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The detection of latent viruses of apples has been an important part of the research programs of experiment stations in apple growing areas of the United States and Europe. Valuable indexing techniques have been reported and preliminary surveys have shown that many of the apple varieties grown commercially are infected with one or more viruses. Nurserymen in Oregon have cooperated in a nursery improvement program to secure virus free foundation plants of stone fruit varieties, but the importance of a similar program for applies has not been demonstrated to them.

The Hopa variety of flowering crab apple (Malus adstringens Zabel) had been found to be a good indicator of latent viruses in apple varieties. A survey was made using the Hopa variety to determine the amount of virus present in apple varieties and rootstocks being used by Oregon nurserymen. These studies showed that 83

percent of the apple trees being used for scionwood and 65 percent of the Malling clonal rootstocks were infected with one or more viruses. During the survey 290 scionwood trees grown on ten different rootstocks and 373 budwood samples from 70 Malling rootstock layering beds were indexed. Only 50 individual variety trees and a few East Malling layering beds gave a negative reation. Most of the Merton Malling beds, however, were negative. Some of the reactions on Hopa were mild while other apple stocks reacted severely.

A high percentage of the scion trees grown on Malling rootstocks were severly infected, whereas 50 percent of the scion trees on seedling rootstocks were either virus free or infected with a mild virus. The degree of virus severity in scion varieties was related to the degree of severity in the rootstocks. Domestic seedling rootstocks gave a negative reaction on Hopa and no other evidence of seed transmission was noted. Scion trees grown on E. M. XVI and M. M. 106. rootstocks showed no reaction on Hopa. Layering beds of these clones also tested negatively for latent virus.

In an experiment to determine the effect of virus on nursery trees the data showed superior budstand and tree growth for trees grown from negatively reacting stock. Apple varieties that were indexed as free of virus by these preliminary studies will be available for commercial propagation.

LATENT VIRUSES IN APPLE VARIETIES IN OREGON NURSERIES

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LATENT VIRUSES IN APPLE VARIETIES IN OREGON NURSERIES

INTRODUCTION

During the last ten years increasing awareness of the presence of latent viruses in stone fruits and strawberries has pointed to the need of investigating latent viruses in pome fruits. Latent viruses show no apparent symptoms on infected host plants and are detected by transmission to sensitive varieties or clones termed "index hosts". The technique is known as indexing.

The effects of viruses on apple production are not well established. Serious fruit disorders may appear in one variety and not in another or both fruit and foliar abnormalities may be uniform within the Malus spp. Proof that a virus is the causal agent is dependent on detection of the virus in a diseased host and transmission of the virus from diseased to healthy plants. Often this is a difficult or a time consuming process.

Detection of latent viruses in apple stocks has become an important consideration in experiment station research programs throughout the apple growing areas of the United States and Europe (10, 12, 13, 14, 15, 21). Most apple virus disease projects in the last five years have been attempts to identify the viruses involved in latent complexes, or demonstrations of the value of certain indicator hosts for determining specific viruses and general freedom from

virus infection. Oregon nurserymen propagate apple trees for commercial plantings in Oregon, Washington, California and elsewhere. Programs whereby every nursery in the state will have access to sources of virus free budwood and rootstocks for all nursery propagation have become essential.

The primary objectives of this thesis have been: (1) to determine, by indexing, the extent and severity of latent virus infection in the apple propagation stock used in Oregon nurseries; (2) to study the relationship of imported rootstock sources to the severity of infection in the commercial scion variety trees that were indexed; and (3) observe the effect of latent viruses on bud stand and tree growth in the nursery row.

The general methods used have been adopted from techniques reported by Reynolds and Milbrath (17). In their investigation Hopa crab apple proved to be, under greenhouse conditions, a rapid indicator for latent apple viruses. This variety was selected over more sensitive ones since it indicated strain differences. They believed their data supported strain reactions rather than different virus reactions as the cause of chlorotic leaf spot and stem pitting. Apple budwood which did not react on Hopa failed to cause symptoms in hosts used to determine chlorotic leaf spot and stem pitting but budwood that caused severe symptoms in Hopa produced severe symptoms in these hosts (17).

