year-old clonally propagated tree of M.II by the usual budding methods. A healthy bud of the Lord

Lambourne variety is inserted a few inches above this bud. When the remaining M.II aerial portion is cut off the inserted buds grow. If the rubbery wood virus is present in the lower bud it will be transmitted to the upper sensitive Lord Lambourne shoot which expresses the symptoms readily. One caution has been emphasized in this procedure. The buds must be vertically aligned so that a portion of the upper bud is directly over the lower bud.

Peach seedlings inoculated with buds from rubbery wood infected plants resulted in foliage symptoms in the form of purple rings and patterns (95). This method of testing apparently has not been expanded. A later note by Harris (52) mentions a latent virus present in Lord Lambourne on M.IX which caused a "green mottle" on peach and has been shown not to be the rubbery wood virus.

The occurrence of rubbery wood at the East Malling Research Station in England has brought to prominence the occurrence of virus diseases in apples. The distribution of these clones throughout the world as dwarfing stock and understocks for apple varieties has considerably increased the concern over virus diseases in apple varieties. The Malling series of rootstocks has been introduced into the United States and has been used widely in promoting the dwarf and semi-dwarf type of tree. Again, through man's manipulations a virus disease has been spread throughout the world. No insect vector is known.

<u>Geographical Distribution</u>: Australia (122), Canada,

England (36), Germany (77), Holland (66), Italy (33), Norway (104), Switzerland (22), United States (96).

Varieties: Golden Delicious, Stayman, Lord Lambourne, Miller's Seedling, James Grieve, Dartmouth Crab, Laxton's Superb, Worcester Pearmain, and more varieties yet to be determined.

Name: Apple Flat Limb Virus Hockey 1943

Disease Name: Apple Flat Limb, Gravenstein Twist, Gravenstein Gnarl, Spindle Wood, Crinkle Wood, Plastomania

Symptoms: In general, contortions, twisting, ribbed trunk, deformed branches, and flattening are terms used to describe the eccentric, sinewy growth of apple flat limb virus infected branches. A cross section of a branch through an affected area shows that the flat areas because they lack xylem and phloem development are accentuated because of the normal development of wood in other portions of the axis. On each side of the flattened areas are longitudinal cracks, the centers of which do not extend to the wood but are covered with bark. Beneath the bark of the flattened areas a pitting condition of the wood appears which is similar to that found in Virginia Crab. The symptoms are variable and may be latent or hardly noticeable in some varieties, more distorted in others, and in extreme cases may be open wounds where the bark has died away exposing the distorted, pitted wood of the xylem. Some workers have reported a reduction in yield of fruit, while others maintain not. There are no foliage symptoms. The apple flat limb virus has been transmitted (59, 61, 70).

Discussion: The distortions of the limb do not usually become discernible until the second or third year in young trees, but it has been observed on the present year's growth. The symptoms start as a slight flattening or depression along one side of the limb from one to several inches in length and may extend half way around the branch. As the age of the tree increases the flattening also increases. Affected limbs become brittle and break under the weight of a crop (12).

Apple flat limb is one of the older and better known diseases of apples, particularly on the variety Gravenstein. It was first noted in Australia (70) in 1905, and was reported in 1907 in Connecticut (59). In 1938 Foster (59), in British Columbia, transmitted the disorder from infected to non-infected Gravenstein trees. Thomas (123) in 1942, grafted diseased material into Pyracantha sp. and obtained severe rough bark symptoms. Hockey in 1943, accumulated the known data on apple flat limb and reported transmission of the virus entity, but since he obtained only 40 per cent transmission in his trials he suggested that the disorder was more involved with the type of rootstock used than with a virus. In Australia (27) certain cultural practices are still recommended to reduce the effects and occurrence of this disorder. Rootstocks which permit rapid growth of the Gravenstein variety have a greater tendency to show severe symptoms than rootstocks which restrict growth. In some varieties the virus is latent.

Kristensen (1956) and Hockey (1943) are good references.

Geographical Distribution: Australia (27, 28), British Columbia (59), Canada (59), Denmark (70), England (73), Germany (115), Holland, Italy (48), New Zealand (82), Norway (104), Nova Scotia (59), Sweden (70), Switzerland (23), United States (15, 124).

Varieties: Gravenstein, Wagener, James Grieve, Filippa, Signe Tillishec, Ontario, Penthalaz, Lord Lambourne;

Latent Varieties: Crimson Beauty, Dudley, Hove Reinet, Ingrid Marie, Golden Russett.

Name: Malus Stem Pitting Virus

Disease Name: Stem Pitting and Necrosis (Smith 1954), Wood Pitting, Tristeza or Quick Decline, Peach Pit.

Symptoms: The Malus stem pitting virus causes a disorder which is most noticeable in the area of the inner phloem and outer xylem in the bodystocks of Virginia Crab, Florence Crab and Red River Crab. The appearance of the outer bark of infected trees offers some indication of the presence of the abnormality. The bark generally has a roughened, scaly appearance and may be ridged, furrowed or cracked depending on the severity of infection. A spiral pattern of scales and ridges ascending in a clockwise direction is imparted to the bark as these demarcations ascend the tree. The thickness of the bark of infected trees is 2 to 3 times that of non-infected trees.

When the outer bark is stripped away, a series of fine lines and small fissures make a wavy, spirally ascending design. In older and in more seriously affected trees peg-like protrusions of the inner bark fit into the deeper pits and fissures in the wood cylinder. The pitting is limited to the bodystock and stops abruptly at the union of bodystock and apple variety. Severely affected trees are considerably dwarfed when compared to trees with unaffected bodystocks.

Generally, symptoms are not expressed on the varietal portion of a tree when the bodystock shows evidence of Malus stem pitting virus infection. However, the McIntosh variety has broad longitudinal cracks in the bark, the centers of which do not extend to the wood but are covered with bark. This bark symptom resembles one of the symptoms of the disorder caused by the apple flat limb virus (15). No extensive sinewy growth has been observed. The Malus stem pitting virus has been transmitted (15, 57, 88, 118, 119).

<u>Discussion</u>: Considerable work has been done with the rootstock selection Virginia Crab. It has been used extensively as a rootstock and bodystock because it possesses the qualities of cold-hardiness, vigorous growth, wooly aphis resistance etc. Early success with the Virginia Crab bodystock caused it to receive much praise and wide dissemination, particularly in the Midwest (78, 79).

Uncongeniality between Virginia Crab and the Blaxtaman apple variety was first reported in 1937 by McClintock (81) although Lantz in 1933 (71) had mentioned this possibility. Since then many varieties have shown uncongeniality which is characterized by lack of graft or bud "take" and poor growth of young trees.

Smith, in 1954 (118) described a stem pitting disorder which befell mature trees on Virginia Crab as well as those on

Florence Crab, and suggested the possibility of a virus disorder. Tukey, later in 1954 (127), and Miller, in 1954 (85), offered additional evidence of the disorder in Virginia Crab and also suggested that it was of virus nature. In 1954 Millikan and Guengerich (87) reported the disorder as a possible virus disease calling attention to the bark splitting aspects. In 1956 (88) they reported transmission to Amelanchier of an agent taken from affected Virginia Crab, and later in the year published experimental evidence indicating the virus-like nature of the stem pitting factor in apple (50).

Hilborn and Hyland, in 1957 (57), reported the following anatomical changes occurring in the cambial area:

> Some cambial initials become multinucleate, and the nuclei become distorted. Some cambial derivatives become abnormal, resulting in disorientation of xylem elements and phloem rays, large parenchyma islands in the xylem, wide xylem rays, degeneration of sieve tubes, and absence of sieve areas and companion cells.

The disorder occurring on Virginia Crab has been referred to as stem pitting, wood pitting and tristeza. Smith used the phrase "stem pitting" in his description and borrowed it directly from the citrus disease Citrus quick decline or tristeza, which causes the same type of disorder with the same pitting symptoms on certain sweet orange and sour orange rootstock combinations (117). The "stem pitting" is adequately descriptive for both types of plants, but since 2 virus entities are probably involved it would be appropriate to give each its own name. In addition there is a stem pitting disorder of arabica coffee trees (111) in Tanganyika, whose symptoms are also like those above. Since the term "stem pitting" is commonly used to best describe the disorder it is suggested that the generic name be used to distinguish between these and future disorders of a similar nature. Thus the disease treated here would be Malus stem pitting and the one on coffee Coffea stem pitting.

The disorder caused by the Malus stem pitting virus resembles no other known apple virus disease except that caused by the apple flat limb virus. Remarks in the literature tend to indicate that there are possible strains of the apple flat limb virus varying in severity much the same as with the apple mosaic virus. Since pitting occurs in both disorders and varietial bark symptoms similar to those on Gravenstein infected with the flat limb virus have been noted in one infected variety, the Malus stem pitting virus may be a strain of the apple flat limb virus.

Geographical Distribution: Canada (130), United States (138).

Varieties: Virginia Crab, Florence Crab, Red River Crab.

Name: USDA 227 Virus

Disease Name: Lethal Effect, Incompatibility, Spy 227 Disorder

Symptoms: The symptoms of plants which are incompatible on the rootstock selection USDA 227, a Northern Spy seedling, are fair growth during the season after budding, followed in the early fall by premature coloring of the leaves and early defoliation. An inspection of the roots reveals that terminal rootlets have died and that no new root tips have been formed. By the following spring the entire root system may be dead or dying. The aerial portions of these plants leaf out in the spring but soon wither and die or are retarded and die in subsequent years. In some trees, severe stunting results. Somewhat the same symptoms are present in the USDA 227 when it is inoculated with buds from some of these lethal varieties (45, 116, 126).

In the foliage of propagated plants of the clonal selection USDA 227, one may discern a speckled spotting similar to the common ring spot markings found in cherry (*Prunus avium*).

Discussion: Several workers have reported the presence of incompatibility between some selections of apple varieties and the clonally propagated USDA 227 (45, 116, 126, 133). Some effort has been made to explain the incompatibility by a genetic variation between apple varieties and their selected sports. However, two different selections of the same variety showed compatibility and incompatibility without any appreciable difference in varietal variation. The experiments performed by Gardner, Marth and Magness (45) indicate that the USDA 227 rootstock is susceptible to lethal entities carried in scionwood of apple varieties. In several instances a single bud inserted into the USDA 227 rootstock caused the death of the plant. Gardner, Marth and Magness proposed that the lethal entity is a virus and that it is present in the buds which are placed into the USDA 227 rootstock. They further postulated that the lethal entity (virus) may be present in some trees selected for scionwood sources and not in others. The observation that of the many USDA selections tested, only USDA 227 and some clones of its progeny show this incompatibility, suggested that a "second factor, perhaps a metabolite developed by certain varieties and not by others, is necessary for the lethal activity of the virus" (45).

Recent work at the University of New Hampshire (16) has shown that this "second factor" is a virus perpetually present in the USDA 227 rootstock clone, and it expresses itself in the leaf by speckled, translucent spots. This entity has been transmitted to McIntosh seedlings by bud inoculation which later resulted in leaf symptoms. It also caused a reaction on the leaves of the indexing hosts _M. floribunda_ and _M. brevipes_ when buds from propagated trees of USDA 227 were inoculated into them.

From the evidence presented in previous papers it would seem that the virus present in USDA 227 has been with it since it was first selected in 1923 (45). It may also explain the incompatibility of some of its progeny, the virus having been seed transmitted to them.

Geographical Distribution: United States (45).

Variety: USDA 227.

Varieties of Some Clonal Selections Containing The Partner Virus Which is Lethal to USDA 227: Rome Beauty, Blackjon, Yellow Transparent, Jonathan, Golden Delicious, Winesap, Delicious, McIntosh, Northern Spy, Baldwin.

Virus Symptoms Expressed in Fruit

Name: Apple False Sting Virus Hockey 1943

Disease Name: Apple False Sting (See Green Crinkle Virus).

Symptoms: The apple false sting virus causes the fruit of infected trees to be stunted, considerably misshapen and distorted. No foliage or bark symptoms have been recorded. The depressed areas or furrows are formed on the fruit surface and seem to radiate from either the stem or calyx end. These furrows may transverse the apple from stem to calyx, may unite with other furrows or discontinue half way over the fruit. On the more pointed varieties the calyx end becomes quite knurled and misshapen. In the early stages of fruit development the disorder resembles stings caused by insects. Russetting may accompany this disorder in meager proportions, sometimes occurring on the surface of the fruit but more often associated with a pit or furrow. Corky areas develop internally beneath the pits or furrows. The causal agent of false sting has been transmitted (59, 61).

Discussion: Apple false sting was first described by Hockey in 1941 and in 1943 he reported the transmission of the virus entity. The disorder has since been observed in other parts of the world.

The disease first appears two or three weeks after full bloom as small depressed areas which resemble the injury which follows some insect punctures. As the fruit matures these depressed areas turn into sharp fissures on the more severely affected varieties and may develop cracks or russet within the furrows. There is no indication of insect transmission, and the spread which does occur is by natural root graft or by grafting techniques. In the latter case a relatively high percentage of transmission occurs.

Hockey has noted that

> a microscopic examination of diseased apples reveals that the normal vascular pattern is disturbed. Main vascular bundles from the core area extend outward to the hypodermal cells adjacent to a depressed area and return to continue their normal path in the tissue. This abnormal vascular pattern is accompanied by a radial elongation of cells in the cortical region below the depressed tissue and adjacent to the distorted vascular bundles.

Apple false sting resembles the apple crinkle virus disease of New Zealand but differs from it in that apple crinkle has 2 fruit symptoms rather than 1. No doubt, these 2 disorders are related possibly with some additional entity present in the apple green crinkle. The disorder has been observed in New Hampshire on the Baldwin variety.

The reviews by Hockey are well illustrated.

Geographical Distribution: Canada (59), Denmark (70), England (73), Holland (70), Norway (70), Nova Scotia (59), United States (15).

Varieties: Gravenstein, Baldwin, Northern Spy, McIntosh, Ben Davis, Golden Delicious, Fuhr, Blenheim Orange Pippin, King of Tompkins, Tolman Sweet, Guldborg.

Name: Apple Green Crinkle Virus Atkinson and Robbins 1951. (Pyrus virus 4 (Smith) van Katwijk)

Disease Name: Apple Green Crinkle (See Apple False Sting)

Symptoms: The symptoms caused by the green crinkle virus are very much like those of apple false sting. Deep fissures or furrows ramify over the surface of the apples causing them to be considerably misshapen and distorted. The disorder may involve fruits on one limb or the whole tree. As with the apple false sting virus disease which shows similar fruit symptoms the apples are stunted and commercially useless. Additional symptoms of the green crinkle disorder include wart-like swellings on the surface of the fruit which are often covered with a rough russet. These swellings may occur on the same tree as the false sting symptoms in the same or different fruits. Also, the 2 types of symptoms may alternate from year to year in the same tree. These wart-like swellings appear with less regularity than the other symptom and do not appreciably reduce the size of the fruit. No foliage or bark symptoms have been noted. Apple green crinkle virus has been transmitted (11, 12).

Discussion: In 1932 Thomas and Raphael (125) reported the occurrence of this disorder under the supposition that it was a physiological disease and associated it with "internal cork and malformed wood growths". The malformed wood growths apparently were of some other nature since Atkinson in 1956 (12) indicated there were no bark or leaf symptoms. McAlpin regarded apple green crinkle as a "confluent form of bitter pit", the latter considered to be a physiological disorder. Atkinson as late as 1947 still considered it to be physiological in origin. However, in 1951 Atkinson and Robbins reported experimental transmission of the virus entity.

Apple green crinkle occurs principally in New Zealand on the variety Granny Smith. Little if any natural spread occurs. No insect vectors are known.

Geographical Distribution: Australia (125), New Zealand (11).

Varieties: Sturmer Pippin, Granny Smith, Winesap, Rome Beauty.

Name: Apple Chat Fruit Virus Luckwill and Crowdy 1950

Disease Name: Chat Fruit

Symptoms: Chat fruit affected trees are characterized by the upright growth of the tree in contrast to the normal spreading habit of healthy trees. All the fruits on infected trees are dwarfed, being about 1/4 the size of normal apples. The fruits remain small and green at maturity and the exposed side is dull brownish-red. The pedicels of the fruit are somewhat elongated, permitting the fruit to swing. The fruit falls off prematurely. The virus entity has been transmitted (75).

Discussion: Apple chat fruit is a disorder most often found in the Lord Lambourne variety and occurs rather often in trees which are also affected with rubbery wood. Apple chat fruit apparently resembles no other virus disorder previously described except that of apple dwarf fruit and decline described by Cation (29). It has recently been said to occur on the Turley variety in Missouri (96). Transmission experiments have shown the virus entity to affect trees of the Lord Lambourne and Jonathan varieties. The Lord Lambourne variety has been used as an indicator host for the presence of the chat fruit virus. In the clonal rootstock beds at Long Ashton, England the M.IV clone was found to be 22 per cent infected, the M.XVI and M.XII clones showed 6 and 4 per cent infection respectively, while the M.I, M.II, M.VII and M.IX clones were free of the disorder (74).

Geographical Distribution: England (75), Switzerland (8), United States (96).

Varieties: Lord Lambourne, Robert Grieve, Jonathan, Turley, Golden Delicious.

Name: Apple Dwarf Fruit and Decline Virus Cation and Gibson 1952

Disease Name: Dwarf Fruit and Decline

Symptoms: Infected fruits of the Jonathan variety retain their normal shape but are reduced in size. The fruit of the Hyslop Crab is dwarfed and deeply lobed or prominently five ribbed longitudinally. The calyx end is characteristically oblate with a very shallow basin. The tree declines (i.e. probably a slow wilting with dieback) until it becomes dead or worthless at the end of 5 or 6 years (29).

Discussion: The expression of this disorder originated when the apple variety Jonathan was grafted on to Hyslop Crab where this latter plant was apparently being used as an interpiece. It is suggested that the virus was located in the Jonathan scions and when transmitted to the Hyslop Crab by grafting caused the decline.

The reduction in Jonathan fruit size may be explained by the interference in movement of the nutrient supply to the variety by the decline effects of the virus in the Hyslop Crab through which the nutrients must come. Another possible explanation is the presence of an unknown latent virus in the Hyslop Crab interacting with the virus in the Jonathan and causing size reduction in both varieties.

The decline symptom on Hyslop Crab is not so severe when the inoculum is from an infected Hyslop Crab as when it is from the infected Jonathan although the fruit symptoms are the same.

Geographical Distribution: Michigan (29).

Varieties: Jonathan, Hyslop Crab.

Name: Apple Scar Skin Virus Millikan and Martin 1955

Disease Name: Scar skin (probably synonymous with rough skin of van Katwijk)

Symptoms: Apple scar skin causes a corky scarring on the fruit skin in linear patterns or patches extending from the stem to the apex. Patches may be small and irregular, coalesced or affecting the whole of one side. Star-shaped radial scarring generally spreads from the apex but occasionally from the stem end. Fruit size is reduced, and ripening is considerably retarded. Apparently no foliage symptoms are present. Transmission of the causal agent of this disorder has not been reported (84, 86).

Discussion: Further study of the developmental sequence of apple scar skin showed that the disorder starts in fruits 1/2 inch in diameter with light, water-soaked blemishes radiating from the calyx end. Scar tissue begins to develop on these water-soaked areas when the fruit is 3/4 inch in diameter, and irregular patches of scar tissue become present on the fruit when it is 1 inch or over. By harvest time the scar tissue may cover 50 per cent of the fruit surface.

This disease resembles apple rough skin, described by van Katwijk (67), and the apple ring spot, described by Atkinson, Chamberlain and Hunter (14). There is little if any difference to be noted between the descriptions for the disorders caused by apple scar skin virus and apple rough skin virus. The same type of disorder occurs in apple ring spot with the exception that circular radiating concentric patterns of corky tissue occur on the skin of fruit affected with apple ring spot. These concentric patterns occur in a rather low percentage of affected fruits. Except for them the descriptions of the disorders would be practically the same.

It has been suggested that there is a similarity between the disorder caused by the apple star cracking virus and scar skin and rough skin symptoms. However, there seems to be a lack of association between these 2 types of disorders. The star-shaped markings of scar skin are composed of corky periderm which radiates from either the stem end or the calyx end. Scar skin apparently does not form actual cracks in the fruit. The star cracking disorder is typified by actual star-shaped cracks extending into the flesh of the apple, and depth of penetration apparently is determined by the size of the crack. Also, in the description of star cracking there is no russetting mentioned. No russetting is discernible in the photographs. This tends to indicate that these two types of disorders are caused by somewhat separate entities.

This disorder occurs in the Delicious apple variety when a Virginia Crab interpiece is used, thus further associating the Virginia Crab with virus disorders of apple trees.

Geographical Distribution: Missouri (84).

Varieties: Delicious with an interpiece of Virginia Crab.