LITERATURE REVIEW

Spy 227, an apple rootstock variety under investigation by the U. S. Department of Agriculture in 1927, either declined or died when budded with certain scion varieties (20). The condition became known as Spy 227 decline and is probably the first case of a sensitive clone reacting to latent virus. At the time investigators did not suggest a virus disease. They believed the poor performance was caused by genetic differences between Spy 227 and the varieties budded on this rootstock (20). Later Shaw and Southwick (18) and Yerkes and Aldrich (22) reported extensive horticultural testing of Spy 227. Shaw and Southwick found the varieties Blackmac, six out of eight McIntosh strains, Stayman, Shotwell, Starking's Delicious, Delicious, Golden Delicious, Winesap, Arkansas Black, and Turley died after the second year when grown on Spy 227. Yerkes and Aldrich (22) over a period of years obtained corresponding results. The inconsistencies noted within McIntosh strains were related to the sources of budwood or to mutations, the mutants differing in their compatibility with clone 227. These authors, however, suggested that possibly a latent virus could also account for the death or retarded growth of certain varieties grown on the sensitive Spy rootstock. In 1946, experimental evidence was lacking to prove such a hypothesis.

Stem Pitting (SPV), a wood-pitting and bark-cracking disease of Virginia crab body stocks, appeared in apple growing areas of the East in 1940 (11, p. 19). This was the second major period of observation of reactive apple types to possible latent virus infection. Guengerich and Millikan (8) demonstrated transmissibility. Their experiments consisted of working affected Virginia crab buds and bark patches into healthy non-pitted specimens, and inoculating other healthy Virginia crab trees with inoculum from known infected scion varieties. Pitting was not found on the scion varieties top worked on Virginia crab rootstocks. The varietal portion acted as the latent scource of virus and the crab as a sensitive indicator (8).

Cation and Gibson (6) considered Hyslop crab apple to be an indicator of latent virus after close observation of two Hyslop orchards reworked to the variety Jonathan. Grafted Jonathan scions grew normally for several years then gradually declined over a period of six years. Jonathan fruits were small but normally shaped. Fruit borne on Hyslop stock on other branches, was dwarfed, deeply lobed, and generally worthless following grafting. These symptoms were produced by a latent virus inoculum present in the Jonathan scions.

Mink and Shay (13) reported that a Russian seedling designated R12740-7A was useful in determining the presence of SPV and a new

virus disease termed Chlorotic Leaf Spot (CLSV). Shay (14) had examined an orchard at Elste, Holland in 1955 for scab resistant selections. Eight of 22 R12740-7A seedlings had either failed to grow following top-working or were growing abnormally. These selections were suspected of being sensitive to virus present in the stock trees. During 1956 and later, thorough testing of the Russian variety was carried out. Sources of apple budwood with known latent virus infection and varieties selected at random disclosed that, besides the CLSV pattern, SPV symptoms appeared in the indicator within four months after inoculation. Association, either in the presence or absence, of CLSV with SPV was noted in 32 of 36 samples tested. Similar experiments in 1959 by Shay and Mink (19) showed that both SPV and CLSV could be transmitted from a majority of severely pitted Virginia crab sources to R12740-7A. Three trees with non-pitted Virginia crab stems did not contain the SPV but all three contained the CLSV. Two trees were infected with the SPV but not the CLSV. The CLSV syndrome was also found to be present in seven of eight isolates of apple mosaic inoculum and in single isolates of Hyslop decline, apple green mottle, Spy 227 decline, and scar skin (19). In general, these experimental results have shown that the virus or virus complex causing reactions in Spy 227, Hyslop crab, and Virginia crab may have been responsible for the symptoms in

R12740-7A and there was some evidence for relating the new disease CLSV with stem pitting.

An additional comparison of SPV and CLSV was made by Cation and Carlson (5). Two hundred and fifteen double- and single-worked 5-year old apple trees in an orchard were indexed on R12740-7A. A select group of 28 of these trees with SPV sensitive 0-524 interpieces were examined for both SPV and CLSV. Of the 28 trees, 14.3 percent gave the CLSV reaction without showing stem pitting, while 21.4 percent showed stem pitting without giving a CLSV reaction.

Another report of the independent occurrence of these viruses was published by Keane and Welsh (9). A source, 9D-90514, induced stem pitting in Virginia crab but had failed to incite CLSV symptoms in R12740-7A in two seasons following inoculation.

Gilmer and Brase (7) studied the association of CLSV of commercial apples with Spy 227 decline. Varieties dying on Spy 227 contained CLSV while 10 of 11 types that grew well on Spy 227 were free of CLSV. A variety, Red Wealthy 13-40, grew very weakly on Spy 227, but did not induce CLSV in R12740-7A. This was explained on the basis that more than one virus may have been present in the test source. They believed that differences among various varieties in rate of decline when propagated on Spy 227 may involve a complex

of viruses, one of which was CLSV.

Comparison of Hyslop decline with CLSV was made by Cation (3). He showed that, when inoculum from typically affected trees was worked into R12740-7A, the CLSV pattern occurred in all cases. Hopa crab seedlings similarly inoculated developed mottle, leaf pucker, and necrosis.

Keane and Welsh (9) compared Hyslop decline and stem pitting. Virginia crab displayed no symptoms in the season following inoculation with buds from Hyslop affected with dwarf fruit, stem pitting of the woody tissue, and decline. They concluded that stem pitting and dwarf fruit could occur separately on Hyslop crab, and either could occur on trees that have never shown decline symptoms.

In summary, evidence for associating Spy 227 decline, Virginia crab stem pitting, Hyslop decline, and apple chlorotic leaf spot with a single causal virus or strains of one virus entity were supported by a few workers but other papers have shown contradictory results.

The question of latent virus causing symptomatic effects in sensitive clones was supported in all cases. As a result a wide range of apple clones and flowering crabs have been tested. In Washington, Bodgett and Aichele (1) subjected numerous flowering crabs and other clones to sources of SPV inoculum. Die-back and

foliage reactions were noted after 43 days in a number of these varieties. The most promising crab types were Irene, Jay Darling, Lemoine Crab, Purple Wave, Hopa, Red Flesh, Eley, Evelyn, Van Eseltine, Carmine, Japanese floribunda, Red Silver, Red Vein, and Rose Tea crab. Some very severely affected clones, i.e. Jay Darling, Lemoine Crab, Purple Wave, and Van Eseltine did not maintain a bud stand when propagated on infected rootstocks and this was used as a diagnostic criterion for virus presence.

A dwarfing and mottling of Hopa crab apple following regrafting on apple rootstocks previously budded to commercial varieties killed by a November freeze suggested to Reynolds and Milbrath (16) the testing of some 88 flowering crab varieties for latent virus sensitivity. This was the first time a large number of crab apple clones were tested under uniform greenhouse conditions as possible indicators for latent apple viruses. Although Jay Darling, M. theifera, Purple Wave, Purple Lemoine, Ming Shing, and Crimson Brilliant reacted rapidly and severely to selected sources of inoculum, Hopa was chosen as the most promising greenhouse indicator because of the ability to distinguish severity of the strains of virus involved. All seven promising varieties showed some variation but Hopa was the most uniform in its reaction. Other more severely reacting clones of crab apple gave no indication of strain of virus involved,

or whether a lack of growth was due to a virus or to graft failure. Five degrees of sensitivity ranging from negative to very severe were noted. This allowed for the selection of infected apple sources reacting uniformly. Reynolds and Milbrath (17) suggested that the Hopa reaction was caused by either CLSV or SPV or strains of the same virus. Hopa, then, appeared to be an ideal general indicator for viruses latent in commercial apple varieties and could be used for studying complexities under empirical conditions.

Other workers in the U. S. and Europe have failed to evaluate Hopa as a good indicator host, possibly because under field conditions the reaction was not nearly as distinct and therefore no improvement over other quickly sensitive clones (1, 2, 3, 9).