Name: Apple Rough Skin Virus van Katwijk 1955
(Pyrus virus 8 (Smith) van Katwijk)

Disease Name: Rough Skin (Probably identical with
Scar Skin of Millikan and Martin)

Symptoms: Apple rough skin virus causes rough, corky brown patches to develop on both the green and colored parts of the apple fruit skin. The smaller patches have a circular shape or may appear in the form of rings or elongated stripes. Band-shaped corky areas may extend from the calyx to the point of pedicel attachment and may be accompanied by rings and lines or by isolated corky spots. Severely affected fruits may have a large part of the fruit surface covered with russetting. In some cases the rough brown patches are cracked, and the fruits may show a slight deformation due to local growth retardation. The rough skin symptoms can be found soon after the first development of the fruit. The cork is formed on the surface after the death of the epidermal cells. A local vein-clearing has been observed on the terminal leaves of the shoots of some varieties. Fruit size is reduced. Transmission of the virus entity was not mentioned (41, 67, 72, 92).

Discussion: The disorder caused by the apple rough skin virus causes considerable losses in the Netherlands (67, 92). Because of the markings and the reduction in the size of the fruit all affected apples are either discarded or are sold in the lowest commercial grade.

The resemblance between apple scar skin, apple rough skin, and apple ring spot has been mentioned under the discussion of apple scar skin virus. Apple rough skin does not resemble the disorder caused by the green crinkle virus.

Geographical Distribution: Denmark (70), France (68), Germany (24), Holland (92), Switzerland (41), Union of South Africa (72), United States (96).

Varieties: Beauty of Boskoop, Glory of Holland, Gravenstein, Golden Delicious, Jonathan, Laxton's Superb, Notaris, Reinette de Champagne, Ontario, Glockenapfel, Baldwin, Canada Pippin, Clocke, Henimuri.

Name: Apple Ring Spot Virus Atkinson, Chamberlain and Hunter 1954

Disease Name: Apple Ring Spot, Henderson Spot, Thumbmark

Symptoms: In mature fruits the symptoms appear as patches of russetted tissue edged with a smooth dark brown band, and occasionally as partial or complete concentric dark brown rings. The patches may be large or small, individual or coalesced. The rough russetted areas may extend from the point of stem attachment to the calyx. The concentric rings are much like a target, its size dependent on the number of rings. They may abut one another but apparently do not overlap. Apple ring spot symptoms may vary in intensity from year to year. The apple ring spot virus has been transmitted (5, 14).

Discussion: The first signs of the disease appear in the early part of the season when the apple is about 3 cm or 1 1/4 inches in diameter. Faint, light brown areas show through the downy covering in these small fruits. These markings develop into irregular shaped patches of varying shades of brown, with a rough russetted surface and a scaly margin. Shortly before harvest a narrow band of smooth, dark brown tissue forms around the margin of many spots, and these markings are considered to be a diagnostic feature. Some development of these marginal tissues has been noted after harvest. The disorder has been transmitted only in the Granny Smith variety. Similar spots on other varieties have been noted.

The disorder caused by the apple ring spot virus resembles very closely the other disorders of rough skin, and scar skin (see Apple Scar Skin Virus). All of these disorders are typified by a russetting of the epidermis in which a corky periderm is formed.

Geographical Distribution: New Zealand (5, 14).

Varieties: Granny Smith, Cox's Orange Pippin, Delicious, Sturmer.

Name: Apple Star Cracking Virus Jenkins and Storey 1955

Disease Name: Star Cracking of Apples

Symptoms: The mature fruits show very characteristic star-shaped cracks which tend to be concentrated at the calyx end of the fruit. Star cracks which occur on the sides of the fruits are considerably larger and deeper than those which occur near the calyx end. The star cracks may be small or large, individual or coalesced. More severely affected fruits are distorted. Dieback of terminal shoots during the winter is another symptom which appears. Cankers form along the dead portion of the shoot, girdling and killing the terminal portion. Associated with this dieback is the production of buds which apparently arise adventitiously. The shoots which develop give an unusual proliferate type of growth. Lesions are present on 1-year-old stems and may persist for several years (14, 63).

Discussion: The star cracks which appear on the surface of the fruits are apparently not associated with any type of russetting or corkiness. It has been explained that the lesions on the young stems start as blister-like formations and develop around the buds and nodes. These affected areas are sharply delineated, and the blistered bark dissappears in time. The ornamental plant, Bechtel's Flowering Crab, has a bark and canker symptom very similar to that described for apple star cracking. To the writer's knowledge, this disorder has not been described or reported in the literature on this variety.

The lack of extensive russetting, the presence of die-back on the vigorous terminal shoots, and the lesions on the stems distinguish this disorder from scar skin, rough bark and apple ring spot. An attempt to associate this disorder with the 3 fruit-russetting types of disorders has been made through the occurrence of the cracks, but the star-crackings are distincitively shaped whereas those with the other disorders have no regular shape.

It is thought that there might be some association between the star cracking and witch's broom disorders since there is a proliferation of branches in both diseases. However, the dieback which occurs in the witch's broom type of disorder takes place so that the terminal portion of the shoot dies by the end of the summer. In star cracking the cankers are formed and death of the shoot takes place during the winter. Also, no fruit symptoms were considered to be worthy of note in the witch's broom disease. The lack of enlarged stipules in star cracking also separates these two disorders. There seem to be too many disputable points for a very close relationship between star cracking and witch's broom.

Geographical Distribution: England (63), Norway (105).

Varieties: Cox's Orange Pippin, Early Victoria, Charles Ross, Laxton's Fortune, Monarch, Bramley.

Name: Dapple Apple Virus Smith, Barrat and Rich 1956

Disease Name: Dapple Apple

Symptoms: The symptoms of dapple apple are restricted to the fruit. At maturity more or less circular patches of the skin remain greenish and interrupt the natural coloration of the fruit. These patches may appear at any place on the surface but tend to concentrate near the calyx end. They may occur individually, but often coalesce to form a larger discolored area. The surface area involved by these patches is slightly flattened, giving the mature fruit a slightly pebbled appearance. The bloom over these areas is reduced. Fruit size is normal. The virus entity causing the disorder has been transmitted to the varieties Cortland, McIntosh, Starking Delicious and Golden Delicious (120).

Discussion: The dapple apple virus causes symptoms to appear on the fruits of affected trees every year, but not always with the same intensity. Some years the discolored spots are quite evident several weeks before harvest, while in other years they are less pronounced and, at times, may be lacking. Generally all the fruit on a tree is affected.

The first sign of the disorder is in mid-July when small circular spots are distinguishable from the yellowish-green color of the skin. These spots enlarge as the fruit matures and do not develop a normal amount of red pigmentation. The spots become more intense the longer the apples are left on the tree. This is due to the greater development and darkening of the red color in normal areas. The greenish color of the dappled areas fades to yellow in storage (15, 120).

The disorder is manifested when the Cortland variety is topworked to Virginia Crab interpieces. In several instances the wood pitting disorder of the Virginia Crab occurs on the same trees as dapple apple but there is evidence that these two disorders occur independently of each other. Additional information concerning the dapple apple disorder will be treated under Materials and Methods.

Geographical Distribution: New Hampshire (120), Massachusetts (109), Missouri (?) (96).

Varieties: Cortland, McIntosh, Starking Delicious, Golden Delicious, Turley (?), Virginia Crab.

Name: Apple Green Mottle Virus Palmiter and Parker 1955

Disease Name: Green Mottle

Symptoms: Discolored rings cover the surface of affected fruits, apparently causing a green mottling type of pattern. No further description or photographs are available in the literature (93).

Discussion: This disease is of relatively minor importance in New York. It has been transmitted to the Duchess variety. It is apparently rather slow moving in the tree and into the fruits. This disorder may be similar to dapple apple.

Geographical Distribution: New York (93).

Varieties: Duchess of Oldenburg.

SECTION III

MATERIALS AND METHODS

1. Experimental Basis of Orchard

In an effort to counteract injury and death to apple trees due to winter injury, a study was undertaken by Dr. W. W. Smith, Horticulturist, New Hampshire Agricultural Experiment Station, at Gilford, New Hampshire, to determine the desirability of using bodystocks in areas of the framework of the trees which are most susceptible to winter injury (118). The severe winter of 1933-34 indicated that the areas of the trees most susceptible to injury were the trunks and crotches of the major branches. McIntosh, in particular, although considered to be rather hardy, showed considerable injury to the trunks, while many Baldwin trees were killed.

Other investigators (106, 124) showed that the greater part of the injury was caused by killing a portion of the cambium or injuring it in such a manner that it failed to differentiate. This type of injury, known as "frost rings", prevents the tree from developing properly in the injured areas. Other symptoms of freezing injury are bark splitting and peeling which result in exposing the wood of the tree at the cambial area. These areas dry out, and the cambium is killed. If left unattended the affected areas remain exposed throughout the life of the tree offering avenues for fungal and bacterial agents of disease. Injury of this nature to the tree causes considerable retardation in growth, weaknesses at the site of injury and misshapen form.

The purpose of incorporating a bodystock between the roots and the varietal limbs is to reduce the prospect of winter injury to a minimum. Of the several hardy stocks available the most commonly used is the selection Virginia Crab. Another variety selected for comparison was the Florence Crab which had proven its winter hardiness in North Dakota. The Virginia Crab has been used extensively in the Midwest as an understock as well as an interpiece. In addition, seedling roots and Malling IV rootstock were used for comparison. The varieties used were McIntosh (Rogers strain, J-2 and B. F. 224, U.N.H. selections), Northern Spy (B. F. 52, a U.N.H. selection), Red Spy (Farley strain) and Cortland.

The source of these varieties warrants some consideration. The Rogers strain of McInotsh and the Farley strain of Red Spy were purchased from nurseries and were on seedling roots. The varieties McIntosh, Northern Spy and Cortland were propagated on M.IV and seedlings on the farm near the orchard.

Two trees, J-2 and B. F. 244, at the University of New Hampshire orchard, were used as scionwood sources for the McIntosh variety. One tree, B. F. 52, was used as the scionwood source for the Northern Spy, and one tree, also in the University orchard was used as a scionwood source for the variety Cortland. At the time of planting, those varieties on seedling roots and M.IV had either been purchased from a nursery or had been propagated at the farm.

The Virginia Crab and Florence Crab trees were purchased as 2-year-old stock and planted in the orchard. These plants, once established, were whip-grafted on the scaffold limbs to the varieties McIntosh (J-2 and B. F. 224), Northern Spy (B. F. 52) and Cortland (from 1 tree). Since the disorders under discussion in this study are concerned with the variety Cortland, an important point is that the scionwood for this variety came from 1 tree.

The general method for the propagation of fruit trees should be mentioned. Apple fruit trees are started from the seeds of the apple, which upon planting germinate and develop into seedlings. Toward the end of the growing year, but while each seedling is still actively growing, a bud from a selected variety, such as Cortland, is cut out of the stem from a branch of the present year's growth. A T-slit is made in the bark of the seedling and the bud inserted into this slit so that the 2 cambial areas are flush against each other. The slit is usually made near the soil line or near the crown on the aerial portion of the seedling. This bud is tied or wrapped securely to the seedling, and in time, usually a matter of 2 weeks or so, callus tissue forms from the inserted bud and from the seedling. These unite and form a firm attachment between the bud and the seedling. In the spring of the following year the aerial portion of the seedling above the bud is cut off, and and this forces the bud to grow. The whole root system which previously fed the aerial portion of the seedling now feeds only the bud; because of this large food supply the shoot from the bud grows rapidly. At the end of this season the varietal shoot on the seedling root has grown several feet, and the tree is ready for transplanting into an orchard.

In some instances the seedling may have some select qualities such as wooly aphis resistance, good root system, straight growth, cold hardiness, or vigorous growth. It may be desired to continue this particular plant so that an orchard of trees would be more uniform and have the select qualities in the root which it has demonstrated. In such cases the seedling is then clonally propagated, usually by layering, rooting of cuttings (76) or in the manner described above. Both Virginia Crab and Florence Crab are propagated by the budding method, and rooting of the bodystock is promoted by deep planting.

Budding and grafting have been mentioned, and it should be pointed out that they differ in a few respects. Budding is usually limited to periods when the plant is in a stage of active growth so that the bud may be easily slipped into the T-slit in the bark; grafting is usually done when the plant is dormant. It is possible, however, to do either one at any time by proper manipulation. Budding involves the use of 1 bud plus a short piece of the stem as it is cut out of the branch. A graft is a section of the branch which may contain 2, 3 or more buds per piece and contains considerably more plant tissue than a bud.

When considering viruses this is important. It is known that in indexing for stone-fruit viruses the use of 3 or 4 buds is much more reliable than the use of 1 bud. This is due to the distribution of the virus within the plant from which the buds are taken. Virus distribution is not always uniform throughout its host, and the amount of plant tissue involved in a bud is small. A bud piece may not contain any of a virus erratically distributed in a plant. However, the chances of missing a virus portion in a bud are not great. A graft, on the other hand, involves a piece of branch which may be several inches long and stands a much greater chance of having the virus present in the portion of the plant tissue used. In virus transmission or propagation, grafts are more reliable than buds, and buds are considered more reliable, per unit, than any other method known. The standard for virus transmission, however, is with buds.

The experimental part of the orchard, see Figure 1, was designed to include 10 rows with 30 trees in each row. Each of the 4 groups of understocks was replicated 15 times within the 10 rows. There were 6 plots of 5 trees each per row. Nine of the 10 rows were designed so that 2 rows of each variety were adjacent to each other, and the single rows of each variety were not adjacent to a row of the same variety. Red Spy and Northern Spy were included in the same rows. The first row consisted of 10 trees of Cortland followed by 10 trees of McIntosh and 10 trees of Red Spy. The rootstocks of this row were planned with the regular series across the orchard.

During the years some of the trees died and were replaced with other varieties and rootstocks not in sequence with the original plan. By 1957, 71 of the 300 original trees had been replaced.

Tree Number		D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
										Row										
1							V	V	V	F	F	V	V	V						
2						V	V	V	V	V	V	4	3	5	4	5	4	1	3	4
3					V	V	V	V	V	V	V		3	5	4	4	4	4	4	4
4					V	V	V	V	V	V	V	4	5	5	4	5	5	5	5	5
5				V	V	V	V	V	V	V	V	4	3	10	4	4	5	16	16	16
6			4	4	4	4	4	4	4	V	4	4	3	10	4	4	4	1	3	4
7		4	V	4	V	4	4	V	4	V	4	4	1	5	4	4	4	1	4	4
8	C	4	V	4	V	4	4	V	4	V	4	4	1	5	1	5	5	5	5	4
9	o	4	V	4	V	4	4	V	4	V	4	4	1	1	1	1	5	16	16	16
10	r	4	V	4	V	4	4	V	4	V	4	4	1	1	1	1	4	1	3	3
11	t	4	V	4	V	4	4	V	4	V	4	4	1	1	1	1	4	1	4	4
12	l	S	F	S	F	S	S	F	S	F	S	4	1	1	1	1	4	16	5	16
13	a	S	F	S	F	S	S	F	S	F	S	4	1	1	1	1	4	16	4	4
14	n	S	F	S	F	S	S	F	S	F	S	4	1	1		1	4	1	4	4
15	d	S	F	S	F	S	S	F	S	F	S	4	1	V	V	1	4	1	4	4
16	—	S	F	S	F	S	S	F	S	F	S		1	V	V	1	4	1	4	4
17		V	4	V	4	V	V	4	V	4	F		1				4	4	4	4
18	M	V	4	V	4	V	V	4	V	4	V		1				4	16	4	4
19	c	V	4	V	4	V	V	4	V	4	V		1							
20	I	V	4	V	4	V	V	4	V	4	V		1							
21	n	V	4	V	4	V	V	4	V	4	V		1							
22	t	F	S	F	S	F	F	S	F	S	F		4							
23	o	F	S	F	S	F	F	S	F	S	F		4				Slough			
24	s	F	S	F	S	F	F	S	F	S	F		4							
25	h	F	S	F	S	F	F	S	F	S	F		4	4						
26	—	F	S	F	S	F	F	S	F	S	F		4	4						
27		V	V	4	V	4	4	V	4	V	4		4	4	F					
28	R	V	V	4	V	4	4	V	4	V	4		4	4	F					
29	e	V	V	4	V	4	4	V	4	V	4		4	4	F					
30	d	V	V	4	V	4	4	V	4	V	4		4	4	F	V				
31		V	V	4	V	4	4	V	4	V	4		4	4	F	V				
32	S	F	F	S	F	S	S	F	S	F	S		4	4	F	V	V			
33	p	F	F	S	F	S	S	F	S	F	S		4	4	F	V	V	V		V
34	y	F	F	S	F	S	S	F	S	F	S		4	4	F	V	V	V	V	V
35		F	F	S	F	S	S	F	S	F	S		4	4	F	V	V	V	V	V
36		F	F	S	F	S	S	F	S	F	S		4	4	F	V	V	V	V	V
37		Mixed Row	Cortland Row	Spy Row	McIntosh Row	McIntosh Row	Cortland Row	Cortland Row	Spy Row	Spy Row	McIntosh Row		4	4	F	V	V	V	V	V

Legend

F-Florence Crab
S-Seedling (*Malus pumila*)
V-Virginia Crab
1-M.I
3-M.III
4-M.IV
5-M.V
10-M.X
16-M.XVI

Figure 1. Rootstock, Interpiece and Varietal Plan of Experimental Portion of Orchard.

A. Orchard Occurrence of Malus Stem Pitting. Despite the fact that trees infected with the Malus stem pitting virus have a roughened bark surface, this symptom alone is not sufficient to determine the presence of the disorder. It is necessary to cut through the bark and to inspect the wood at the surface of the xylem in order to be certain whether or not the pitting symptom is present.

This was done by selecting a portion of the trunk stock either at the union of the variety and bodystock or at a rough area on the trunk. With the aid of a sharp knife an inverted V-shapedcut was made. The sides of the V were cut about 2 to 4 inches long and the bark pried away from the wood at the apex. By bending the flap back one was able to see the vertical surface of the xylem at the cambial area as well as the inner phloem. Pitting was readily seen when present. After inspection the apex of the bark was fitted back into the crevice and the flap nailed to the tree.

In the experimental block all Virginia Crab and Florence Crab bodystocks were examined in the spring of 1957 as described above, to determine the presence of pitting. Trunks displaying a rough bark condition plus many other trees of all stocks were examined to determine the presence of pitting. No pitting was ever seen in M.IV or seedling roots.

In addition all Virginia and Florence Crab trees outside of the experimental block at the head of the rows were examined. These trunk portions as well as those in the experimental block showed pitting. A few trees of Virginia Crab which had not been topworked were also present in the orchard.

These were inspected but showed no symptoms of pitting.

There were 71 trees in the experimental block in the spring of 1956 with Virginia Crab bodystocks. Forty-three trees or 60.5 per cent were infected with the Malus stem pitting virus and expressed symptoms. Forty trees remained of the original 75 trees with Florence Crab as an interpiece, and 8 of these had symptoms of stem pitting. Of the 5 Red River Crabs present in the experimental block 4 showed stem pitting symptoms. In fact, all the top-worked Red River Crabs in the orchard showed infection and 1 (M-28) showed symptoms similar to apple flat limb. See Figure 2 for the occurrence and distribution of the stem pitting disorder.

B. <u>Orchard Occurrence of Dapple Apple</u>. Dapple apple was first noted about 1951 when a few boxes of fruit showing the symptoms were observed in the packing shed. During the following years some tree records were kept as the fruit was picked, and several affected trees were located. In 1954 a general survey was made. At this time 11 affected trees were located in the experimental block and a few trees outside of the block were also noted. All trees except 2 were on Virginia Crab bodystocks, and these 2 were replacement trees on Robusta V bodystock. The scionwood for these trees is suspected to have come from an infected tree. In addition all trees affected were the Cortland variety except 3 McIntosh trees outside of the experimental block. Again, it is suspected that these trees may have had some Cortland budwood grafted into them or that they were originally Cortland trees grafted over to McIntosh.