Whether or not latent viruses were carried in seedling rootstocks routinely used to propagate index host plants was a problem needing investigation. Cation (4) set up experiments to determine if seedlings were infected when grown from seed produced by declined Hyslop trees showing CLSV on R12740-7A. Seeds were collected from a specimen tree which had shown virus symptoms for ten years and seedlings were grown to sufficient size for budwood. Both random and selected inocula were worked into R12740-7A indicators. Of the 116 seedlings tested, none gave symptoms, even after six months. He also used 600 domestic seedling apple

rootstocks to propagate R12740-7A. Under greenhouse conditions, most of the buds grew normally, but in the few cases of failure the original seedlings were sampled and found normal. This presents evidence from 716 plants studied that the CLSV was not seed transmitted.

Within the last three years certain of the East Malling Research Station rootstock clones were found to be infected with latent viruses while others were apparently free. Campbell (2) and Shay and Mink (19) reported virus freedom in Merton Malling 104, 106, and 111. They found some Indiana M. M. 106 layering beds and many M. M. 109 sources infected. Mink and Shay (14) and Cation and Carlson (5) showed East Malling I to be carrying CLSV. Further testing added E. M. 2, 9, and 7 to the list (5, 19) Keane and Welsh (9) also found E. M. II to be virus infected. Among the hosts used to make determinations were both R12740-7A and Hopa crab.

Apparently most commercial orchard varieties of apples are infected with one or more latent viruses. Shay and Mink (19) reported the following infection percentages in six different varieties: Delicious 69, Golden Delicious 75, Jonathan 91, Rome 90, Stayman Winesap 60, and Turley 81. From various reports and communications[1/] at least one orchard tree of the following varieties has been

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1/ Personal communication with Gaylord I. Mink and Merrit D. Aichele.

found free of CLSV and possibly other latent viruses: Cortland, Crandall, Delcon, Delicious-Starking, Delicious-Richared, Fireside, Greensdale, Grimes, Jonathan-Black Jon, Jonathan-Jonared, Lakeland, Macoun, McIntosh-Black mac, McIntosh-Double Red, Melrose, Red Gold, Rome-Double Red, Ruby, Spartan, Stayman Red, Turley, Wealthy, Winesap, and Wrixparent.

MATERIALS AND METHODS

Hopa crab apple (Malus adstringens Zabel) was used exclusively in this study as the indicator host for all latent virus determinations when apple varieties and rootstocks were indexed. Budwood was obtained from a group of scionwood source trees grown at the Botany and Plant Pathology experimental plots near Corvallis. These trees originated from a symptomless clone maintained in a flowering crab collection at the Lewis Brown Horticultural Farm, east of Corvallis.

Hopa crab index trees were prepared from the budwood by whip-and-tongue grafting 2-bud scions of Hopa on one-quarter inch caliper Winesap apple seedlings obtained from Pacific Coast Nursery Company, Portland. Parafilm M cut to 3/4 inch spools on a bandsaw was used to cover all cut surfaces of the scion graft except the tip which was sealed with hot paraffin base grafting wax. Lots of 150 Hopa index trees were prepared and stored in polyethylene bags until the time of inoculation.

Inoculations were accomplished by chip-budding. Two chip-buds, cut from a budstick source to be tested for latent virus, were placed two to four inches below the indicator graft on opposite sides of the seedling. Cut surfaces were protected with Parafilm M.

The inoculated trees were planted in cans filled with soil to

within an inch of the top seam. Number 10 cans were used when indexing the commercial varieties; 46 oz. juice cans were used for the Malling rootstock index because they required less space for each unit. The soil mixture consisted of sandy loam and peat moss fertilized with sheep manure, ammonium sulphate and commercial lime. Each can was placed six inches apart on ground beds in a greenhouse with an approximate 70° F. air temperature during the test interval February 1 to May 25. Painted wooden labels coded with variety or clonal line, grower, and time of inoculation identified every plant.

EXPERIMENTAL PROCEDURE AND RESULTS

EXPERIMENT I

Purpose: This experiment was to determine the incidence and severity of latent viruses in commercial apple varieties grown in Oregon nurseries.