Tree Number	Row D	E	F	G	H	I	J	K	L	M
7	CRA[1]	CV	Y4	MVP	M4	C4	CVA	Y4	YV	M4
8	C4	CVP	Y7	MS	M4	C4	CV	YV	YV	M4
9	CR	CV	Y4	MVPA	M4	C4	CVA	Y7	YV	M4
10	CR	CVP	Y7	MVP	M4	C7	CV	Y4	YVP	M4
11	CR	CVA	Y4	MVP	M4	C4	CVA	Y4	YVP	M4
12	CDA	CF	YS	MF	MSA	CS	CF	YS	YF	MS
13	CS	CFPA	YS	M7	MSA	CS	CF	YS	YFP	MS
14	C7	CFP	YS	MF	MS	CS	CF	YS	Y7	MS
15	CS	CF	YS	MR	MS	CS	CF	YS	YF	MS
16	C7	CR	YS	MR	MS	CS	CF	YS	YF	MS
17	MVP	C4	YVP	M7	MVP	CVPA	C4	YVP	YR	MVP
18	M7	C4	YV	M4	MVP	CVPA	C4	YVP	Y7	MVP
19	MVP	CRA	YVP	M4	MVP	CV	C4	YVP	Y4	MVP
20	MR	C4	YVP	M4	MVP	CVA	C7	YVP	Y4	MVP
21	MKA	C4	YV	M4	MVP	CVP	C4	YV	YR	MVP
22	MK	CS	YF	MS	M7	CFPA	CS	YF	YS	MDP
23	MK	CS	YF	MS	MR	CFPA	CS	YFP	YS	MK
24	MR	CS	Y7	MDP	MR	CF	CS	YR	YS	MF
25	MS	CS	YF	MS	MV	C7	CS	YK	YS	M7
26	MVP	CS	YF	MS	MK	CF	CS	YR	YS	MDP
27	YVP	CV	Y7	MV	M4	C4	CVP	Y7	YVP	M4
28	YV	CR	Y4	MV	M7	C4	CVP	Y7	YV	MDP
29	YV	CVPA	Y4	MV	MK	C4	CVP	Y4	YV	M7
30	YVP	CVPA	Y7	MV	MR	C4	CVPA	Y4	YVP	M4
31	YVP	CRA	Y4	MV	M4	C4	CVPA	Y4	Y7	M4
32	YR	CF	YS	MF	MS	CS	CR	YS	YF	MS
33	YFP	CF	YS	MS	MS	CS	CR	YS	YF	MS
34	YR	CF	YS	MFP	MS	CS	CR	YS	YF	MS
35	YS	CF	YS	MR	MS	CS	CR	YS	YF	MS
36	YS	CS	YS	MF	MS	CS	CF	YS	YF	MS

Legend

A- Dapple Apple
C- Cortland
D- Red River Crab
F- Florence Crab
K- *Malus sikkimensis*
M- McIntosh
P- Malus stem pitting
R- (Malus) Robusta V
S- Seedling
V- Virginia Crab
Y- Northern or Red Spy
4- M.IV
7- M.VII

1/ The first letter indicates variety, the second denotes type of bodystock or rootstock, and the remaining letters indicate type of disorder.

Figure 2. Experimental Orchard Indicating Variety, Bodystock or Rootstock and Occurrence and Distribution of Virus-Like Disorders, 1956 and 1957.

In 1956 an additional survey was made, and more trees were located. In 1957 symptoms were not fully developed at harvest, and the detection of infections even on previously recorded Cortland trees was difficult. Optimum growth conditions for symptom expression were apparently lacking during 1957. Several records of new occurrences were made, but these should be verified in succeeding years.

The presence of dapple apple virus infection is detectable only on the fruit of the tree. Inspections are best carried out at the time when fruits are mature for maximum symptom expression.

During the course of the year Cortland trees which were known to have expressed symptoms during previous years were watched carefully for early symptoms. The first indication of dapple apple symptoms was noted about the middle of July on the immature Cortland fruits. At the same time other trees were inspected for similar symptoms. It was found that early symptoms were quite variable from tree to tree.

In 1956 12 trees had symptoms of dapple apple in the experimental block. In addition to these, 10 trees had symptoms in such meager proportions that they were to be checked for symptom expression the following year. In 1957, a poor year for disease expression, 14 trees showed definite symptoms. Two of these were new infections. None of the 10 questionable infections in 1956 was noted as infected in 1957. In the area outside of the experimental block previously mentioned, 5 trees were infected in 1956 and 1957. See Figure 2 for the occurrence and distribution of dapple apple infected trees.

C. Orchard Occurrence of Other Diseases Noted. During the course of taking notes on the Malus stem pitting and dapple apple disorders, 3 other abnormalities were noted in the orchard which were virus-like in nature.

Two trees (K-41, L-42) which had been blown over in the fall of 1956 were inspected, and the rootstock shoots exhibited leaves with apple mosaic symptoms. Upon inspecting the aerial portion which was of the Northern Spy variety, very mild and scarce symptoms of apple mosaic could be found in the leaves of the plant. The rootstocks expressed the symptoms of apple mosaic more clearly than the Northern Spy top. It is believed that the presence of the virus in the rootstock weakened it, and, thus, the trees were more susceptible to separation at the union of rootstock and variety.

Further occurrence of apple mosaic was seen in 1 or 2 trees (F-12, F-14) where the disorder was limited to a few leaves in the center of the tree. The variety in which this occurred was again Northern Spy. The symptoms were extremely mild, but the markings were distinctly those of apple mosaic. In this same row another seedling (F-27) was observed which showed apple mosaic symptoms, but since the varietal portion had been blown over and removed it was not available for inspection.

These occurrences of apple mosaic in 2 widely separated areas of the orchard indicate that the variety Northern Spy, whose scionwood source was from 1 tree (B. F. 52), may be infected with the mild strain of apple mosaic and that the symptoms of this particular form are obscure. Following the observation of the disease in 1 tree, several trees were inspected, but no further symptoms of apple mosaic could be seen. There is the question of chance occurrence of seed transmission of the apple mosaic virus, but there is no evidence that this virus is seed transmitted. Its ability to be seed transmitted has been questioned by Posnette (12). It is not uncommon, however, to overlook the mild strain of apple mosaic in infected trees because of the scarcity of its symptoms. Due to time limitations no further investigation of apple mosaic was made.

Another disorder which may be a genetic variation was the appearance of lobed fruit on 2 McIntosh trees (H-11, H-23). This disorder occurs on 1 branch on H-23 on the east side of the tree. In tree H-11 it occurs also on the east side of the tree but is not limited to 1 branch. The apples on the other parts of the tree are more or less normal and do not exhibit the accentuated 5 lobes that appear on the abnormal fruit. Apple dwarf fruit and decline, described by Cation (29), is the only disorder reviewed in the literature which resembles this abnormality. Since the 2 McIntosh trees were apparently in good vigor in all other respects, and the fruit was not noticeably dwarfed this probably was the result of some genetic variation rather than a virus.

Virginia Crab apples on the tree J-30 show dapple apple symptoms. In addition to these symptoms, there is a severe cracking on some of the fruits which follows the pattern of running from the stem end to the calyx end. The cracks may be restricted to the sides of the fruit. These cracks are lined with a black, corky periderm and offer a sharp contrast to the reddish-yellow skin. Russetting also occurs on numerous Virginia Crab fruits on the same tree. The russetted areas are usually limited to the basin at the stem end and around the calyx. It may appear in conjunction with the cracks, the cracks occurring within the borders of the russet. These symptoms are somewhat similar to the borders around the rough russet spots of apple ring spot, and the cracking is similar to that of apple rough skin and apple scar skin.

A tree (M-28) with a McIntosh variety topworked on to a Red River Crab bodystock showed Malus stem pitting symptoms. In addition, symptoms very similar to those of apple flat limb were also present on the Red River Crab bodystock.

No mention has been made of the presence of the rubbery wood virus disease of apples in the experimental orchard because of the lack of familiarity with this disorder and the dependence of symptom recognition on the qualitative measure of bending. The writer has seen trees with the weeping habit and extreme flexibility in the branches. However, gradations of these symptoms occur in different varieties and only experience with known infections can aid in determining the presence of the disorder in other areas. It is suspected, however, that rubbery wood may be present in the experimental orchard in some of the trees with rootstocks of the Malling series and possibly on other combinations. The forms of some trees in the experimental orchard closely resemble photographs of trees infected with the rubbery wood virus. No particular tests were made concerning this disorder.

2. Orchard Transmission Studies

A. <u>Mature</u> <u>Trees</u>. It has been mentioned that dapple apple symptoms occur on trees of the Cortland variety on Virginia Crab bodystocks. In some cases the Virginia Crab bodystocks showed stem pitting, and others did not. At the time of inoculation in the spring of 1956, it was thought with the meager information available, that it would be better to include the Malus stem pitting virus in the inoculation series rather than to duplicate inoculations with other material and, thus, to use additional trees in the transmission studies. The tree J-30 was selected because it showed severe symptoms of the Malus stem pitting virus on the Virginia Crab bodystock as well as clear cut symptoms of the dapple apple virus on the Cortland variety. Later it was observed that the Virginia Crab apples also showed symptoms of dapple apple. None of the trees inoculated had ever shown symptoms of dapple apple. Some trees with Virginia Crab bodystocks which had not shown symptoms of the Malus stem pitting virus were inoculated.

Furthermore, at the time the symptoms of dapple apple were first observed, there was some discussion as to the effect of the rootstock and its ability to carry and transmit the disorder to the variety. J-30, because of its stunted growth and restricted stempiece, had sent forth many root suckers from which buds could be taken to be used as inoculum. The Virginia Crab bodystock in J-30 had been permitted to grow, and several limbs were available from which to take inoculating material.

The varietal portion of the tree, Cortland, had several limbs from which inoculating material was available. Thus, the source of inoculating buds was the tree J-30 with buds taken from the Cortland variety, Virginia Crab bodystock and seedling shoots. Because of the restricted occurrence of dapple apple in the orchard on the variety Cortland and because McIntosh had shown symptoms of dapple apple these two varieties were chosen for the experiment. In addition, the four rootstocks Virginia Crab, Florence Crab, M.IV and seedlings were chosen to see if they imparted any modification to the virus entity which could be detected by symptom expression. The effect of the stem pitting virus on these rootstocks and bodystocks was also to be observed.

The method of inoculation was by budding, the most common method of inoculating trees. Two limbs, one on the south side and one on the north side of the tree, were chosen for inoculating-bud placement, and 3 or 4 buds were placed on each limb. Each tree was inoculated with buds from only 1 source of J-30 rootstock, Virginia Crab bodystock or Cortland variety. The initial inoculation was done in the spring of 1956. Since symptoms did not develop on any of the trees inoculated and since some of the buds on each of the trees had died, all trees were reinoculated by buds in the fall from the same source and in the same manner. To insure that the virus would be introduced into the trees, 2 grafts of at least 3 or 4 buds were placed in close proximity to each of the 2 limbs used for budding. This grafting was done in the spring of 1957. No further inoculations of these trees were made. A total of 20 trees, all approximately 15 years old, were used for this experiment.

The plan of inoculation was as follows:

Inoculum Source	Tree Number	Type of Tree: Variety / Bodystock / Root
J-30 Cortland variety	E-7	Cortland on Virginia Crab on seedling root
Same	E-9	Same
Same	E-11	Same
Same	E-12	Cortland on Florence Crab on seedling root
Same	E-22	Cortland on seedling root
Same	E-17	Cortland on M.IV rootstock
Same	G-7	McIntosh on Virginia Crab on seedling root
Same	H-12	McIntosh on seedling root
J-30 Virginia Crab Bodystock	J-9	Cortland on Virginia Crab on seedling root
Same	E-13	Cortland on Florence Crab on seedling root
Same	E-24	Cortland on seedling root
Same	E-18	Cortland on M.IV rootstock
Same	G-9	McIntosh on Virginia Crab on seedling root
Same	H-14	McIntosh on seedling root
J-30 Seedling Shoot	J-27	Cortland on Virginia Crab on seedling root
Same	E-14	Cortland on Florence Crab on seedling root
Same	E-26	Cortland on seedling root
Same	E-20	Cortland on M.IV rootstock

Inoculum Source	Tree Number	Type of Tree Variety	Bodystock	Root
Same	G-11	McIntosh on Virginia Crab on seedling root		
Same	H-16	McIntosh on seedling root.		

In addition to the above trees, the following trees were inoculated with 10 buds from the J-30 Cortland variety in the spring of 1956. These buds were scattered over the tree at shoulder height rather than concentrated on any particular branches. No buds were placed on these trees in the fall of 1956. In the spring of 1957 3 grafts from the same source were placed in these trees on random branches.

Inoculum Source	Tree Number	Type of Tree Variety	Bodystock	Root
J-30 Cortland Variety	R-15	McIntosh on M.VII rootstock		
Same	S-15	Macoun on M.IV rootstock		
Same	T-15	Starking Delicious on M.I rootstock		
Same	U-15	Golden Delicious on M.IV rootstock		
Same	P-14	Baldwin on M.I rootstock.		

B. Immature Trees. This portion of the field studies is of long duration because fruit symptoms are required. Normally an apple tree bears no fruit before it is 4 or 5 years old. This period may be shortened by girdling the trunks of the young trees, by inverting a strip of bark on the trunk or even by tying knots in the stems of young shoots (114). In order to encourage fruiting as early as possible the trees used in this experiment were girdled. This was accomplished by making a circumferential cut in the trunk of the trees with the edge of a dull knife in June, 1957, the recommended time for such girdling.

Fruit symptoms are required for the determination of dapple apple virus infection. It is believed by the writer that the cause of dapple apple is not the action of 1 virus but the action of 2 virus entities which are individually latent within the host when by themselves. When they occur together in the same host dapple apple symptoms are expressed. One component of the virus complex is believed to be present in the Cortland variety while the other component is believed to be present in the Virginia Crab bodystock. The explanation for this theory will be found in the Discussion and Conclusions. In order to find out if the virus entity is composed of the action of more than 1 virus the following experiment was undertaken.

The Cortland tree I-12 was chosen as the varietal carrier of the component suspected of being present in the Cortland variety. This tree was chosen because it was on a seedling root, produced fruit without dapple apple symptoms and had thrifty growth.

The other component is suspected of being in the Virginia Crab. It is known only in trees with dapple apple symptoms. There is no known way to separate it from the Cortland component. Therefore, a search had to be made in other Virginia Crab trees which were not topworked to Cortland. The other available varieties were Northern Spy and McIntosh both of which were grafted to Virginia Crab. Several trees were chosen which contained Virginia Crab interpieces and were selected with the intention of avoiding the Malus stem pitting virus, although it probably was not avoided in all cases.

The experiment was designed to include representatives of the 3 elements of J-30: J-5 which shows dapple apple without the stem pitting virus; D-19 which shows the symptoms of the Malus stem pitting virus but no dapple apple; I-12 which has the Cortland component but none of the others. Check trees were also included.

One hundred and twenty trees on seedling roots, 60 Cortland and 60 McIntosh, were purchased from the Kelley Brothers Nursery. In the spring of 1957 30 trees of each variety were planted in available orchard space in Gilford, N.H., and 30 trees of each variety were planted at the lower end of the Plant Pathology orchard at the University of New Hampshire in Durham.

These trees were grafted in the spring of 1957. No fruit was observed in the fall of 1957 on any of the grafted trees. Outlines of the plantings showing tree number, variety and source of inoculum are on the following pages.

Table 1. Nursery Stock Planting at Gilford, New Hampshire, 1957.

Tree Number	Row 1 Variety	Inoculum
1	Cortland	I-12 and G-31
2	Cortland	I-12 and G-30
3	Cortland	I-12 and G-29
4	Cortland	I-12 and G-28
5	Cortland	I-12 and G-27
6	Cortland	I-12 and M-17
7	Cortland	I-12 and K-17
8	Cortland	I-12 and I-4
9	Cortland	I-12
10	Cortland	I-12
11	Cortland	I-12
12	Cortland	check
13	Cortland	check
14	Cortland	check
15	Cortland	check
16	Cortland	check
17	Cortland	J-5 Dapple Apple
18	Cortland	J-5 Dapple Apple
19	Cortland	J-5 Dapple Apple
20	Cortland	J-5 Dapple Apple
21	Cortland	J-30 Seedling
22	Cortland	J-30 Seedling
23	Cortland	J-30 Virginia Crab
24	Cortland	J-30 Virginia Crab
25	Cortland	J-30 Virginia Crab
26	Cortland	J-30 Cortland var.
27	Cortland	J-30 Cortland var.
28	Cortland	J-30 Cortland var.

(Continued on page 62)

(Table 1. continued)

Tree Number	Row 2 Variety	Inoculum
1	McIntosh	I-12 and G-31
2	McIntosh	I-12 and G-30
3	McIntosh	I-12 and G-29
4	McIntosh	I-12 and G-28
5	McIntosh	I-12 and G-27
6	McIntosh	I-12 and M-17
7	McIntosh	I-12 and K-17
8	McIntosh	I-12 and I-4
9	McIntosh	I-12
10	McIntosh	I-12
11	McIntosh	I-12
12	McIntosh	check
13	McIntosh	check
14	McIntosh	check
15	McIntosh	check
16	McIntosh	check
17	McIntosh	J-5 Dapple Apple
18	McIntosh	J-5 Dapple Apple
19	McIntosh	J-5 Dapple Apple
20	McIntosh	J-30 Seedling
21	McIntosh	J-30 Seedling
22	McIntosh	J-30 Seedling
23	McIntosh	J-30 Virginia Crab
24	McIntosh	J-30 Virginia Crab
25	McIntosh	J-30 Virginia Crab
26	McIntosh	J-30 Cortland var.
27	McIntosh	J-30 Cortland var.
28	McIntosh	J-30 Cortland var.
29	McIntosh	J-30 Cortland var.
30	McIntosh	J-30 Cortland var.
31	Cortland	J-30 Cortland var.
32	Cortland	J-30 Cortland var.

Table 2. Nursery Stock Planting at Durham, New Hampshire, 1957.

Tree Number	Variety	Inoculum
	Row 1	
1	McIntosh	J-30
2	McIntosh	J-30
3	McIntosh	D-19 Va. C.
4	McIntosh	D-19 Va. C.
5	McIntosh	J-5
6	McIntosh	J-5
7	McIntosh	I-12 and L-28
8	McIntosh	I-12 and L-6
9	McIntosh	I-12 and L-10
10	McIntosh	I-12 and L-29
11	McIntosh	I-12 and M-6
12	McIntosh	I-12 and L-3
13	McIntosh	I-12 and F-21
14	McIntosh	I-12 and M-21
15	McIntosh	I-12 and L-7
16	McIntosh	I-12 and M-4
17	McIntosh	I-12 and K-21
18	McIntosh	I-12 and L-4
19	McIntosh	I-12 and L-5
	Row 2	
1	Cortland	
2	Cortland	J-30 Cortland
3	Cortland	J-30 Cortland
4	Cortland	D-19 Va. C.
5	Cortland	D-19 Va. C.
6	Cortland	J-5
7	Cortland	J-5
8	Cortland	check
9	Cortland	check
10	Cortland	check
11	Cortland	check
12	Cortland	check
13	McIntosh	check
14	McIntosh	check
15	McIntosh	check
16	McIntosh	check
17	McIntosh	check
18	McIntosh	I-12
19	McIntosh	I-12 and M-5
20	McIntosh	I-12 and L-11

(Continued on page 64)

(Table 2. continued)

Tree Number	Variety	Inoculum
	Row 3	
1	Cortland	I-12 and L-28
2	Cortland	I-12 and L-6
3	Cortland	I-12 and L-10
4	Cortland	I-12 and L-29
5	Cortland	I-12 and M-6
6	Cortland	I-12 and L-3
7	Cortland	I-12 and F-21
8	Cortland	I-12 and M-21
9	Cortland	I-12 and L-7
10	Cortland	I-12 and M-4
11	Cortland	I-12 and M-21
12	Cortland	I-12 and L-4
13	Cortland	I-12 and L-5
14	Cortland	I-12 and L-11
15	Cortland	I-12 and M-5
16	Cortland	I-12
17	Cortland	----
18	Cortland	----

3. Greenhouse Studies

A. Non-Rosaceous Plants. Bawden states "No virus that infects rosaceous plants has yet been transmitted by sap-inoculation" (17). Essentially this is true. The exception so far has been made by Yarwood who transmitted a virus which causes apple mosaic symptoms in apple; this virus is considered to be different from the true apple mosaic virus which has not yet been transmitted mechanically (see literature review, apple mosaic virus). Wilborn reported that he was able to obtain a local lesion type of reaction to cucumber and bean from the Malus stem pitting virus. However, he was not able to duplicate these reactions with other sources (56, 58). In an effort to obtain an indexing host for apple viruses several hosts were tried for mechanical inoculations via the Yarwood method (131). Little if any information is available in the literature other than the above concerning mechanical transmission of apple virus diseases.

Preparation of Plant Material. Seeds were usually started in a wooden flat filled with sand. The seeds were scattered over the moistened sand and then covered with another thin layer of sand. A piece of paper was then placed over the sand to prevent disturbing when watering and to prevent drying out. Small holes were punctured in the paper with a pencil point to let the water through. The flat was then placed in the greenhouse and watered daily. After the seeds had germinated and had grown to an inch or so in height they were transplanted to 2- 3- or 4- inch clay flowerpots depending on the size of the seedlings and permitted to grow until ready for inoculation. In some instances the seed was planted directly in flowerpots and the plants permitted to develop.

In some instances only the cotyledons or only the leaves were used, particularly on older plants. The cotyledons or leaves were then lightly dusted with a fine (600 mesh) grade of carborundum by using a DeVilbiss atomizer with the nozzle tip removed. The plant was then ready for inoculation.

The following technique described by Yarwood was used with slight modification:

Leaf Tissue. A leaf from an apple or other plant being used as an inoculum was folded 3 or 4 times and placed in a pair of forceps so that one side of the leaf projected from the base of the forceps. The folds of the leaf were cut off with a sharp razor, exposing several layers of cut surface. The upper surface of the leaf blade was rubbed immediately with the cut surface of the inoculum. Sometimes the leaf tissue was dipped in a 1.0 per cent solution of K_2HPO_4 just before rubbing.

Fruit Tissue. Fruit tissue was prepared by cutting a wedge-shaped piece of flesh from the fruit with a sharp razor and by rubbing the leaf surface with the exposed flesh.

Stem Tissue. Inoculum from stems was prepared by shaving the bark away from the stem and exposing the tissues at the cambial area. The stem portion was then rubbed on the leaf surface.

In all sources of inoculum the tissue was repeatedly cut during inoculating procedure to expose fresh tissue to the surface of the leaf being inoculated.

The plants were maintained in the greenhouse after inoculation.

Most plants were prepared in the above manner. In one instance the plants were heated in a 1.0 per cent solution of K_2HPO_4 at 45^{o} C for 60 seconds immediately before inoculation. This was accomplished by heating the solution to the desired temperature in a partially filled 1000 ml beaker. The pot containing the plants was then inverted and the plants immersed in the warmed solution. Upon removal they were dusted immediately with carborundum, and the leaves were rubbed with the inoculum.

<u>Cucumis sativa</u> (Cucumber). National Pickling Cucumber seed was obtained from Associated Seed Growers, New Haven, Connecticut. The plants were raised in the manner described above. In this particular variety of cucumber a flecking of the cotyledons and of the leaves as well as some deformation of these parts was present. The selection was not considered to be the most desirable from this standpoint, but since it has been widely used for this purpose it was not changed.

<u>Series 1</u>. Young leaves or fruit from propagated trees from the experimental orchard were obtained and used in the leaf inoculation procedure described above. Generally, 5 pots of 4 plants each were used. Four check plants for each inoculum were also maintained. The inoculum came from the following sources: J-30 Cortland[1] leaves, J-30 Virginia Crab fruit, E-31 Cortland leaves, F-6 Virginia Crab leaves, L-4 Virginia Crab

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1. See Appendix B for tree variety, bodystock, rootstock and disorder.

leaves, G-31 Virginia Crab fruit, USDA 227 leaves, Prunus tomentosa/J-30 leaves, P. tomentosa/J-31 leaves, P. tomentosa/E-31 leaves, and checks which were rubbed with a pot label. The local lesion readings were made 4 and 10 days after inoculation. The systemic reaction readings were made 30 days after inoculation. These plants were maintained for 8 weeks after inoculation.

Series 2. The above experiment was repeated. These plants were immersed in hot water immediately previous to inoculation. Readings were made 4 days after inoculation. The plants were maintained for 20 days.

Series 3. Since some reactions to inoculations were obtained in Series 2, cotyledons of these plants were used as sources of inoculum 7 days after inoculation. Five pots of 4 plants each were used per inoculum and 5 check pots were maintained. Local lesion readings were taken after 4 and 6 days. The plants were maintained for 15 days. Leaves of following cucumber plants were sources of inoculum:

Cucumber/P. tomentosa/J-30 Cortland, leaves
Cucumber/P. tomentosa/J-31 Cortland, leaves
Cucumber/P. tomentosa/E-31 leaves
Cucumber/USDA 227, leaves
Cucumber/J-30 Virginia Crab, fruit
Cucumber/G-31 Virginia Crab, fruit
Checks.

Raphanus raphanistrum (Wild Radish). Wild radish plants were collected from a cultivated field and placed in 2 inch pots. They were permitted to become established for a few days and then inoculated by the modified Yarwood method with leaf tissue, after which at 4 and 8 day intervals they were read for a local lesion reaction. Systemic reaction readings were taken 12 days after inoculation. These plants were maintained in the greenhouse for 30 days. About 10 plants were used per source of inoculum. Leaves from the following plants were sources of inoculum: J-30 Virginia Crab[1], I-12 Cortland, D-19 Virginia Crab, G-31 Virginia Crab, J-5 Cortland, and checks which were rubbed with a pot label.

Datura stramonium (Jimson Weed). These plants were raised from seeds in the greenhouse. The plants were inoculated in the usual manner. Local lesion readings were taken on the 4th and 8th day and systemic reaction readings on the 20th day.

It is apparent that a disorder causing virus-like, ring-spot type symptoms is visible in the leaves of this host and is seed transmissible, but because there was some confusion in determining true inoculation symptoms, its occurrence is not officially recorded. Seven plants were used per inoculum. Five check plants were maintained. Leaves from the following trees were sources of inoculum: J-30 Virginia Crab, I-12 Cortland, D-19 Virginia Crab, G-31 Virginia Crab, J-5 Cortland, and checks which were rubbed with a pot label.

Chrysanthemum leucanthemum (White Daisy). Seedlings from a weed belonging to the Compositae family growing in the vicinity of the greenhouse were found to germinate readily in some flats which had been outside. These plants were trans-

1. See Appendix B for tree variety, bodystock, rootstock and disorder.

ferred to pots and inoculated in the usual manner. Five plants were used for each source of inoculum. Local lesion readings were taken on the 4th, 8th, and 15th days. Systemic reaction readings were taken 30 days after inoculation. Leaves from the following trees were used as sources of inoculum: J-30 Virginia Crab, I-12 Cortland, D-19 Virginia Crab, G-31 Virginia Crab, J-5 Cortland, and checks which were rubbed with a pot label.

Gomphrena globosa (Globe amaranth). *G. globosa* is used in potato indexing to determine the presence of the potato X virus. Seeds were available at the greenhouse. Plants were reared, transferred to pots and inoculated by the Yarwood method. Ten plants per inoculum were used. Ten checks were also maintained in the greenhouse. Readings were taken on the 10th day after inoculation. The plants were held in the greenhouse for 30 days after inoculation.

Series 1. The plants were inoculated with leaves from the following trees: J-30 Virginia Crab, I-12 Cortland, D-19 Virginia Crab, G-31 Virginia Crab, J-5 Cortland, and checks which were rubbed with a pot label.

Series 2. The plants were prepared in the usual manner. Stem pieces were used instead of leaves. Two or 3 plants were used per inoculum. Leaves were inoculated instead of cotyledons since these plants were about a month old at the time of inoculation. Stem pieces with 1 exception were used as sources of inoculum from the following trees: J-30 seedling shoot, J-30 Virginia Crab, J-30 Virginia Crab, leaves, J-30 Cortland, D-19 Virginia Crab, G-31 Virginia Crab, and checks which were rubbed with a pot label.

Series 3. It was thought that some reaction may have been expressed in previous experiments with G. globosa using leaves and stem pieces. In this experiment fruit tissue was used as inoculum. The plants were prepared in the usual manner. Three plants were used per inoculum. Local readings were taken on the 7th and 12th day after inoculation. Systemic reactions failed to develop after the 20th day. Fruit tissue was used from apples from the following trees: J-30 Cortland, J-5 Cortland, I-12, Cortland, H-11 McIntosh, H-18 McIntosh, G-14 McIntosh, and checks which were rubbed with a pot label.

Citrus aurantifolia (Key Lime). Descriptive names which have been used for the Malus stem pitting disorder are "Tristeza" by Smith and "quick decline" by Hilborn. Tristeza or quick decline is a virus disease of Citrus which has symptoms of stem pitting. It has been suggested that the virus entity causing the stem pitting in each disorder is one and the same thing.

The plant, Key Lime, C. aurantifolia, is used as an indexing host for the Tristeza disorder. The response to inoculation with Tristeza infected buds is the development of interveinal flecks and clearing in the leaves 4 to 6 weeks after inoculation.

Key Lime seedlings were obtained from Dr. F. O. Holmes, Rockefeller Institute for Medical Research, New York. These seedlings were planted in 10 inch pots in the greenhouse, and at the end of 4 weeks they were established and actively growing.

Four plants were budded to each of the following sources of inoculum: D-19 Virginia Crab and Gravenstein I. All buds remained alive in the rubber wraps for a period of at least 2 weeks. The plants were closely watched for 10 weeks.

Miscellaneous Hosts. In addition to the above the following plants were also tried as possible hosts for mechanical transmission of apple viruses. They were raised and prepared in the usual manner. In general the usual inoculating sources were used for all plants. They were observed closely and readings were recorded, generally within 8 days after inoculation. Fruit and leaves were from the following trees: J-30 Virginia Crab, leaves; I-12 Cortland fruit and leaves; D-19 Virginia Crab, leaves; J-5 Cortland fruit and leaves; and checks which were rubbed with a pot label. The hosts were: Sting nettle (*Urtica sp.*), Pigweed (*Amaranthus retroflexus*), Leaf Mustard (*Brassica juncea*), Lamb's Quarters (*Chenopodium album*), Onion, variety Utah Valencia; Pepper, variety Merrimack Wonder; Celery, variety Emerson Pascal; Beets, variety Detroit Dark Red; Tomato, variety Bonny Best; Tobacco, White Burley variety Judy Pride; Aster, variety Burpeeana Early; and Carrot, variety unknown.

B. Rosaceous Plants.

a. Non-Malus Group. The plants used in this group were the alpine strawberry (*Fragaria vesca*), black raspberry seedlings (*Rubus occidentalis*), Korean cherry (*Prunus tomentosa*), ninebark (*Physocarpus opulifolius*), *Cotoneaster multiflora*, red chokeberry (*Aronia arbutifolia*), and peach seedlings (*Prunus persica*).

Fragaria vesca plants were available because they are used extensively as an indexing host for strawberry virus diseases at the University of New Hampshire.

It is known that some viruses introduced into *F. vesca* exhibit symptoms more readily and vigorously if the receptor plants are infected with the strawberry latent virus A. The *F. vesca* (hereafter called vesca) plants available for use were infected with the strawberry latent virus A.

The method of inoculation was patterned after that adopted for use with the strawberry indexing method proposed by Bringhurst and Voth (26). Longevity of the excised leaf from the donor plant was increased by lengthening the center petiole cut of the receptor and placing the excised leaf nearer the base of the petiole. The leaf area of the vesca host and the apple leaf was reduced by cutting off about 2/3 of each leaf blade.

Apple leaves were obtained from the trees in the experimental orchard as well as from trees propagated in the greenhouse. The method of preparing the leaves for grafting is as follows: individual leaves were separated from the apple stem, with care being taken to leave as much of the petiole attached to the leaf as possible. About 2/3 of the leaf blade was then cut with a sharp razor. The cut was made on 1 side of the petiole by starting about 3/4 of the way to the blade and drawing the razor in a slantwise direction toward the petiole base. The angle of cut was such that it penetrated only to the center of the petiole. A similar cut was made on the other side of the petiole, thus forming a long, tapering wedge.

A different type of cut was made on the vesca leaf. The center leaflet of the three leaflets was removed, and the other 2 leaves were reduced in area by cutting off about 2/3 of each leaf. The petiole was split down the center with a sharp razor blade starting at a point between the 2 remaining leaflets. This cut extended from the leaflets to about 3/4 of the way down the petiole.

The petiole-wedge of the apple leaf was carefully fitted into the split vesca petiole. The sides of the vesca petiole were fitted along the apple petiole and wrapped with Stericrepe (a crepe rubber adhering only to itself). The wrap started at the base of the vesca petiole cut and continued up the petiole, including the apple petiole, and leaving the apple leaf exposed. Two leaves were grafted for each plant. The apple leaves remained green on the vesca petiole for more than 2 months.

The plants were then placed in a plastic screen cage and permitted to grow for 6 months. The plants were watered daily and fertilized about every 3 to 4 weeks. Four or 5 plants were used per inoculum source. Leaves from the following plants were used as sources of graft inoculum: J-30 Virginia Crab, I-12 Cortland, J-5 Cortland, D-19 Virginia Crab, G-31 Virginia Crab, USDA 227, propagated plant, leaf graft, Baldwin 2, apple mosaic, from a branch brought in by a representative of a chemical company.

Rubus occidentalis (Black Raspberry). Because black raspberry is susceptible to many virus disorders in cultivation, such a plant should offer some possibilities as an indexing host for virus disorders within the Rosaceae.

Black raspberry seeds were obtained from Mr. E. M. Meader, Associate Horticulturist, New Hampshire Agricultural Experiment Station. They were grown in the manner described. When the canes were large enough to bud, each of 5 or 6 plants was inoculated with 1 or 2 buds from each of the sources of inoculum. These plants were maintained in the greenhouse for a period of 4 months. During this time they were cut back to permit new growth and the development of possible evidence of virus reaction. Readings were taken 8 weeks after inoculation.

The bud-inoculum was taken from the following trees: J-30 Virginia Crab, I-12 Cortland, J-5 Cortland, D-19 Virginia Crab.

<u>Prunus tomentosa</u> (Korean Cherry).

<u>Series 1</u>. <u>P. tomentosa</u>, the Korean Cherry, has been used as an indexing host for stone fruit virus diseases (110). Since some information concerning the use of this plant as an indexing host is available in the literature, it seemed to be a good starting point in the search for an indexing host for apple virus diseases. <u>P. tomentosa</u> seeds were obtained from F. W. Schumacher, Sandwich, Massachusetts, and a few seeds were obtained from local plants in Durham.

These seeds were placed in a can containing moist sand and stored for 90 days in a refrigerator at a temperature range of 32° -- 38° F. The sand was moistened periodically. After stratification the seeds were placed in a sand flat, as previously described, and placed in the greenhouse for germination. As the seed germinated the plants were transferred to 4-inch pots.

Originally 1/4 pound of seed was purchased which contained several hundred seeds. Germination was extremely low, and only enough seedlings were available for preliminary indexing trials. Because of the lack of plants, inoculations were made as the seedlings became large enough to bud. All inoculations were by buds. Three or 6 plants were used for each. The following trees were sources of inoculating buds: J-30 Cortland, J-30 Virginia Crab, J-31 Cortland, E-31 Cortland, L-4 Virginia Crab, F-6 Virginia Crab, and USDA 227. These plants were maintained in the greenhouse for a period of 2 years. The initial reading was taken 4 months after inoculation.

Series 2. Seedlings of P. tomentosa were obtained from a local nurseryman, planted in 4-inch pots and grown in the greenhouse. The sources of inoculum were from the same trees used in the orchard transmission studies on immature trees. Some of the standard sources of inoculum were also used. Two inoculating buds were used for each plant. In most cases only 1 plant was used for each source of inoculum. Most of the trees had a Virginia Crab bodystock. One P. tomentosa seedling, unless otherwise indicated, was inoculated with buds from the following trees: J-30 Seedling root, J-30 Cortland, J-5 Cortland 3 plants, I-12 Cortland 3 plants, I-4 McIntosh, K-41 M.IV rootstock 3 plants, E-31 Cortland, G-27 McIntosh, G-28 McIntosh, G-29 McIntosh, G-30 McIntosh, G-31 Virginia Crab, F-21 Virginia Crab, L-5 McIntosh, L-6 Northern Spy, L-11 Red Spy, L-28 Northern Spy, L-29 Northern Spy, M-4 Virginia Crab, M-5 McIntosh, and M-17 McIntosh.

Physocarpus opulifolius (Ninebark). Seeds of an ornamental P. opulifolius were collected in Durham, cleaned and stratified for 90 days in the refrigerator. The seeds were germinated in sand flats and transferred to 4-inch pots. When these seedlings had attained sufficient size they were inoculated. Symptom readings were taken 3 months after inoculation. Three plants, unless otherwise indicated, were budded with the following sources of inoculum: J-30 Virginia Crab, I-12 Cortland, J-5 Cortland, D-19 Virginia Crab, Gravenstein 1 2 plants, Baldwin 1 2 plants, and check plants.

Cotoneaster multiflora. Seedlings of Cotoneaster multiflora were obtained from Mr. Robert Kennedy, Associate Professor of Horticulture, Thompson School of Agriculture, and were grown in the greenhouse in 4-inch pots. Four seedlings were inoculated with each source of inoculum. Symptom readings were taken 12 weeks after inoculation. The following trees were the sources of inoculating buds: J-30 Virginia Crab, I-12 Cortland, J-5 Cortland, D-19 Virginia Crab, G-31 Virginia Crab, Gravenstein 1, Baldwin 1, and check plants.

Aronia arbutifolia (Red Chokeberry). Seeds of A. arbutifolia were collected locally, stratified, germinated and transferred to 4-inch pots and grown in the greenhouse. Three seedlings were inoculated with each source of inoculu. Readings were taken 7 weeks after inoculation. The following trees were the sources of inoculating buds: J-30 Virginia Crab, D-19 Virginia Crab, G-31 Virginia Crab, I-12 Cortland, J-5 Cortland, Gravenstein 1, and check plants.

These plants were kept in the greenhouse for 2 weeks after inoculation and were then transferred to available space under artificial light. The temperature in this room was around 60°F which was too low for adequate growth of the plants. The light intensity was 20 foot candles at pot height and at 1 foot nearer the light was 60 foot candles. Readings of symptom expression were taken 5 weeks after placement under incandescent light.

<u>Prunus persica</u> (Peach). Twenty-four peach seedlings growing under an abandoned peach tree were transferred to 4-inch pots and maintained in the greenhouse. Half of these plants were used in the initial trial while the remaining seedlings were saved for later use. One or 2 seedlings were inoculated for each source of buds. Symptom readings were taken after 14 weeks. The following trees were the sources of inoculating buds: J-30 Cortland, J-5 Cortland, I-12 Cortland, G-31 Virginia Crab, K-41 Seedling root and check plants.

b. Malus Group. Since viruses do not always produce visible symptoms it may be necessary to demonstrate their presence in a plant by transmission to another plant. A plant which will accept a virus from another host and respond to the infection by displaying symptoms may be called an indexing host. These reactions may be in the form of leaf spots, mottling, distortion, necrotic areas, wilting, and stem necrosis, dieback, dwarfing or even death of the plant. One of the objectives of this study was to try to find an indexing host for apple virus detection. It was thought that the development of an indexing host closely related to the cultivated apple would stand a greater chance of being susceptible to more or even all of the viruses present in the cultivated apple rather than one distantly related. There is some evidence that certain plants will react to apple viruses. Millikan and Geungerich (88) have used an Amelanchier sp. which reacted to the Malus stem pitting virus by leaf mottling. Thomas (124) used a Pyracantha sp. to demonstrate a bark canker reaction to the apple flat limb virus. These reactions indicate that the prospect for finding an apple virus indexing host is possible. To date, there is no indexing host of this nature developed for apple viruses.

The present methods of indexing are designed for specific reactions to rubbery wood, apple mosaic and chat fruit. The latter disorder requires 4 to 6 years for fruit development and symptom expression. The indexing method for rubbery wood and apple mosaic has been described in the literature review. These methods have been adopted by workers in England (53) and Holland (83) for their indexing procedures. In general they are for specific reactions and would not detect other entities which are latent.

<u>Malus</u> <u>Seed</u> <u>Collections</u>. Clonally propagated indexing material is desirable because it is uniform and when inoculated with like virus entities gives uniform reactions. However, in

stone fruits it has been shown that some of the indexing plants of Shirofugen and Kwanzan after having been widely dispersed contained a serious virus entity. At the outset, then, in order to avoid any similar occurrence the writer decided that seedlings should be used in preference to clonal material.

The host range study was greatly aided by the location of many species of the genus Malus at the Arnold Arboretum, Jamaica Plain, Massachusetts. Several species of Malus were collected in 1956 with the aid of Dr. Karl Sax, director in the Arnold Arboretum. Seeds of the following Malus taxons were collected and used: _Malus brevipes_[1] (1850-2-A)[2], _Malus floribunda_ (21497-A), _Malus halliana spontanea_ (10796-3), _Malus prunifolia rinki_, _Malus robusta_ (2553-1-B), _Malus sargenti rosea_ (11045), _Malus sieboldii arborescens_ (10094), _Malus sikkimensis_ (50-36-A), _Malus toringoides_ (180-52-A). These Malus taxons were selected because they possessed the quality of apomixis in some degree. In older trees they tend to breed true and are genetically identical with the mother plant. This is, then, a near substitution for clonally propagated plants.

Culture. The seeds from these plants were cleaned by hand. Later collections were cleaned in a Waring Blendor with a leather baffle substituted for the metal baffle. The seeds were stratified in sand in the refrigerator and at the end of 90 days were placed in the greenhouse to germinate. Seed germination was poor for the amount of seed collected. This

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1. See Appendix A for scientific name.