Procedure: During the winter of 1960-61, 15 nurseries representing the significant Oregon growers, furnished budwood for virus indexing from 290 apple scion wood trees. Most of the trees were commercial fruiting types, but a few were considered fruiting crab apples. This collection included 101 named varieties, some of which may be synonymous. At the time of budwood collection, accurate data covering the original source, history, and understock of every scion tree to be tested was compiled from records taken during an interview with either the owner or field manager. In February 1961, the scion trees were indexed by inoculating groups of three Hopa crab index trees with buds from each scion tree source furnished by the nurseries. Nine hundred and five Hopa crab index trees were used, of which 35 remained uninoculated as controls.

Results: Virus symptoms were apparent on some Hopa crab index trees 28 days following inoculation, but the final uniform readings

were made after five to seven weeks.

Each group of three trees inoculated with buds from a single scion source compared favorably in degree of reaction as a group and was given group rating on the basis of being placed in one of five categories.

The rating categories used were: (Figure 1)

NEGATIVE	--	no apparent symptoms.
MILD	+	slight leaf flecking with purplish necrotic spots.
MODERATE	++	numerous purplish necrotic spots on leaves but no stunting.
SEVERE	+++	purplish necrotic spots coalescing, plants stunted, but leaves of normal shape.
VERY SEVERE	++++	severe dwarfing, epinasty and necrosis of terminal shoots or apices.

Table I shows the relationship of the number of scion varieties on a specific rootstock to the severity of the reaction on Hopa. In Table II the data are summarized to show a comparison between the results of indexing scion varieties on seedling rootstocks and indexing scion varieties on Malling rootstocks. Degrees of Hopa reaction were produced from 240 scion sources or 83 percent of the total trees indexed. The ratio of mild-moderate to severe-very severe ratings was greater for the scion varieties on seedling rootstocks than for those on Malling roots. This indicates milder infection in

Figure I. Hopa crab apple showing five degrees of severity produced by viruses in apple inocula. The increase in severity is shown from normal on the right to very severe on the left.

Table I. Relationship of Rootstock to the Severity of the Virus Reaction on Hopa.

Total Number of trees indexed*	Rootstock	Severity of the Hopa reaction				
		--	+	++	+++	++++
170	Domestic Seedling	35	23	31	45	36
14	Clark Dwarf Seedling	6	0	0	0	8
7	Black Twig-Domestic Seedling Combination	0	0	4	2	1
13	East Malling I	0	2	0	1	10
7	East Malling II	0	0	3	0	4
11	East Malling IV	0	3	2	1	5
35	East Malling VII	0	4	10	12	9
18	East Malling IX	0	1	3	4	10
6	East Malling XVI	3	1	0	0	2
9	Merton Malling 106	6	1	0	0	2
290		50	35	53	65	87

* Includes a random distribution of the 101 different varieties used in Oregon nurseries.

Table II. Summary of the Reaction of Hopa Crab Apple to Latent Viruses from Different Propagating Stock.

Plants indexed	Severity of Hopa reaction --	+	++	+++	++++	Total trees	Percent infected
Variety trees on seedling rootstock	41	23	35	47	45	191	79%
Variety trees on Merton Malling clonal rootstocks	6	1	--	--	2	9	33%
Variety trees on East Malling clonal rootstocks	3	11	18	18	40	90	97%
Total trees indexed	50	35	53	65	87	290	83%

varieties on seedling roots. More than 50 percent of the varieties on Malling rootstocks were on East Malling VII and IX and the severity ratings for these trees differed markedly. Varieties on E. M. VII produced 11 percent mild to 26 percent very severe reactions, while those on E. M. IX showed 5.5 percent mild and 55 percent very severe reactions on Hopa.

Twenty-one percent of the scion varieties on seedling rootstocks gave a Hopa negative reaction whereas only nine percent of the scion varieties on Malling rootstocks reacted negatively. All uninoculated Hopa index trees used as controls were negative.

Table III reports the percent of the more popular Oregon scion varieties that were infected with latent virus. The most widely

sampled varieties were Delicious, Gravenstein, Jonathan, Rome, Winesap and Yellow Delicious. All were over 70 percent infected but none were uniformly infected. Jonathan showed the largest percent negative reacting scions. Six of the 16 varieties listed in Table III were uniformly infected. Of these six varieties, two, King and Yellow Newtown, are grown in quantities by Oregon nurseries.

Table III. Relative Occurrence of Latent Viruses in Popular Apple Varieties in Oregon.