2. Arnold Arboretum identification number.

limited the extent of experimentation to the number of plants available in each taxon. Furthermore, genetic or virus abnormalities occurred in some of the seedlings, thus limiting again the number of seedlings available for use. Only plants which were free from visual symptoms of disorder were used.

The plants were kept in the greenhouse, watered daily and fertilized every 2 or 3 weeks. No light restrictions were placed upon them, and the temperature was variable.

After a period of time readings were taken and the plants were subjected to low artificial light intensity at day temperatures of 70°F and night temperatures of 60°F. Commercial 60 watt incandescent light bulbs were placed about 2 1/2 feet above the bench in aluminum reflectors. The Malus seedlings were in 4-inch pots, and the light intensity at pot level was adjusted to 20 foot candles. At the height of 12 inches which was also the height of most of the plants the light intensity was 60 foot candles. The plants were exposed to the light for 6 hours daily. As the plants grew toward the source of light the light intensity increased at the terminal leaves. The full range of light intensity for a plant 18 inches tall was from 20 foot candles to about 150 foot candles.

The plants were budded with a standard series of buds which represented the various combinations of viruses occurring in the experimental orchard.

Plant growth is modified under low artifical light intensities. The shoots become etiolated, the leaves become more succulent, and growth is reduced. Some virus disorders are more pronounced under these conditions (17, 121).

The plants under light were fertilized every 2 or 3 weeks. The liquid fertilizer solution was prepared by adding 1 tbsp of ammonium nitrate (33 per cent nitrogen) pellets and 1 tbsp of 16-32-16 starter fertilizer to a 12 quart pail of water. Frequent fertilization was necessary to maintain adequate growth.

After the plants had been grown in the greenhouse and the readings taken, at least part of each series was moved to the artificial light room. Some plant groups were moved to the light room without waiting for greenhouse symptoms to develop. In the greenhouse adventitious shoots tended to show symptoms of distortion. Therefore, the plants were induced to promote new growth by cutting back and leaving a few buds above the point of inoculation.

<u>Malus floribunda</u>. The seeds germinated readily and in quantity. Seedling growth in 4-inch pots was good. The plants were not uniform, however, having leaves varying from entire to 2-, 3-, or 5-lobed. These plants were in a state of vigorous growth when inoculated with buds from the following source trees: J-30 Virginia Crab, I-12 Cortland, J-5 Cortland, G-31 Virginia Crab, D-19 Virginia Crab and Gravenstein 1. Check plants were maintained, and observations were made at daily intervals to note symptom development. Greenhouse and artificial light readings were made over a 3 month period.

<u>Malus sieboldii arborescens</u>. The seeds germinated readily but in less quantity than <u>M</u>. <u>floribunda</u>. The plants

were not uniform and the leaves were either entire or 3- or 5-lobed. The plants were in a state of vigorous growth at the time of inoculation with buds from the following source trees: J-30 Virginia Crab, D-19 Virginia Crab, I-12 Cortland, J-5 Cortland, G-31 Virginia Crab, Gravenstein 1 and Baldwin 1. Half of the plants in this and other series were left in the greenhouse while the other half was placed under artificial light. All symptoms occurred within a 3 month period in the greenhouse and within 5 months in the light room.

Malus brevipes. These seedlings grew fairly well. The leaves were either 2-, 3- or 5-lobed. The plants were in a state of moderate growth when inoculated with buds from the following source trees: J-30 Virginia Crab and Gravenstein 1. Readings were recorded during a 2 month period.

Malus sikkimensis. These seedlings were uniform in growth with leaves mostly entire or 3-lobed. The seedlings are considered to be apomictic. Seed germination was only fair. These plants were inoculated with buds from the following source trees: J-30 Virginia Crab, I-12 Cortland, D-19 Virginia Crab, J-5 Cortland, G-31 Virginia Crab and Gravenstein 1. Readings were taken over a period of 1 1/2 months.

Malus prunifolia rinki. The leaves were fairly uniform being either entire or 2- or 3-lobed, and the plant is said to be apomictic. Seed germination was poor, and various disorders occurred in the leaves thus reducing the number of plants available. The seedlings were inoculated with buds from the following tree sources: J-30 Virginia Crab, I-12 Cortland, J-5 Cortland, D-19 Virginia Crab, G-31 Virginia Crab and Gravenstein 1. Readings were taken over a period of 2 months.

Malus robusta. Very few seeds germinated. The leaves of this taxon were uniform, being either entire or slightly 3-lobed. The serrations may be fine or coarse. These plants were actively growing when inoculated. The seedlings were inoculated with buds from the following tree sources: J-30 Virginia Crab, I-12 Cortland, D-19 Virginia Crab, J-5 Cortland and G-31 Virginia Crab. Readings were taken over a period of 2 1/2 months.

Malus toringoides. These seedlings were the most uniform of all the Malus taxons inoculated. The leaves were very uniform, being 5-, 7-, or more lobed. The seedlings were inoculated with buds from the following tree sources: J-30 Virginia Crab, I-12 Cortland, J-5 Cortland, D-19 Virginia Crab, G-31 Virginia Crab and Gravenstein 1. Readings were taken over a period of 2 months.

The seeds of *M. halliana spontanea* and *M. sargenti rosea* did not germinate well, and very few seedlings were available for inoculation. They were moved to the light room 1 week after inoculation. Their growth was poor, and readings are not recorded.

SECTION IV

RESULTS

1. Orchard Transmission Studies

A. Mature Trees. These apple trees were inoculated in the spring of 1956 with tree number J-30. The inoculum source was from each of the 3 components of the tree: Cortland variety, Virginia Crab bodystock and seedling root shoots. The trees were inspected in the fall of 1956 at harvest time. No symptoms of the dapple apple virus disorder were present on any of the trees. The trees were reinoculated in the fall of 1956 in the same manner, and grafts were placed on them in the spring of 1957. The trees were inspected once on July 17; no symptoms were discernible at that time. The trees were watched closely, and at harvest time dapple apple symptoms were evident on 5 trees.

Inoculum Source J-30 Cortland Variety

Tree Number	Symptoms	Type of Tree
E-7	None	Cortland on Virginia Crab on seedling root
E-9	None	Same
E-11	Dapple Apple	Same
E-12	None	Cortland on Florence Crab on seedling root
E-22	None	Cortland on seedling root
E-17	None	Cortland on M.IV rootstock
G-7	None	McIntosh on Virginia Crab on seedling root

Tree Number	Symptoms	Type of Tree
H-11	Dapple Apple	McIntosh on seedling root
		Inoculum Source J-30 Virginia Crab Bodystock
J-9	Dapple Apple	Cortland on Virginia Crab on seedling root
E-13	Dapple Apple	Cortland on Florence Crab on seedling root
E-24	None	Cortland on seedling root
E-19	None	Cortland on M.IV rootstock
G-9	Dapple Apple	McIntosh on Virginia Crab on seedling root
H-14	None	McIntosh on seedling root
		Inoculum Source J-30 Seedling Shoot
J-27	None	Cortland on Virginia Crab on seedling root
E-14	None	Cortland on Florence Crab on seedling root
E-26	None	Cortland on Seedling root
E-20	None	Cortland on M.IV rootstock
G-11	None	McIntosh on Virginia Crab on seedling root
H-16	None	McIntosh on seedling root.

In addition to these trees, the following trees which were inoculated on random branches in the spring of 1956 and with 3 grafts in the spring of 1957 were watched closely for symptoms of dapple apple.

During the summer of 1957 symptoms of the dapple apple virus were observed on 1 apple of the Golden Delicious variety. A word of explanation is in order about the Golden Delicious.

Most mature red colored apples start out as immature green fruits which turn yellowish green during the summer and then assume the red color. Golden Delicious, however, is not red at maturity but a yellowish green. It may have a slight blush on 1 cheek. During its developmental period, however, there is a stage during the summer when the cheek of the Golden Delicious has a pronounced blush to its surface. It was on this surface that the first transmitted symptoms of the dapple apple virus were observed. This blush faded away later in the season, as is customary, and so did the symptoms of dapple apple.

The Starking Delicious at harvest time gave good symptoms.

Inoculum Source J-30 Cortland Variety

Tree Number	Symptoms	Type of Tree
R-15	None	McIntosh on M.VII rootstock
S-15	None	Macoun on M.IV rootstock
T-15	Dapple Apple	Starking Delicious on M.I rootstock
U-15	Dapple Apple	Golden Delicious on M.VII rootstock
P-14	None	Baldwin on M.I rootstock.

D. <u>Immature Trees</u>. No fruit appeared on any of the trees during 1957. It is expected that some fruit may appear in 1958 as a result of the girdling technique. The following trees died in 1957 in Gilford: Row 2, tree 19 and row 2, tree 30. No trees in this experiment died in Durham. It is expected that these experiments will be continued until all trees produce fruit.

2. Greenhouse Studies

A. Non-Rosaceous Plants

Cucumber Inoculations, Series 1. The plants were maintained for 8 weeks after inoculation. The local lesion readings were made 4 and 10 days after inoculation. The systemic readings were made 30 days after inoculation.

Source of Inoculum	No. Plants Inoculated	Reactions	
		Local Lesions	Systemic
J-30 Cortland	20	none	none
F-6 Virginia Crab	20	none	none
E-31 Cortland	20	none	none
USDA 227	19	none	none
L-4 Virginia Crab	18	none	none
J-30 Virginia Crab fruit	20	none	1 constricted leaf tip
P. tomentosa/J-30 Cortland	20	none	none
P. tomentosa/J-31 Cortland	16	none	none
P. tomentosa/E-31 Cortland	20	none	none
P. tomentosa/USDA 227	20	none	2 constricted leaf tips
G-31 Virginia Crab	20	none	none
Check	18	none	1 constricted leaf tip

Series 2. These plants were heat treated prior to inoculations. They were immersed in a solution of 1.0 per cent K_2HPO_4 at 45°C for 60 seconds.

Source of Inoculum	No. Plants Inoculated	Reaction Local Lesion
J-30 Cortland leaves	18	Fine stippling on cotyledons, 7/18 plants with symptoms.
J-30 Virginia Crab fruit	20	Fine stippling on cotyledons, meager. Blotch area at site of inoculation, 5 with symptoms.
E-31 Cortland leaves	14	Fine stippling on cotyledons, meager. Blotch area at site of inoculation, small. 6 with symptoms.
F-6 Virginia Crab leaves	17	Fine stippling on cotyledons. Blotch area at site of inoculation, 3 with symptoms.
L-4 Virginia Crab leaves	20	Fine stippling on cotyledons, meager, 7 with symptoms.
G-31 Virginia Crab fruit	20	Blotch area at site of rubbing, 5 with symptoms.
USDA 227 leaves	12	Fine stippling on cotyledons, 2 with symptoms.
P. tomentosa/J-30 leaves	19	Fine stippling on cotyledons. Blotch areas at site of inoculation, 10 with symptoms.
P. tomentosa/J-31 leaves	18	Fine stippling on cotyledons. Blotch areas at site of inoculation, 4 with symptoms.
P. tomentosa/E-31 leaves	20	Fine stippling on cotyledons. Blotch areas at site of inoculation, 13 with symptoms.
Checks	20	Stippling, blotches, or scratches on 17 plants, none severe.

Series 3. Cotyledons from some plants in Series 2 were used to see if any of the disorders could be transmitted. Readings were taken 4 and 6 days after inoculation.

Source of Inoculum	No. Plants Inoculated	Reaction
Cucumber/*P. tomentosa*/J-30 Cortland	20	none
Cucumber/*P. tomentosa*/J-31 Cortland	20	none
Cucumber/*P. tomentosa*/E-31	12	none
Cucumber/USDA 227	20	none
Cucumber/J-30 Virginia Crab fruit	20	none
Cucumber/G-31 Virginia Crab fruit	20	none
Checks	12	none

Raphanus raphanistrum (Wild Radish)

J-30 Virginia Crab	10	none
I-12 Cortland	10	none
D-19 Virginia Crab	10	none
G-31 Virginia Crab	13	none
J-5 Cortland	12	none
Checks	4	none

Datura stramonium (Jimson Weed). A peculiar vein clearing occurred in some of these leaves, but the presence of other disorders previously mentioned reduces the possibility of considering it related to the inoculum.

Source of Inoculum	No. Plants Inoculated	Reaction	
		Local Lesions	Systemic
J-30 Virginia Crab	7	scratches	none
I-12 Cortland	7	none	none

Source of Inoculum	No. Plants Inoculated	Reaction Local Lesions	Systemic
D-19 Virginia Crab	7	none	none
G-31 Virginia Crab	7	none	none
J-5 Cortland	7	scratches	none
Checks	7	scratches	none

Gomphrena globosa (Globe amaranth). Necrotic areas were present at the site of inoculation and did not vary with the scratches and injury on the checks.

Series 1:

Source of Inoculum	No. Plants Inoculated	Reaction Local Lesions	Systemic
J-30 Virginia Crab	10	8 injured	none
I-12 Cortland	9	4 injured	none
D-19 Virginia Crab	10	9 injured	none
G-31 Virginia Crab	10	2 injured	none
J-5 Cortland	10	4 injured	none
Checks	10	10 injured	none

Series 2. The fact that these plants were older led to some difficulty. Uninoculated plants of G. globosa at a certain age develop red circular spots on the leaves similar to those which appear after inoculating with potato X virus. Since older plants were used in this series it is probable that the symptoms which appeared were the results of this maturing factor. Injury due to inoculation was also present.

Source of Inoculum	No. Plants Inoculated	Reaction Local Lesions	Systemic
J-30 seedling shoot	2	2 injured	none
J-30 Virginia Crab	2	2 injured	none

Source of Inoculum	No. Plants Inoculated	Reaction Local Lesions	Systemic
J-30 Cortland	2	2 injured	none
D-19 Virginia Crab	2	2 injured	none
G-31 Virginia Crab leaves	3	2 injured	none
J-30 Virginia Crab leaves	3	none	none
Checks	2	none	none

<u>Series 3</u>.

J-30 Cortland	3	none	none
J-5 Cortland	3	none	none
I-12 Cortland	4	none	none
H-11 McIntosh	3	none	none
H-18 McIntosh	3	none	none
G-14 McIntosh	3	none	none
Checks	3	none	none

<u>Chrysanthemum</u> <u>leucanthemum</u> (White Daisy).

J-30 Virginia Crab	5	none	none
I-12 Cortland	5	none	none
D-19 Virginia Crab	5	none	none
G-31 Virginia Crab	5	none	none
J-5 Cortland	5	none	none
Checks	5	none	none

<u>Citrus</u> <u>aurantifolia</u> (Key Lime). The 8 inoculated Key Lime plants were watched closely for a period of 10 weeks. Readings were taken at the end of 8 weeks. No symptoms appeared which could be related to the usual virus reactions

of Key Lime seedlings when inoculated with Tristeza infected buds.

Source of Inoculum	Leaf Symptoms
D-19 Virginia Crab	none
Gravenstein	1 leaf with a single fleck 1 leaf with tip constriction
Check Plants	none

Miscellaneous Hosts. The series of plants listed below were inoculated in the usual Yarwood method with slight modification. No local lesions or systemic reactions were ever observed in these plants. Sting nettle (*Urtica* sp.); Pigweed (*Amaranthus retroflexus*); Leaf mustard (*Brassica juncea*); Onion, variety Utah Valencia; Pepper, variety Merrimack Wonder; Celery, variety Emerson Pascal; Beets, variety Detroit Dark Red; Tomato, variety Bonny Best; Tomato, variety Window Box; Tobacco, White Burley variety Judy Pride; Aster, variety Burpeeana Early; Carrot, variety unknown.

Rosaceous Plants

a. Non-Malus Group.

Fragaria vesca (Alpine Strawberry). These plants were

a. Non-Malus Group.

obse

Fragaria vesca (Alpine Strawberry). These plants were

week observed frequently. Symptom readings were taken after 8 reactions occurred, and no other symptoms were observed. However, some plants were set aside for further observation. The leaves of these plants exhibited no definite or mild symptoms, but they did not impress the writer as being quite normal. No further symptom development was observed in these plants for another 4 weeks. June yellows appeared in a runner plant from a parent plant which had been inoculated with a leaf showing apple mosaic symptoms. June yellows is a genetical variation which occurs spontaneously. Leaves from this plant were grafted onto other vesca plants, but the disorder did not appear again.

Inoculum Source	Number of Plants grafted	Reaction	No. Plants Saved
J-30 Virginia Crab leaf graft	5	none	2
I-12 Cortland leaf graft	5	none	0
J-5 Cortland leaf graft	5	none	1
D-19 Virginia Crab leaf graft	5	none	2
G-31 Virginia Crab leaf graft	2	none	0
USDA 227, leaf graft	3	none	0
Baldwin 2, leaf graft	3	June Yellows	1

Rubus occidentalis (Black Raspberry). Considerable variation was noted among the seedling plants of the black raspberry. The leaf shape varied from plant to plant; slight distortions were apparent in different leaves of the same plant. There were slight color variations accompanying the distortions, and it was necessary to discard many of the plants. By far the most discouraging thing about the black raspberry plant is its susceptibility to mite infestation. Despite having received much care, these plants developed mite populations that interfered greatly with their use. Heavier applications of a miticide caused injury to the leaves of the plant. The variation in leaf shape, coloration, spray injury and distortions made readings of the black raspberry plant difficult, and further testing was discontinued.

Source of Inoculum	No. of Plants Grafted	Reactions	No. Plants Reacting
J-30 Virginia Crab	6	marginal reddening on older leaves,	2
		slight necrotic areas,	3
		yellowing of older leaves,	4
		basal shoots forced	2
I-12 Cortland	6	yellowing and veination,	2
		purplish spots,	1
		lower leaf abscissions	4
J-5 Cortland	6	reddening of lower leaves,	4
		stunted growth,	1
		yellow blotches	3
D-19 Virginia Crab	6	yellowing of lower leaves,	3
		reddening of leaves,	3
		purplish spots	2

Prunus tomentosa.

Series 1. Due to the inconsistency in seed germination and growth these plants were inoculated in a series of 3 over a period of 2 months, and readings were taken 4 months after the first inoculations. Consequently the checks were among the last plants to be selected although they were with a late group of inoculations.

Source of Inoculum	No. Plants	Symptoms
J-30 Cortland	6	Fine necrotic spots, mottling, color variation; 1 with no symptoms

Source of Inoculum	No. Plants	Symptoms
J-30 Virginia Crab	3	Necrotic spots (1/4 to 3/8 inch diameter) interveinal, color variation
J-31 Cortland	3	Fine mottling, fine necrotis spots
E-31 Cortland	3	Fine mottling, necrotic spots, shot holes
E-31 Robusta V	3	1 fine mottling, 2 no symptoms
F-6 Virginia Crab	3	Color variation, no mottling, tertiary veins netted, 1 no symptoms
L-4 Virginia Crab	3	Color variation, no mottling, few fine necrotic spots, 1 no symptoms
USDA 227	3	Fine mottling, color variation, 1 no symptoms
Checks	3	Fine mottling, few fine necrotic spots, 1 no symptoms

The plants in Series 1 were maintained for an additional 2 months. The mottling and necrotic spots became more prevelent on all plants including the check plants. The plants were cut back, and in most cases new shoots appeared which remained symptomless until terminal growth ceased. At this point necrotic spots appeared on the lower leaves and later on the upper leaves. Mottling also appeared in the leaves. All plants appeared to show the same symptoms even though inoculated with seemingly different virus material. Somewhat later, seedlings were obtained from a local nurseryman, planted in 4-inch pots and permitted to grow. They were maintained in the greenhouse under conditions similar to those in Series 1. Mottling and necrotic spotting developed in the usual manner. It had been suspected that spray materials applied to these plants for the control of mites and aphids had some effect upon the symptoms which appeared. Therefore, half-strength and full-strength concentrations of the miticide Aramite were applied to the plants. The full-strength applications caused the usual symptoms to develop, whereas the half-strength applications reduced the intensity of the necrotic spots. The mottling on the lower leaves was a result of shading, and the subsequent color changes were due to the onset of abscission.

Series 2. Seedlings of *P. tomentosa* were obtained from a local nurseryman and grown in the greenhouse. Three weeks after inoculation these plants were cut back and new shoots soon appeared. These plants were sprayed with the miticide at half-strength concentration to reduce spray injury. Symptom readings were taken 15 weeks after inoculation.

Source of Inoculum	Symptoms
J-30 Seedling Root	None
J-30 Cortland	None
J-5 Cortland	Necrotic spots and color variation, 1 no symptoms
I-12 Cortland	Marginal chlorosis, necrotic spots, 1 no symptoms, 1 with ring spot symptoms
D-19 Virginia Crab	Necrotic spots, mottle
I-4 McIntosh	None
K-41 M.IV rootstock	2 necrotic spots, 1 terminal die-back, next lower leaves distorted
E-31 Cortland	Necrotic spots
G-27 McIntosh	None
G-28 McIntosh	Necrotic spots
G-29 McIntosh	Necrotic spots

Source of Inoculum	Symptoms
G-30 McIntosh	Necrotic spots
G-31 Virginia Crab	Necrotic spots
F-21 Virginia Crab	Necrotic spots
L-5 McIntosh	Necrotic spots, mottling
L-6 Northern Spy	None
L-11 Red Spy	None
L-28 Northern Spy	None
L-29 Northern Spy	Necrotic spots
M-4 Virginia Crab	Necrotic spots
M-5 McIntosh	Necrotic spots
M-17 McIntosh	None
Checks	None and necrotic spots

Plants of Series 2 showed little appreciable difference from the responses that occurred in Series 1. Consequently, no further trials with *P. tomentosa* were made.

Physocarpus opulifolius (Ninebark). Distinct virus symptoms were not observed on the *P. opulifolius*. The plants were maintained for an extended period. During this time they were cut back and permitted to grow. Three months after the initial inoculation plants failed to show any variation from the initial reading.

Source of Inoculum	Symptoms
J-30 Virginia Crab	Terminal leaves slightly flattened
I-12 Cortland	None
J-5 Cortland	None
D-19 Virginia Crab	Leaves slightly rugose

Source of Inoculum	Symptoms
Gravenstein 1	Leaves slightly distorted
Baldwin 1	None
Check plants	None

These plants were cut back and new growth appeared. No symptoms were observed on this new growth. Although these plants were cut back a second time no symptoms were observed on the new growth.

<u>Cotoneaster multiflora</u>. During the course of the experiment some of the inoculating buds died. These plants were rebudded. In addition all plants were rebudded 6 weeks after the initial inoculation to insure exposure to virus inoculum.

Source of Inoculum	Condition of Buds 1st	Condition of Buds 2nd	Symptoms
J-30 Virginia Crab	4 dead	1 dead	Veinal and interveinal mottling
I-12 Cortland	4 alive	4 alive	Mild mottling and spots
J-5 Cortland	4 alive	4 alive	Leaves apparently normal
D-19 Virginia Crab	4 alive	4 alive	Leaves mostly normal, some slight mottling and spots
G-31 Virginia Crab	4 alive	2 dead	Leaves mottled on some
Gravenstein 1	4 alive		Russetting on lower leaf surface, some mottling and veinal necrosis
Baldwin 1	1 alive		Terminal leaves with flecks
Check plants			Normal leaves, few with slight mottle.

<u>Aronia arbutifolia</u> (Red Chokeberry). Leaf symptoms were noted on only 1 group of plants inoculated. The inoculation

site was the area of host reaction.

Source of Inoculum	Condition of Bud
J-30 Virginia Crab	3 living
D-19 Virginia Crab	2 living; 1 dead with girdling canker at inoculation site; stem above canker dead
G-31 Virginia Crab	3 living
I-12 Cortland	2 living; 1 dead with girdling canker at inoculation site; stem above canker dead
J-5 Cortland	3 living
Gravenstein 1	2 dead; on 1 a girdling canker at inoculation site; stem above canker dead; small orange spots on midrib of some leaves; necrotic wilting of terminal leaf
Check plants	2 normal; 1 defoliated.

The low light intensity and cool temperature apparently caused the lower leaves to absciss on most of the plants. No leaf distortion was observed.

Prunus persica (Peach).

Source of Inoculum	Symptom Reaction
J-30 Cortland	None
J-5 Cortland	None
I-12 Cortland	None
G-31 Virginia Crab	Marginal yellowing and interveinal spots on lower leaves
K-41 seedling root	None
Check plants	Marginal yellowing.

After the initial reading the plants were set aside, and excessive growth was cut off when necessary. The only change noted was in the peach seedling inoculated with buds of J-30

Cortland. This plant had not put forth new growth, and cutting back was not necessary. Approximately 6 months after the initial reading an excessive enlargement of the bud base or node was noted. No further leaf symptoms occurred on any of the plants.

Table 3. Malus floribunda. Greenhouse. Symptom development on inoculated seedlings.

Symptoms

	Source of Inoculum: J-30				I-12				D-19				J-5				G-31				Grav.1				Check			
Replications	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Main shoot																												
Bud dead	x	x	x	x a/																	x		x	x				
Leaves																												
normal	x	x	x	x		x	x	x			x			x		x	x	x							x	x	x	x
distorted					x				x	x		x	x		x													
spotted			x			x				x			x b/						x									
mottled									x																			
colored					x											x	x											
necrosis																												
Lobes																												
distorted													x															
Serrations																												
distorted																												
Adventitious shoots																												
Leaves																												
normal			x					x																				
distorted	x	x		x	x	x	x				x																	
spotted		x																										
mottled	x																											
colored																												
necrosis																												
Lobes																												
distorted																												
Serrations																												
distorted																												

a/ Symptom expressed
b/ Bright orange spots appeared 4 days after inoculation

Table 4. _Malus_ _floribunda_. Artificial light. Symptom development on inoculated seedlings.

		Source of Inoculum																											
		J-30				I-12				D-19				J-5				G-31				Grav.1				Check			
	Replications	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	Bud dead			x	x							x																	
	Leaves																												
	normal				x	x				x			x		x			x			x			x			x	x	x
	distorted	x	x	x			x	x	x		x	x		x		x	x		x	x		x	x		x				
S	spotted												x											x					
y	mottled																												
m	colored																												
p	rounded			x																									
t	epinasty		x				x	x				x														x			
o	necrosis			x			x					x	x											x					
m	wilt		x				x					x												x					
s	Lobes																												
	distorted																												
	Serrations																												
	distorted																												

Table 5. Malus sieboldii arborescens. Greenhouse. Symptom development on inoculated seedlings.

Source of Inoculum

Symptoms	J-30				I-12				D-19				J-5				G-31				Grav. 1				Bald. 1				Checks			
Replications	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Main shoot																																
Bud dead	x	x							x																							
Leaves																																
normal	x	x			x	x			x	x							x	x							x				x	x		
distorted														x							x	x										
spotted	x	x b/			x	x a/			x	x b/			x	x							x				x							
mottled																																
colored	x					x			x																x				x			
necrosis													x	x															x			
Lobes																																
distorted																						x										
Serrations																																
distorted																					x											
Adventitious shoots																																
Leaves																																
normal																																
distorted	x				x								x																			
spotted																																
mottled		x							x																							
colored	x	x								x											x											
necrosis																																
Lobes																																
distorted					x																											
Serrations																																
distorted										x																						

a/ Faint spot depressions appeared 2 days after inoculation
b/ Bright orange spots appeared 6 days after inoculation

Table 6. <u>Malus sieboldii arborescens</u>. Artificial Light.

Symptom development on inoculated seedlings.

Symptoms	Source of Inoculum	J-30				I-12				D-19				J-5				Grav.1				Bld.1		G-31		Check	
	Replications	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	1	2	1	2
	Bud Dead	x a/	x		x						x																
	Leaves																										
	normal		x	x								x				x	x					x		x	x	x	x
	distorted	x			x	x	x	x	x	x			x	x	x			x	x				x				
	spotted						x				x			x													
	mottled														x			x	x b/	x	x	x					
	colored		x																								
	rounded																										
	epinasty			x		x	x																				
	necrosis			x			x			x				x					x								
	wilt												x	x													
	Lobes																										
	distorted					x							x		x			x									
	Serrations																										
	distorted																										

a/ Canker girdling stem at site of 1st bud. Necrosis at site of 2nd bud.
b/ Lower surface of older leaves with brown veinal and interveinal necrosis.

Table 7. Malus brevipes. Greenhouse. Symptom development on inoculated seedlings.

		Source of Inoculum													
		J-30		I-12		D-19		J-5		G-31		Grav.1		Check	
	Replications	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Symptoms	Main Stem														
	Bud Dead	x				x						x			
	Leaves														
	normal	x		x	x	x	x	x		x		x			
	distorted		x						x						x[a]
	spotted														
	mottled		x											x[a]	x
	colored	x		x		x	x								
	necrosis														
	Lobes														
	distorted														
	Serrations														
	distorted														
	Adventitious shoots														
	Leaves														
	normal														
	distorted		x												
	spotted														
	mottled		x												
	colored														
	necrosis		x												
	Lobes														
	distorted														
	Serrations														
	distorted														

a/ Check plants had visual symptoms of mottling which developed after other plants had been inoculated

Table 8. Malus brevipes. Artificial Light. Symptom development in inoculated seedlings.

Source of Inoculum

		J-30				I-12				D-19				J-5				G-31				Grav. 1				Check		
	Replications	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
	Bud Dead												x					x										
	Leaves																											
	normal	x			x			x	x	x		x	x		x		x			x				x		x	x	x
S	distorted		x	x		x	x				x			x		x			x		x	x	x		x			
y	spotted																											
m	mottled														x													
p	colored									x					x			x	x				x	x				
t	rounded																		x		x							
o	epinasty																					x						
m	necrosis		x			x			x																	x		
s	wilt		x			x			x																	x		
	Lobes																											
	distorted																											
	Serrations																											
	distorted																x	x			x							

Table 9. Malus sikkimensis. Greenhouse. Symptom development on inoculated seedlings.

	Source of Inoculum																				
	J-30			I-12			D-19			J-5			G-31			Grav 1			Check		
Replications	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Symptoms																					
Main Stem																x[a]					
Bud Dead														x[b]					NONE		
Leaves																					
normal		x		x	x		x	x		x	x		x			x	x				
distorted	x		x			x			x			x			x	x[c]					
spotted	x		x																		
mottled																					
colored					x							x	x		x		x				
necrosis									x					x							
Lobes																					
distorted																					
Serrations																					
distorted																					
Adventitious shoots																					
Leaves																					
normal																					
distorted						x	x	x													
spotted								x													
mottled							x														
colored																					
necrosis							x														
Lobes																					
distorted						x															
Serrations																					
distorted						x		x													

a/. Stem had sinewy appearance
b/ Very small plant
c/. Tumors on leaf petiole.

Table 10. <u>Malus sikkimensis</u>. Artificial Light. Symptom development on inoculated seedlings.

		Source of Inoculum																			
		J-30			I-12			D-19			J-5			G-31			Grav1		Check		
	Replications	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	1	2	3
	Bud Dead														x				NONE		
	Leaves																				
	normal			x	x			a/							a/		x b/				
S	distorted	x	x				x			x	x	x	x			x					
y	spotted																				
m	mottled																				
p	colored																				
t	rounded															x					
o	epinasty																				
m	necrosis					x		x		x	x							x			
s	wilt					x		x		x	x										
	Lobes																				
	distorted																				
	Serrations																				
	distorted										x					x					

a/ No leaves
b/ Galls formed on midrib and leaf petioles.

Table 11. <u>Malus prunifolia rinki</u>. Greenhouse.

Symptom development on inoculated seedlings.

		Source of Inoculum													
		J-30		I-12		D-19		J-5		G-31		Grawl		Check	
	Replications	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Symptoms	Main Stem									-					
	Bud Dead		x										x		
	Leaves														
	normal	x	x	x	x	x	x			x			x	x	x
	distorted	x[a]						x	x		x	x			
	spotted														
	mottled														
	colored			x											
	necrosis														
	Lobes														
	distorted										x				
	Serrations														
	distorted							x			x	x			
	Adventitious shoots														
	Leaves														
	normal														
	distorted											x			
	spotted														
	mottled														
	colored														
	necrosis														
	Lobes														
	distorted														
	Serrations														
	distorted											x			

<u>a</u>/ Lower leaves cupped downward.

Table 12. <u>Malus</u> <u>prunifolia</u> <u>rinki</u>. Artificial Light. Symptom development on inoculated seedlings.

		Source of Inoculum																											
		J-30				I-12				D-19				J-5				G-31				Grav.1				Check			
	Replications	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	Bud dead																												
	Leaves																												
	normal			x	x			x	x				x		x	x							x	x	x	x			
S	distorted	x	x			x	x			x	x	x		x			x	x	x			x					x	x	x
y	spotted																												
m	mottled																												
p	colored																												
t	rounded																												
o	epinasty																												
m	necrosis																												
s	wilt																												
	Lobes																												
	distorted																		x										
	Serrations																												
	distorted																												

Table 13. <u>Malus</u> <u>robusta</u>. Greenhouse. Symptom development on inoculated seedlings.

	Source of Inoculum													
		J-30		I-12		D-19		J-5		G-31		Grav.1		Check
	Replications	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Symptoms	Main Stem														
	Bud Dead	x	x												
	Leaves														
	normal	x	x	x	x	x	x		x	x				x	
	distorted							x							
	spotted				x										
	mottled														
	colored				x	x		x	x	x				x	
	necrosis				x	x								x	
	Lobes														
	distorted														
	Serrations														
	distorted														
	Adventitious shoots														
	Leaves														
	normal														
	distorted														
	spotted														
	mottled														
	colored														
	Lobes														
	distorted														
	Serrations														
	distorted														

Table 14. *Malus robusta*. Artificial Light. Symptom development on inoculated seedlings.

	Source of Inoculum													
	J-30		I-12		D-19		J-5		G-31		Grav.1		Check	
Replications	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Symptoms														
Bud dead														
Leaves														
normal													x	
distorted	x	x	x	x	x	x	x	x	x		x			
spotted														
mottled														
colored														
rounded														
epinasty														
necrosis				x					x					
wilt				x					x					
Lobes														
distorted	x	x									x			
Serrations														
distorted											x			

Table 15. <u>Malus toringoides</u>. Greenhouse. Symptom development on inoculated seedlings.

	Source of Inoculum										
	J-30		I-12		D-19		J-5		G-31	Grav.1	Check
Replications	1	2	1	2	1	2	1	2	1	1	None
Symptoms											
Main Stem											
Bud dead											
Leaves											
normal	x	x	x	x	x	x	x	x	x	x	
distorted											
spotted											
mottled											
colored											
necrosis											
Lobes											
distorted											
Adventitious shoots											
Leaves											
normal											
distorted											
spotted											
mottled											
necrosis											
colored											
Lobes											
distorted											
Serrations											
distorted											

Table 16. <u>Malus</u> <u>toringoides</u>. Artificial Light. Symptom development on inoculated seedlings.

		Source of Inoculum												
		J-30		I-12		D-19		J-5		G-31		Grav.1		Check
	Replications	1	2	1	2	1	2	1	2	1	2	1	2	None
	Bud dead	x					x							
	Leaves													
	normal													
S	distorted	x	x	x	x	x	x	x	x	x		x		
y	spotted						x							
m	mottled													
p	colored													
t	rounded											x		
o	epinasty													
m	necrosis													
s	wilt													
	Lobes													
	distorted		x	x	x	x	x	x	x					
	inhibited	x	x	x	x	x			x					
	Serrations													
	distorted													

SECTION V

DISCUSSION AND CONCLUSION

The experimental portion of the orchard was designed for a bodystock study. The virus disorders which appeared in the trees were incidental to the main purpose of the experiment, but proved to be most interesting. There were several features of tree structure in the orchard which made this study possible and promoted a reasonable theory for the cause of disease occurrence.

The foremost feature is that all grafting wood used for each of the varieties came from 1 tree of that variety. If this had not been so the disorder dapple apple could only have been described as a disease, and proof of its virus-like nature demonstrated by transmission. The opportunity for tracing and understanding the means of infection would have been missed. This would have greatly reduced the possibility of demonstrating that dapple apple is a complex of viruses rather than a single virus entity. Also the single tree source of scionwood eliminates the contention that the Malus stem pitting virus is carried in its entirety in all the varieties as has been suggested, because if this were true all topworked Virginia Crabs in the orchard would have shown the disorder, since it occurred on some trees of each variety. The virus, then, must be located in the Virginia Crab or in the seedling root as the perpetuating agent. Florence Crab on seedling rootstock is also susceptible to the Malus stem pitting virus but few trees are affected when compared with Virginia Crab on seedling rootstock. By this deduction the Virginia Crab remains as the main agent of dissemination of the Malus stem pitting virus. How and when the initial infection occurred is not known, but a knowledge of nursery practices indicates that it occurred unintentionally and by any of several various methods. An excellent article by Posnette (94) considers some of the methods involved.

Virginia Crab scionwood when propagated in large quantities on seedlings must come from several trees. The occurrence of infection in some bodystocks and not in others indicates that some of the Virginia Crab scionwood source trees are infected while others are not. The virus (or viruses) is perpetuated with the scions as the plant is propagated. The Malus stem pitting virus is latent within the Virginia Crab and is not expressed in the bodystock until a variety is top-worked to it. The disorder has not been reported in the literature nor seen by the writer on trees which have not been top-worked. Why symptoms appear in the Virginia Crab only when top-worked with a variety has not been investigated.

The bodystock varieties Virginia Crab, Florence Crab and Red River Crab are susceptible to infection by the Malus stem pitting virus. Symptoms occur only on trees which have been top-worked with a variety. Mature 15-year-old trees were inoculated with the Malus stem pitting virus. Symptom readings will be taken in subsequent years.

Much the same reasoning can be proposed for the occurrence of dapple apple; however, it is more complicated. The theory is proposed that the disorder dapple apple is a complex disease consisting of 2 virus entities which, when coming together in 1 apple tree, cause the symptoms of dapple apple. Either factor alone within a single plant is latent. Dapple apple first occurred on the Cortland variety trees which had Virginia Crab as their bodystocks. Also it has not occurred spontaneously on any other variety unless suspected of having been grafted with infected material. The fact that it occurred originally on the Cortland variety and only with the bodystock Virginia Crab indicates an interaction of some entities between these 2 units. However, it does not occur on all Cortland/ Virginia Crab combinations. Neither does it always occur with the Malus stem pitting virus; therefore, it must be considered a separate virus entity. Since we know that the Cortland scionwood was grafted from 1 tree we can say with some assurance that any virus composition in the Cortland is the same throughout the orchard. This, then, leaves the Virginia Crab as the variable factor. Some of the scionwood source trees used to propagate the Virginia Crab may contain 1 component while the Cortland contains the other component. When they are brought together in 1 tree dapple apple is expressed. The orchard experiment on immature trees is designed to substantiate or to defeat this theory. Seedlings can be eliminated as the perpetuating agent because the disorder does not occur on other Cortland trees where seedlings have been used.

The virus entities which cause the symptoms of dapple apple have been transmitted to varieties other than Cortland. In the inoculation series inoculating buds were placed on limbs on the opposite sides of each tree and in other trees they were placed on random limbs. The occurrence of symptoms more widely spread on the random limb inoculations indicated that several points of inoculation cause more rapid symptom spread within the tree than more intensive localized inoculations.

Apples in the vicinity of the inoculating buds in either direction along the limb remained symptomless. However, those fruits growing on the first or second side branches centripetally to the buds developed symptoms, indicating an unusual pattern of virus movement through the tree.

The conclusion offered is that the Malus stem pitting virus is perpetuated in the Virginia Crab bodystocks by the use of scionwood material from infected Virginia Crab trees. It is also concluded that the most reasonable explanation for the occurrence of the dapple apple disorder is the presence of 1 component in the Cortland variety and that the other component is in some of the Virginia Crab bodystocks. The Malus stem pitting and dapple apple disorders occur independently of each other.

The relationship between the Malus stem pitting and apple flat limb symptoms has been mentioned. Preliminary inoculations have been made; G-31 Virginia Crab scions were inoculated with flat limb infected Gravenstein 1. At the end of 18 months sinewy growth and longitudinal fissures in the wood as well as tumerous swellings above the bud node appeared in the Virginia Crab. The reverse inoculation with Malus stem pitting infected Virginia Crab to symptomless Gravenstein has not been made.

The leaf inoculation technique proposed by Yarwood works with many plant virus diseases and is more or less a standard method of inoculating many herbaceous hosts. In the attempt to develop an indexing host this method was thought to be the most desirable for transfer of viruses to herbaceous plants. Many plants were tried in which the Yarwood method was used as the method of inoculation. In all cases no symptoms resulted from inoculation which could be classified as a reaction to virus inoculation. Mechanical transmission of viruses in the Rosaceous group does not take place readily, if at all (17).

The Rosaceous plants which were tried, including the Malus group, did not respond in any consistent fashion to the apple virus inoculations. _F. vesca_ was a disappointment in that the leaf grafting technique could be readily adapted to this plant but the responses were lacking or very meager. On those plants which were saved vague leaf distortions were noted. However, these were single leaves which could not be definitely associated with the inoculation. It is thought that more extensive testing of _F. vesca_ may lead to further knowledge of apple viruses.

Prunus tomentosa was difficult to grow in the greenhouse for the purposes intended. In the beginning considerable time was spent in obtaining and germinating seed. The initial responses obtained were the result of spray injury and resembled very closely the necrotic ring spot type of injury associated with the Prunus group. In some cases a mottling of the ring spot type was noted from some sources of apple inoculation, but the lack of seedlings prevented further study. The possibility exists that the purchased seedlings which had spent a year in the field may have been exposed to natural virus infection, which resulted in expression of symptoms in the greenhouse.