Variety	Percent Scion Sources Reacting on Hopa					Total Trees Indexed	Total Percent Infected
	--	+	++	+++	++++		
Beacon	0	33.3	33.3	0	33.3	3	100
Delicious	15.9	11.1	25.4	20.6	27.0	63	84.1
Gravenstein	10.0	10.0	21.0	27.4	31.6	19	90.0
Jonathan	28.5	4.9	19.0	28.6	19.0	21	71.5
King	0	12.5	37.5	12.5	37.5	8	100
Lady	25.0	0	25	0	50.0	4	75.0
Lodi	16.6	16.6	0	50.0	16.6	6	83.4
Mc Intosh	25.0	12.5	12.5	25.0	25.0	8	75
Melrose	0	50.0	0	25.0	25.0	4	100
Red Spy	12.5	12.5	0	12.5	62.5	8	87.5
Rome	11.1	16.7	0	22.2	50.0	18	88.9
Spitzenburg	0	42.9	14.2	28.7	14.2	7	100
Winesap	7.7	23.0	23.0	30.8	15.5	13	92.3
Yellow Delicious	23.5	5.9	17.7	23.5	29.4	17	76.5
Yellow Newtown	0	16.6	16.6	49.8	16.6	6	100
Yellow Transparent	0	0	40.0	0	60.0	5	100

EXPERIMENT II

Purpose: This experiment was to determine the incidence and severity of latent virus in East Malling and Merton Malling series of clonal apple rootstocks grown in Oregon nurseries.

Procedure: In December 1961 and January 1962, 373 budwood samples for indexing were furnished from 70 Malling layering beds established in 13 nurseries located in Clackamas, Hood River, Jackson, Linn, Marion, and Washington counties, Oregon. The term "layering bed" as used here means a special area where a single numbered clonal line of Malling understock was established. Oregon Malling layering beds were established by planting one-year old rooted shoots about three feet apart in rows four to eight feet apart. These shoots are wired down horizontally and in the spring the soil is hilled up so that new shoots developing along the length of the layer may root. During the following November the bed is opened and all the rooted shoots removed. A few of the stronger shoots that have not rooted are layered to produce a new crop.

Samples were collected by selecting a budstick at 50 foot intervals from beds totaling less than 700 feet and having their origin from a single source. Layering beds over 700 feet were sampled at random if established from a single source. Beds from mixed

sources or those established by budding or grafting the clonal number desired on some other rootstock for the first year were sampled in a manner to insure separate identity.

In March, each budstick collected was indexed on a single Hopa crab index tree. Four hundred and one Hopa crab index trees were used during the season; 373 were inoculated and 28 served as controls.

Results: Virus symptoms and severity of reactions were rated and recorded 54 days after inoculation when a uniform reading could be taken. For summary comparisons, in Tables IV and V, the mild and moderate ratings were combined and the severe and very severe ratings were combined. Two hundred forty four inoculated Hopa index trees showed symptoms. Twenty nine percent of the Merton Malling clones and 82 percent of the East Malling clones reacted. All uninoculated controls remained symptomless.

Nearly all Merton Malling 109 beds reacted uniformly severe. M. M. 104, 106 and 111 samples were negative from all layering beds indexed except those established at a single nursery where the original stock was "nurse rooted" on East Malling VII. "Nurse rooting" refers to the practice of budding or grafting a clone on an established layered understock for the purpose of maintaining it until large enough to layer.

Table IV. Severity and Extent of Virus Infection in Merton Malling Clones as Shown by the Hopa Crab Index.

M. M. clone	Number tested	No reaction	Mild or moderate	Severe or very severe	Percent infected
104	39	33	3*	3*	15
106	36	34	1*	1*	5
109	19	0	2	17	100
111	20	14	1*	5*	30

* Nurse rooted by grower.

Table V. Severity and Extent of Virus Infection in East Malling Clones as Shown by the Hopa Crab Index.