Physocarpus opulifolius was not found to be a responsive plant to apple virus inoculation. It is not considered to be satisfactory for use as an indexing host.

Cotoneaster multiflora showed symptoms of virus reaction only after the plants had been held for an extensive time. Some veinal and interveinal mottling occurred in the leaves. As the mature leaves aged the mottling became more pronounced. Although this plant responded to Gravenstein 1 flat limb inoculation it is thought that better plants are available for indexing work.

Aronia arbutifolia plants were in a room at relatively cool temperatures for plant growth due to lack of space in the warmer light room. The plants did respond to inoculation by developing necrosis and cankers at the site of bud-inoculation with Gravenstein flat limb. For this particular virus it is probably a better plant than C. multiflora.

Prunus persica did not respond readily to any of the inoculations made. Only after considerable time did the enlarged bud condition become manifest. The limited trials with peach seedlings are inconclusive, but they indicate that this host is not adaptable to apple virus indexing.

It was thought that the plants in the Malus group because of their close relation to the cultivated apple should offer greater potential for expressing virus reactions than any of the species previously mentioned. The initial series of greenhouse inoculations showed considerable variation in symptom response but these responses were not uniform. In an effort to standardize the reactions, low light intensities for short periods of time were tried and found to be conducive to leaf distortion as a direct result of virus inoculation.

The leaves under the light intensities of the greenhouse did not express any deformation on the main shoots. The leaves on lower adventitious shoots, however, being somewhat shaded, did show distortion and gave responses which indicated virus reaction. Also there is a time element involved after inoculation. The more vigorously the plant was growing the more rapidly the leaf spotting and necrosis appeared in the terminal leaves. When these plants were placed under artificial light the terminals would keep growing but distorted leaf growth did not appear readily. When these plants were cut back, the new growth, either adventitious or from buds formed after inoculation readily showed distortion symptoms in the new leaves, but shoots from buds formed prior to inoculation grew normally for a while and then showed leaf distortion. The virus entity apparently enters the bud more readily when it is being formed rather than after it has been formed.

It is thought that the leaf distortions are the result of simultaneous growth of the leaf meristems and virus multiplication. Apparently the leaf meristems of apple plants grow faster than the virus multiplication under high light but under low light the leaf meristems are retarded enough to permit virus multiplication within the area of leaf meristems and thus influence their behavior. The modifications resulting from the virus activity in these meristems is enough to cause distortion and create a symptom. In some instances the meristems were inhibited and growth of this particular area ceased. This reaction is most noticeable in _M. toringoides_ where leaf lobes are inhibited and distortion occurs.

Seedlings of _M. floribunda_ offered major responses to 2 sources of inoculum. The J-30 Virginia Crab and Gravenstein 1 inoculating buds responded in death of the bud and necrosis at the site of inoculation. This would seem to further substantiate a former assumption that the apple flat limb and Malus stem pitting viruses are related. However, D-19 which is the tree selected to represent the Malus stem pitting disorder did not respond with bud death, although in 3 out of 4 plants the leaves were distorted. Various leaf colorations, spottings, and mottlings in several of the plants resulted from the different sources of inoculum. These reactions are adequate for indexing reactions. Generally leaf spotting reactions appear within 2 weeks. The most rapid appearance of symptoms in this group was 6 days after inoculation. Under artificial light leaf distortion of the new shoots was the most prominent reaction. It occurred in plants from all sources of inoculum. The reactions were general and the symptoms could not be placed in categories which would aid in separating the virus entities. It is concluded that *M. floribunda* may be used as an indexing host for general screening of apple virus detection. It is suggested that at least 5 replicates be used for each source of inoculum.

M. sieboldii arborescens gave the impression of being slightly more sensitive to virus inoculations than *M. floribunda* by responding with more leaf coloration and spotting symptoms. The bright orange spots appeared readily in some seedlings, and, as with *M. floribunda* orange spots were present after 6 days. The most rapid reaction of the whole series was with *M. sieboldii arborescens* in which fine spot depressions were apparent 2 days after inoculation. These spots appeared in the terminal leaves 9 inches away from the site of inoculation. Under artificial light distortion of the leaves occurred in some seedlings from all sources of inoculum. An attempt has been made to propagate the Number 1 seedling inoculated with J-30 Virginia Crab buds. It is realized that this probably contains a virus in it since it was inoculated, but later developments may offer some way to eliminate the virus from this clone. This particular plant would be very useful for necrotic stem reactions similar to Shirofugen in stone fruit indexing.

It is concluded that seedlings of *M. sieboldii arborescens* would make a good general indexing host for apple viruses. It is also suggested that at least 5 seedlings be used for each source of inoculum.

The apparent lack of vigorous growth in seedlings of

Malus brevipes at the time of inoculation probably prevented these seedlings from expressing better symptom reaction under greenhouse conditions. Under artificial light those plants which grew well showed good symptoms. Again, distortion in the leaves of these seedlings occurred in some plants from all sources of inoculum. Further work with M. brevipes is encouraged.

Seedlings of M. robusta were moved to the light room 2 weeks after they were inoculated. They did show some leaf coloration symptoms. Under lights the leaves of the plants showed distortion, but the symptoms were not very pronounced.

Seedlings of M. prunifolia rinki were not vigorously growing when bud-inoculated. The plants grew slowly and symptoms were meager. It is thought that the maintenance conditions for these seedlings were not the best. Once the plants had lost their vigorous growth they never regained it. Very little growth occurred under artificial lights. The check plants in this group showed leaf distortion and enlarged serrations.

Seedlings of M. sikkimensis showed distortion in the greenhouse on at least one plant from each of the groups inoculated. Other symptoms were also expressed which indicated that these seedlings could be used in the greenhouse as indexing hosts. An unusual symptom occurred on the leaf petioles of 1 plant in this group. Galls were formed on the leaf petioles of the number 1 plant inoculated with apple flat limb. The seedling later showed leaf distortion symptoms under the lights.

Under artificial light those plants which grew displayed distinct symptoms of leaf distortion. The greenhouse and artificial light responses of the few plants used of this taxon indicate that M. sikkimensis would be a good indexing host for apple virus diseases. One definite drawback is the lack of adequate seed source of this species.

The leaves of M. toringoides on separate plants were strikingly uniform. Despite the fact that very few plants were available, the writer considers M. toringoides to offer the st possibilities of the plants tested for use with the artificial light indexing technique. The reactions are probably the most distinct of any of the species tested. The lack of greenhouse symptoms is due to the fact that they were moved to the light room after 2 weeks.

It is concluded that M. toringoides offers considerable possibility as an indexing host and would be highly recommended for further trial in any indexing investigations of apple viruses under artificial light.

See Appendix C for photographs of some symptoms expressed.

SECTION VI

SUMMARY

Trees in an apple orchard experiment, in which the main consideration was a hardy bodystock study, developed virus-like disorders in the bodystocks (Malus stem pitting) and the fruits (dapple apple) of some varieties. These virus disorders were studied to determine their occurrence and distribution, and one new virus disease (dapple apple) was demonstrated by transmission. Field inoculations were made in mature and immature trees in an attempt to determine the complex of viruses involved.

Greenhouse studies were undertaken to determine the transmissibility of viruses causing several types of symptoms. No transmissions were obtained with the leaf rub method of inoculation on any of the plants tried outside of the Rosaceae. Grafting inoculations within the family Rosaceae offered some promise for developing an indexing host for apple virus diseases. Within the genus Malus seedlings of several hosts were bud-inoculated and grown under greenhouse and low intensity artificial light conditions. Seedlings of the species *Malus floribunda*, *M. sieboldii arborescens* and *M. toringoides* are considered to be promising as indexing hosts for apple virus detection.

BIBLIOGRAPHY

1. Anderson, H. W. 1956. Diseases of fruit crops. McGraw-Hill, New York.

2. Anonymous. 1948. Report of the Science Service, Dominion Department of Agriculture, for the year ending March 31, 1948.

3. Anonymous. 1949. Twenty-third annual report of the Department of Scientific and Industrial Research. New Zealand.

4. Anonymous. 1951. Department of Agriculture, Kenya, annual report 1949. II. Record of Investigations.

5. Anonymous. 1951. New diseases attack fruit: Spread of "Henderson Spot". Fruit and Prod. 5: 7-8.

6. Anonymous. 1956. Current research, investigations, experiments. Orchard. N. Z. 29:13.

7. Anonymous. 1956. Second symposium on virus diseases of fruit trees in Europe, Aug. 23-27, 1955, Wageningen, Netherlands. Tijdschr. Plantenziekten 62: 33-88.

8. Anonymous. 1956. Stations federales d'essais agricoles, Lausanne. Rapport d'activite 1955. Annu. Agr. Suisse, N. S. 5: 399-513.

9. Anonymous. 1957. Common names of plant virus diseases used in the review of applied mycology. Rev. Appl. Mycol 35: Sup. 1-78.

10. Anonymous. 1957. Crop Production. United States Crop Summary as of October 1, 1957. Crop Rept. Board, Agr. Markt. Ser., U. S. Dept. Agr., Washington, D. C.: 48.

11. Atkinson, J. D. 1947. A note on Crinkle in New Zealand apples. N. Z. J. Sci. Tech. A 28: 332-334.

12. Atkinson, J. D. 1956. Unusual features of some New Zealand fruit tree viruses. Tijdschr. Plantenziekten 62: 39-42.

13. Atkinson, J. D. and E. E. Chamberlain. 1948. Apple mosaic in New Zealand. N. Z. J: Sci. Tech. 30: 1-4.

14. Atkinson, J. D., E. E. Chamberlain and J. A. Hunter. 1954. Apple ring spot. N. Z. J. Sci. Tech. 35: 478-482.

15. Barrat, J. G. Unpublished data.

16. Barrat, J. G., A. E. Rich and W. W. Smith. Unpublished data.

17. Bawden, F. C. 1950. Plant viruses and virus diseases. Third Ed. Chronica Botanica, Waltham, Mass.

18. Beakbane, A. B. and E. C. Thompson. 1945. Abnormal lignification in the wood of some apple trees. Nature 156: 145.

19. Blodgett, F. M. 1923. A new host of mosaic. Plant Disease Reptr. 7: 11.

20. Blodgett, F. M. 1938. The spread of apple mosaic. Phytopathology 28: 937-938.

21. Blumer, S. 1951. Line pattern in Zwetschen. Schweiz. Z. Obst-u. Weinb. 60: 451-454.

22. Blumer, S. 1955. Viruskrankheiten an Obstbaumen. Schweiz. Z. Obst-u. Weinb. 63: 516-519.

23. Blumer, S. 1956. Uber die flachastigkeit (rillenkrankheit) bei apfelbaumen. Schweiz. Z. Obst-u. Weinb. 65: 148-153.

24. Bomeke, J. H. 1954. In 29 Pflanzenschutz-Tagung der Biologischen Bundesanstalt Braunschweig in Heidelberg 5-9 October, 1953. Mitt. biol. ZentAnst. Berl. 80.

25. Bradford, F. C. and L. Joley. 1933. Infectious varigation in the apple. J. Agr. Res. 46: 901-908.

26. Bringhurst, R. S. and V. Voth. 1956. Strawberry virus transmission by grafting excised leaves. Plant Disease Reptr. 40: 596-600.

27. Broadfoot, H. and E. C. Connor. 1956. Control of "twist" in the Gravenstein apple. Agr. Gaz. N. South Wales. 67: 180-188.

28. Brough, C. R. 1958. Work with Gravenstein gnarl. Fruit World and Market Grower. 59: 21.

29. Cation, D. and R. E. Gibson. 1952. Dwarf fruit and decline of apple, a virus disease. Abstr. Phytopathology 42: 4.

30. Chamberlain, E. E., J. D. Atkinson and J. A. Hunter. 1953. Note on the systemic nature of apple mosaic virus in apple trees. N. Z. J. Sci. Tech. A 34: 551-552.

31. Christoff, A. 1934. Mosaikkrankheit oder virus chlorose bei appeln. Eine neue viruskrankheit. Phytopath. Z. 7: 521-536.

32. Christoff, A. 1935. Mosaicfleckigheit, chlorose und steppenfleckigkeit bei apflen, bernen und quitten. Phytopath. Z. 8: 285-296.

33. Ciferri, R. 1956. Recent progress in fruit tree research in Italy. Tijdschr. Plantenziekten. 62: 69-72.

34. Ciferri, R., D. Rui and G. Scaramuzzi. 1954. Reloziano sommoria su alcune malattie da virus e da corenza di alberi fruttiferi nell 'Italia sellentrionale. Riv. Ortoflorofruttic, Ital. 38: 7-8.

35. Cochran, L. C. 1950. Infection of apple and rose with ringspot virus. Abstr. Phytopathology 40: 964.

36. Crane, M. B. 1944. The mystery of Lord Lambourne. The Grower. 22: 10-12.

37. Dyer, R. A. 1949. Botanical surveys and control of plant diseases. Fmg. S. Afr. 24: 119-121.

38. Esau, K. 1953. Plant anatomy. John Wiley, New York.

39. Fernald, M. L. 1950. Gray's manual of Botany. Eighth Ed. American Book, New York.

40. Fernandez Valiela, M. V., M. Bakarcic and A. Turica. 1954. Manual de enfermed ades y plagas de los frutales y forestales en el Delta del Parana. Publ. misc. Minist. Agr., B. Aires. 400.

41. Fisher, H. 1955. Ungewohnliche Berostungen und Rissebildungen bei Boskoop Glockenapfel und anderen apfelsorten eine Viruskrankheit ? Schweiz. Z. Obst-u. Weinb. 64: 125-131.

42. Fisher, H. 1957. Die virose Triebsucht der apfelbaume. Pflanzenarzt 10: 1-2.

43. Fogliani, G. 1952. Segnalazione della virosi a scopazzi del melo. Notiz. Malatt. Piante 21: 10-11.

44. Fulton, R. W. 1956. Non Identity of apple mosaic and tobacco streak viruses. Phytopathology 46: 694.

45. Gardner, F. E., P. C. Marth and J. R. Magness. 1946. Lethal effects of certain apple scions on Spy 227 stock. Proc. Amer. Soc. Hort. Sci. 48:195-199.

46. Gaumann, E. 1950. Principles of plant infection. Hafner, New York.

47. Gilmer, R. M. 1956. Probable coidentity of Shiro line pattern virus and apple mosaic virus. Phytopathology 46: 127-128.

48. Goidanich, G. and E. C. Branzanti. 1954. Le virosi delle piante da frutto in Emilia e Romagna. Ital. Agr. 91: 603-606.

49. Guba, E. F. 1924. New diseases. Plant Disease Reptr. 33: 82.

50. Guengerich, H. W. and D. F. Millikan. 1956. Transmission of the stem pitting in apple. Plant Disease Reptr. 40: 934-938.

51. Harris, R. V. 1954. Plant pathology. Rep. E. Malling Res. Sta. for 1953: 34.

52. Harris, R. V. 1957. Plant pathology. Rep. E. Malling Res. Sta. for 1956: 27.

53. Harris, R. V. and A. F. Posnette. 1956. The production and distribution of virus-free fruit trees at East Malling. Rep. E. Malling Res. Sta. for 1955: 115-119.

54. Harvey, H. L. 1957. Apple mosaic. The Fruit and Market Grower 58: 21.

55. Heald, F. D. 1933. Manual of plant diseases. Second Ed. McGraw-Hill, New York.

56. Hilborn, M. T. Personal communication.

57. Hilborn, M. T. and F. Hyland. 1957. Anatomical changes associated with wood pitting, a suspected virus disease of Virginia Crab apple. Abstr. Phytopathology 47: 16.

58. Hilborn, M. T. and R. Bonde. 1956. Datura sp. as indicator plants for apple and blueberry virus diseases. Abstr. Phytopathology 46: 241.

59. Hockey, J. F. 1943. Mosaic, false sting, and flat limb of apple. Sci. Agr. 23: 633-646.

60. Hockey, J. F. 1955. 35th Annual report of the Canadian plant disease survey 1955. Science Service, Canada Dept. Agr.

61. Hockey, J. F. 1957. Further observations of flat limb of Gravenstein. Canadian J. Pl. Sci. 37: 259-261.

62. Holmes, F. O. 1948. Order virales the filterable viruses. In Bergey's Manual of Determinative Bacteriology. Sixth Ed. Williams and Wilkins, Baltimore: 1125-1296.

63. Jenkins, J. E. and I. F. Storey. 1955. Star cracking of apples in East Anglia. Pl. Path. 4: 50-52.

64. Jossifovic, M. 1937. Plum mosaic a virus disease of plum. Arch. Ministarstva poljoprivred. 7: 131-143.

65. Katwijk, W. van. 1953. Rozet, ein nieuwe virusziekte bij appels. Tijdschr. Plantenziekten. 59:233-236.

66. Katwijk, W. van. 1953. Virusziekte in de vruchtboomwekerij. Versl. PlZiekt. Dienst Wageningen 119.

67. Katwijk, W. van. 1955. Ruwschillegheit bij appels, ein virus-ziekte. Tijdschr. Plantenziekten 61: 4-6.

68. Katwijk, W. van. 1956. Rough skin of apples. Tijdschr. Plantenziekten 62: 46-49.

69. Kirkpatrick, H. C. 1955. Infection of peach with apple mosaic virus. Phytopathology 45: 292-293.

70. Kristensen, H. R. 1956. Flat limb of apple trees. Tijdschr. Plantenziekten 62: 42-46.

71. Lantz, H. L. 1933. Hardy stocks for fruit trees. Trans. Iowa Hort. Soc.

72. Louw, A. J. 1948. Investigations on the cracking of Ohenimuri apples. Fmg. S. Afr. 270: 596-602.

73. Luckwill, L. C. 1950. Some virus diseases of fruit trees in England. Fruit Yearbook 4: 84-88.

74. Luckwill, L. C. 1954. Virus diseases of fruit trees: IV. Further observations on rubbery wood, chat fruit and mosaic in apples. Rep. Agr. Hort. Res. Sta. Bristol 1953: 40-46.

75. Luckwill, L. C. and S. H. Crowdy. 1950. Virus diseases of fruit trees. II. Observations on rubbery wood, chat fruit, and mosaic in apples. Progress report. Rep. Agric. Hort. Res. Sta. Bristol 1949: 69-79.

76. Mahlstede, J. P. and E. S. Haber. 1957. Plant propagation. John Wiley, New York.

77. Mallack, N. 1956. Die wirtschaftliche Bedeutung des apfelmosaiks. Frakt. Bl. PflBau. 51: 225-229.

78. Maney, T. J. 1925. The propagation of own rooted apple stocks. Proc. Amer. Soc. Hort. Sci. 22: 211-217.

79. Maney, T. J. and H. H. Plagge. 1934. Three apple stocks especially well adapted to the practice of double working. Proc. Amer. Soc. Hort. Sci. 32: 330-333.

80. McCallan, S. E. A. 1946. Outstanding diseases of agricultural crops and uses of fungicides in the United States. Contrib. Boyce Thompson Inst. 14: 105-116.

81. McClintock, J. A. 1938. The affinity of varieties other than Grimes on Virginia Crab stocks. Proc. Amer. Soc. Hort. Sci. 36: 131-132.

82. McKenzie, D. W. 1953. The problem of "gnarling" or "flat limb" in Gravenstein apples. Orchardist. New Zealand 26: 2-3.

83. Meijneke, C. A. R. 1956. An indexing scheme in the Netherlands. Tijdschr. Plantenziekten 62: 83-85.

84. Miller, P. R. 1956. Plant disease situation in the United States. F. A. O. Pl. Prot. Bull. 4: 136-139.

85. Miller, V. J. 1954. A trunk disorder of Virginia Crab interstocks. Proc. Amer. Soc. Hort. Sci. 64: 159-164.

86. Millikan, D. F. 1957. Symptoms of scar skin in apple. Abstr. Phytopathology 47: 25.

87. Millikan, D. F. and H. W. Guengerich. 1954. Bark splitting, a possible virus disease in apple. Abstr. Phytopathology 44: 498.

88. Millikan, D. F. and H. W. Guengerich. 1956. Transmission to Amelanchier of an agent causing a disorder on apple. Phytopathology 46: 130.

89. Morse, R. 1916. Maine Agr. Exp. Sta. Bul. 252.

90. Mulder, D. 1953. De proliferatieziekte van appel, een virusziekte. Tijdschr. Plantenziekten 59: 72-76.

91. Mulder, D. 1955. Het eerste symposium over virusziekten van vruchtbomen en Europa te Wadenswil (Zwitserland). Meded. Div. Tuinb. 18: 446-448.

92. Mulder, D. 1955. Ruwschillige vruchten in een bladsymptoom bij appel. Tijdschr. Plantenziekten 61: 11-14.

93. Palmiter, D. H. and K. G. Parker. 1955. Transmission of the causal agent of apple green mottle. Abstr. Phytopathology 45: 186.

94. Posnette, A. F. 1953. Virus diseases and the propagation of fruit trees. Rep. E. Malling Res. Sta. for 1952: 131-135.

95. Posnette, A. F. 1953. Virus transmissions between Prunus and Malus species. Rep. E. Malling Res. Sta. for 1952. 131-135.

96. Posnette, A. F. and D. F. Millikan. 1958. Some virus-like disorders of pome fruit trees in Missouri. Plant Disease Reptr. 42: 200-201.

97. Posnette, A. F. and R. Cropley. 1952. A preliminary report on strains of the apple mosaic virus. Rep. E. Malling Res. Sta. for 1951: 128-130.

98. Posnette, A. F. and R. Cropley. 1952. The rubbery wood virus and apple propagation. Rep. E. Malling Res. Sta. for 1951: 131-132.

99. Posnette, A. F. and R. Cropley. 1954. Distribution of rubbery wood virus in apple varieties and rootstocks. Rep. E. Malling Res. Sta. for 1953: 150-153.

100. Posnette, A. F. and R. Cropley. 1956. Apple mosaic viruses. Host reaction and strain interference. J. Hort. Sci. 31: 119-133.

101. Posnette, A. F., R. Cropley and C. E. Ellenberger. 1953. Progress in the heat treatment for strawberry virus diseases. Rep. E. Malling Res. Sta. for 1952: 128-130.

102. Posnette, A. F. and C. E. Ellenberger. 1957. The line pattern virus disease of plums. Ann. Appl. Biol. 45: 74-80.

103. Prentice, I. W. 1949. Experiments on rubbery wood disease of apple trees. A progress report. Rep. E. Malling Res. Sta. for 1948: 122-125.

104. Ramsfjell, T. 1950. Virusjukdommer pa eple. Gartneryrket 20: 7.

105. Ramsfjell, T. 1952. Virusjukdommer opa hagebruksplanter. Frukt of Baer 5:44-53.

106. Rawlings, C. O. and G. F. Potter. 1936. Unusual severe winter injury to the trunks of McIntosh apple trees in New Hampshire. Proc. Amer. Soc. Hort. Sci. 34: 57-66.

107. Refatti, E. and R. Ciferri. 1954. La virosa del tipo "scopazzi" in vivai di melo. Ann. Sper. Agr. N. S. 8: 1543-1556.

108. Rehder, A. 1940. Manual of cultivated trees and shrubs. Second Ed. MacMillan, New York.

109. Rich, A. E. Personnal communication.

110. Richards, B. L. and L. C. Cochran. 1956. Virus and virus-like diseases of stone fruits in Utah. Utah State Agr. Exp. Sta. and U. S. Dept. Agr. Handbook.

111. Riley, E. A. 1957. Stem pitting of coffee. Commonw. Phytopath. News 3:29-30.

112. Roland, G. 1954. Le problems des viroses des arbers fruitiers. Fruit Belge: 151.

113. Rui, D. 1950. Una malattia inedita: La virosi a scopazzi del melo. Humus 6: 7-10.

114. Sax, K. 1957. The control of vegetative growth and the induction of early fruiting of apple trees. Proc. Amer. Soc. Hort. Sci. 69: 68-74.

115. Schlums, W. and G. Baumann. 1956. Die "flachastigkeit" des apfels in Mitteldeutschland. NachrBl. dtsch. Pfl-SchDienst. Berl., N. F. 10: 56.

116. Shaw, J. K. and L. Southwick. 1943. Certain stock-scion incompatibilities and uncongenialities in the apple. Proc. Amer. Soc. Hort. Sci. 44: 239-246.

117. Smith, K. M. 1957. A textbook of plant virus diseases. Little, Brown, Boston.

118. Smith, W. W. 1954. Occurrence of "stem pitting" and necrosis in some body stocks for apple trees. Proc. Amer. Soc. Hort. Sci. 63: 101-113.

119. Smith, W. W. 1955. Tresteza, a virus disease infecting some body stocks of apple trees. J. N. H. Hort. Soc. 18: 11-14.

120. Smith, W. W., J. G. Barrat and A. E. Rich. 1956. Dapple apple, an unusual fruit symptom of apples in New Hampshire. Plant Disease Reptr. 40: 765-766.

121. Stakman, E. C. and J. G. Harrar. 1957. Principles of plant pathology. Ronald Press, New York.

122. Stubbs, L. L. 1957. Virus diseases of apples and pears and the production of healthy stocks. The Fruit World and Market Grower 58: 41.

123. Thomas, H. E. 1937. Apple Mosaic. Hilgardia. 10: 581-588.

124. Thomas, H. E. 1942. Transmissible rough-bark diseases of fruit-trees. Phytopathology 32: 435-436.

125. Thomas, P. H. and T. D. Raphael. 1932. Internal cork in apples associated with malformed wood growths. Tasmanian J. Agr. N. S. 2: 69-73.

126. Tukey, H. B. and K. D. Brase. 1944. Differences in congeniality of two sources of McIntosh apple budwood propagated on rootstock USDA 227. Proc. Amer. Soc. Hort. Sdi. 45: 190-194.

127. Tukey, H. B., R. L. Klackle and J. A. McClintock. 1954. Observations on the uncongeniality between some scion varieties and Virginia Crab stocks. Proc. Amer. Soc. Hort. Sci. 64: 151-155.

128. Vlasveld, W. P. N. 1952. Wat is er met die apples aan de hand. De Fruitteelt 42: 642-643.

129. Watson, R. D. and A. R. Al-Adhami. 1957. Notes on diseases of fruit trees in Iraq. F. A. O. Pl. Prot. Bull. 5: 104-107.

130. Welsh, M. F. and F. W. L. Keane. 1955. 35th Annual report of the Canadian plant disease survey 1955. Science Service, Canada Dept. Agr.

131. Yarwood, C. E. 1953. Quick virus inoculation by rubbing with fresh leaf discs. Plant Disease Reptr. 37: 501-502.

132. Yarwood, C. E. 1955. Mechanical transmission of an apple mosaic virus. Hilgardia 23: 613-628.

133. Yerkes, G. E. and W. W. Aldrich. 1946. Behavior of apple varieties on certain clonal stocks. Proc. Amer. Soc. Hort. Sci. 48: 227-235.

APPENDIX A

The names of the following plants are in accord with Gray's Manual of Botany (39) or Manual of Cultivated Trees and Shrubs (108).

Amaranthus retroflexus L. Pigweed

Aronia arbutifolia Elliott. Red Chokeberry

Brassica juncea (L.) Coss. Leaf Mustard

Chenopodium album L. Lamb's Quarters

Chrysanthemum Leucanthemum L. var. Pinnatifidum LeCoq and Lamotte. White Daisy

Citrus aurantifolia Swingle. Key Lime

Cotoneaster multiflora Bge.

Cucumis sativa L. Cucumber

Datura stramonium L. Jimson Weed

Eriobotrya japonica Lindl. Loquat

Fragaria vesca L. Alpine strawberry

Gomphrena globosa L. Globe Amaranth

Malus brevipes Rehd.

Malus floribunda Sieb.

Malus halliana Koehne. var. spontanea Rehd.

Malus prunifolia Borkh. var. rinki Rehd.

Malus pumila Mill. Common Apple

Malus robusta Rehd.

Malus sargenti Rehd. var. rosea Rehd.

Malus sieboldii Rehd. var. arborescens Rehd.

Malus sikkimensis Koehne.

Malus toringoides Hughes.

Physocarpus opulifolius Maxim. Ninebark

Prunus avium L. Sweet Cherry

Prunus persica Batsch. Peach

Prunus tomentosa Thunb.

Raphanus raphanistrum L. Wild Radish

Rubus occidentalis L. Black Raspberry

Sorbus pallescens Rehd.

APPENDIX B

A list of trees used as sources of inoculum giving tree components and disorders.

Experimental Orchard Trees

Tree Number	Variety	Bodystock	Rootstock	Disorder
D-19	McIntosh	Virginia Crab	seedling	Malus stem pitting
E-31	McIntosh		Robusta V (A clonal selection of *Malus robusta*)	Dapple apple
F-6		Virginia Crab	seedling	none
F-21	Northern Spy	Virginia Crab	seedling	none
G-14	McIntosh	Virginia Crab	seedling	none
G-27	McIntosh	Virginia Crab	seedling	none
G-28	McIntosh	Virginia Crab	seedling	none
G-29	McIntosh	Virginia Crab	seedling	none
G-30	McIntosh	Virginia Crab	seedling	none
G-31	McIntosh	Virginia Crab	seedling	none
H-11	McIntosh		M.IV	none
H-18	McIntosh	Virginia Crab	seedling	Malus stem pitting, McIntosh fruit lobed
I-4	Cortland & McIntosh	Virginia Crab	seedling	none
I-12	Cortland		seedling	none
J-5	Cortland	Virginia Crab	seedling	none
J-30	Cortland	Virginia Crab	seedling	Malus stem pitting, dapple apple
J-31	Cortland	Virginia Crab	seedling	Malus stem pitting, dapple apple

Tree Number	Variety	Bodystock	Rootstock	Disorder
K-41	Northern Spy		M.IV	Apple mosaic
L-4	Cortland& McIntosh	Virginia Crab	seedling	none
L-5	McIntosh	Virginia Crab	seedling	Malus stem pitting
L-6	Northern Spy	Virginia Crab	seedling	none
L-11	Red Spy	Virginia Crab	seedling	Malus stem pitting
L-28	Northern Spy	Virginia Crab	seedling	none
L-29	Northern Spy	Virginia Crab	seedling	none
M-4	McIntosh	Virginia Crab	seedling	Malus stem pitting
M-5	McIntosh	Virginia Crab	seedling	none
M-17	McIntosh	Virginia Crab	seedling	Malus stem pitting

Miscellaneous Sources

Baldwin 1 From D. McCloud Orchard Wilton, New Hampshire		seedling	Apple mosaic
Baldwin 2 Branch and leaf samples brought in by a representative of a chemical company.		seedling	Apple mosaic
Gravenstein 1 From George Parker Orchard Wilton, New Hampshire		seedling	Apple flat limb
USDA 227 Propagated tree, scionwood obtained from University of Massachusetts.		seedling	USDA 227 virus (latent)

P. tomentosa/J-30 — A *P. tomentosa* seedling had been inoculated buds from J-30, necrotic lesions on older leaves.

P. tomentosa/J-31 — A *P. tomentosa* seedling had been inoculated

	with buds from J-31, necrotic lesions on older leaves.
P. tomentosa/E-31	A *P. tomentosa* seedling had been inoculated with buds from E-31, necrotic lesions on older leaves.

APPENDIX C

Photographs of symptom expression.

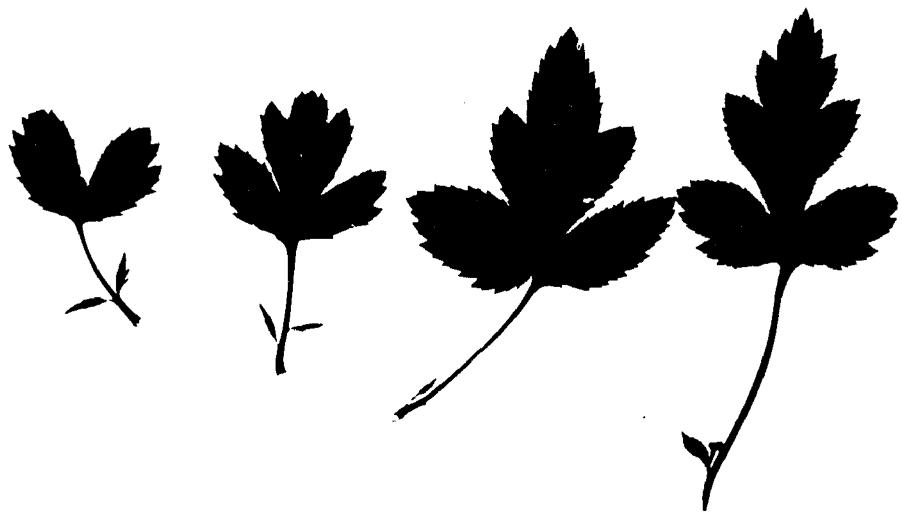

Figure 3. Leaves from a seedling of *M. toringoides* showing distortion symptoms. Normal leaf on right. Plant grown under incandescent light. Inoculum: J-5 Cortland buds.

Figure 4. A seedling of *M. toringoides* showing distorted leaves. Plant grown under incandescent light. Inoculum: D-19 Virginia Crab buds.

Figure 5. Leaves from a seedling of M. floribunda showing distortion symptoms. Normal leaves are on the left. Plant grown under incandescent light. Inoculum: D-19 Virginia Crab buds.

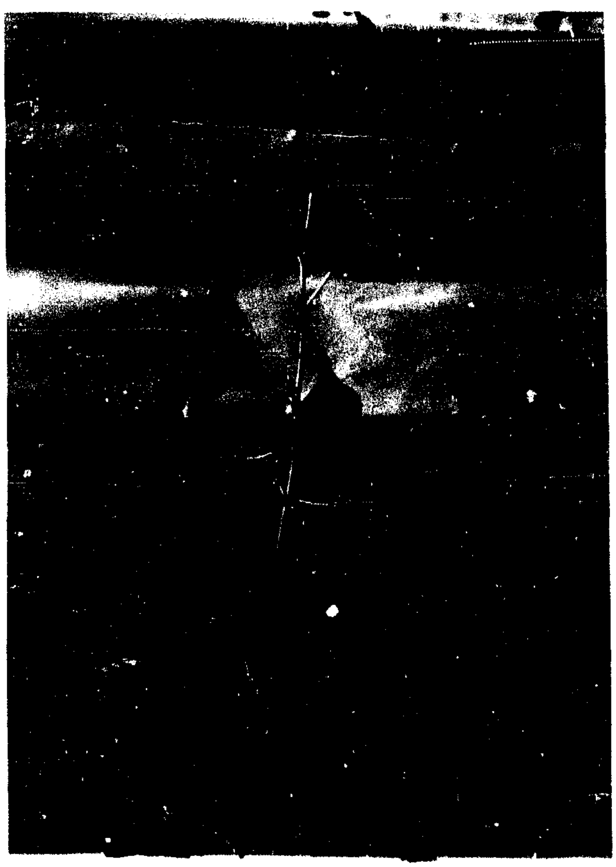

Figure 6. A seedling of M. floribunda showing epinasty of the leaves. Plant grown in the greenhouse. Inoculum: J-5 Cortland buds.

Figure 7. Leaves from a seedling of M. brevipes showing distortion and mottling symptoms. Normal leaf on the right. Plant grown under incandescent light. Inoculum: Gravenstein 1 buds.

Figure 8. A seedling of M. brevipes showing distortion of the leaves. Plant grown under incandescent light. Inoculum: J-5 Cortland buds.

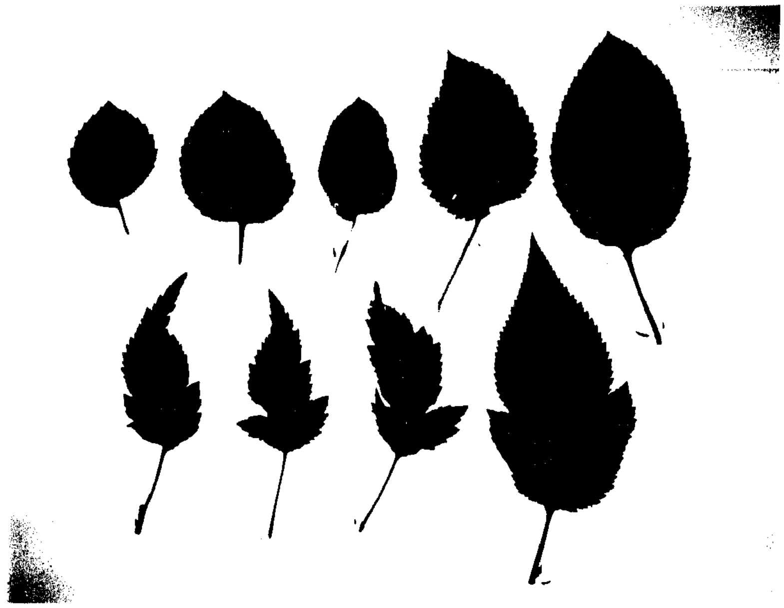

Figure 9. Leaves from a seedling of M. sikkimensis showing distortion and mottling symptoms. Normal leaves on the right. Plant grown under incandescent light. Inoculum: J-5 Cortland buds.

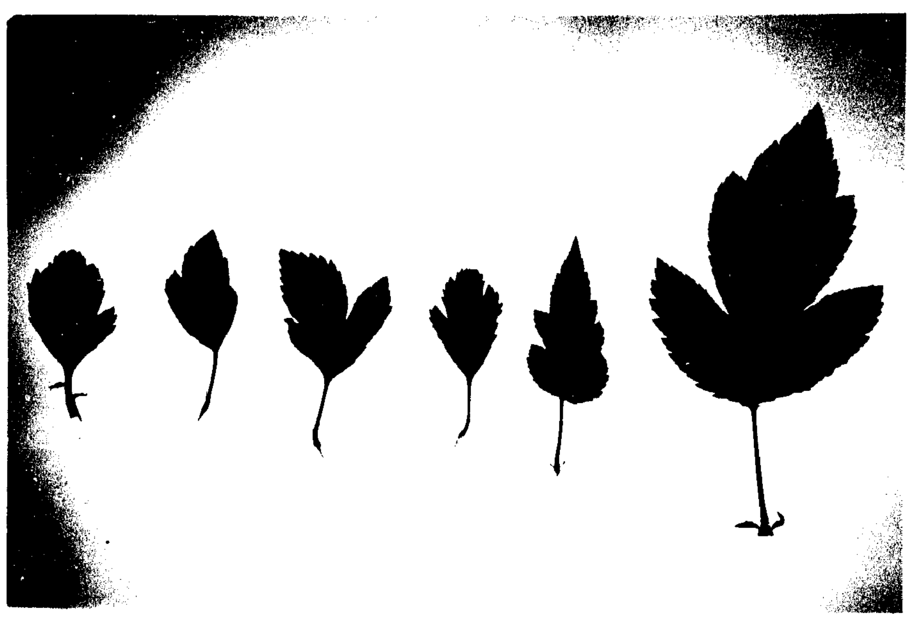

Figure 10. Leaves from a seedling of M. sargenti rosea showing distortion symptoms. Normal leaf on the right. Plant grown under incandescent light. Inoculum: J-5 Cortland buds.

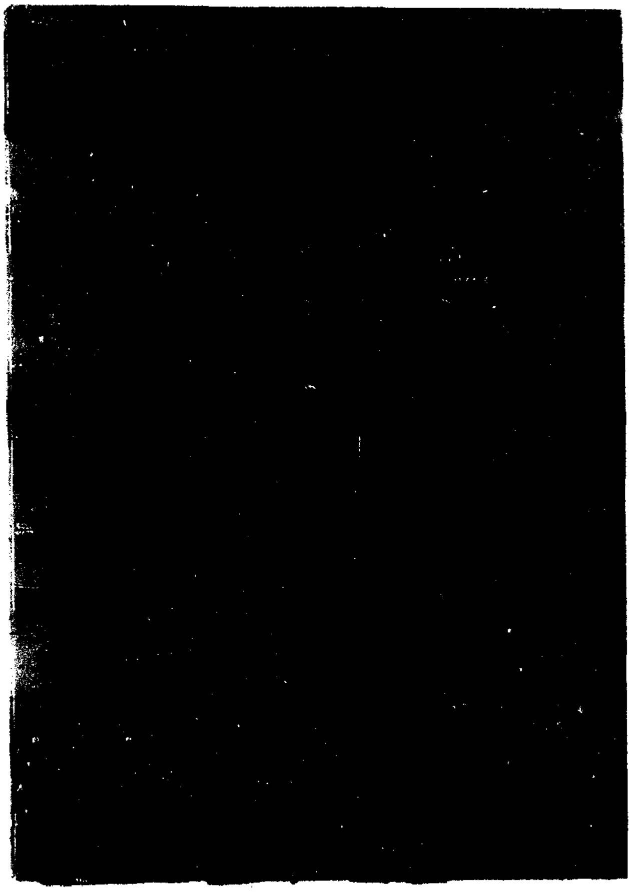

Figure 11. A seedling of M. floribunda showing an elongated type of distortion. Plant grown under incandescent light. Inoculum: J-30 Virginia Crab buds.

Figure 12. A seedling of M. brevipes showing greater distortion on the longer adventitious shoot. Plant grown under incandescent light. Inoculum: J-30 Virginia Crab buds.

Figure 13. Abnormal swellings above the buds on Virginia Crab which had been inoculated with a bud from an apple flat limb virus infected Gravenstein tree.