E. M. clone	Number tested	No reaction	Mild or moderate	Severe or very severe	Percent infected
I	19	6	1	12	69
II	41	2	13	26	95
IV	6	0	5	1	100
V	5	0	1	4	100
VII	52	0	21	31	100
VIIa	11	1	3	7	91
IX	57	0	12	45	100
XVI	26	25	0	1	4
XXV	28	0	12	16	100
XXVI	14	14	0	0	0

Twelve of 15 samples of East Malling I from four layering beds reacted severe or very severe. Of the remaining three samples one was mild and two were negative. Samples from another layering bed all tested negative. The samples from the first four layering beds were of E. M. I stock originally obtained from Canada and Oregon State University in 1950. Samples from the negatively

reacting bed were of E. M. I clones obtained at Oregon State University in 1957.

Over 60 percent of the samples from layering beds of East Malling II, V, VII, VIIa, IX and XXV reacted severely or very severely on Hopa but only 16 percent of E. M. IV samples gave a severe or very severe reaction.

EXPERIMENT III

Purpose: This experiment was performed to determine if negatively reacting apple propagation stock showed superior growth in the nursery row.

Procedure: In September 1961, trial plot spaces were provided by four nurseries and the Botany and Plant Pathology Farm near Oregon State University. A total of 332 rootstock liners were budded one inch above the soil line with budwood from indexed commercial scion varieties reacting negative, mild, severe, or very severe on Hopa crab. The three rootstock types, domestic seedling, East Malling XVI and Merton Malling 104, were chosen because of previous reports that these stocks indexed negative for latent virus at several experiment stations in the United States and Europe. Later indexing of the layering bed sources from which the rootstocks were used showed M. M. 104 to be infected.

All variety sources but Bisbee and the Gravenstein sources on domestic seedling rootstocks were lined out as follows: a given number of infected buds followed by a given number of negative buds of the same variety on identical rootstocks were propagated in alternating groups of 8, 10 or 17. The Bisbee was budded in the following manner: ten alternating groups of three trees each of negative, mild and very severe varieties were propagated in a row on E. M. XVI rootstocks. The Gravenstein trees on domestic seedling rootstocks were planted in alternating sets of three, negative, severe and mild reactors.

Results: Measurements and evaluations were recorded October - December 1962 and the results are shown in Table VI. All trees in the experiment were evaluated for bud stand, stem caliper, and height except the series grown on M. M. 104 rootstocks. Height measurements for this series could not be taken because the trees were pruned to force the terminal budshoots to branch. Caliper dimensions were taken with a tape calibrated in centimeters by extending the tape around the circumference of the budded variety one inch above the bud union. Height determinations were made with a standard steel tape calibrated in inches by extending the tape from the bud union to the apex of the highest terminal bud.

Successful bud stand was noted in 94. 3 percent of the trees

Table VI. Effect of Latent Apple Virus on Budstand and Nursery Growth.

Rootstock	Variety Name	Variety Hopa Rating	No. Trees Budded	Budstand %	Average Height (in.)	Average Caliper (cm)
E. M. XVI	Bisbee	++++	10	80	50.2	4.5
"	"	+	10	100	49.6	4.4
"	"	--	10	100	52.2	4.6
E. M. XVI	Gravenstein	++++	10	100	49.6	3.9
"	"	--	10	100	58.9	4.7
Seedling	"	+++	10	100	53.0	4.5
"	"	+	10	78	56.3	5.1
"	"	--	10	100	57.1	5.1
M. M. 104	"	++++	10	100	-	4.9
"	"	--	10	100	-	4.6
E. M. XVI	Jonathan	++++	8	63	54.0	4.7
"	"	--	8	100	51.0	4.5
Seedling	"	++++	10	70	66.7	5.3
"	"	--	10	90	69.0	5.4
E. M. XVI	Lodi	+++	8	88	53.3	4.8
"	"	--	8	100	56.6	5.1
M. M. 104	"	++++	10	90	-	4.8
"	"	--	10	100	-	5.1
E. M. XVI	Red Spy	++++	8	75	56.8	5.3
"	"	--	8	100	52.2	5.2
E. M. XVI	Ruby	++++	10	100	46.2	3.7
"	"	--	10	100	53.6	4.3
Seedling	"	++++	10	100	56.1	5.2
"	"	--	10	100	64.4	6.0
E. M. XVI	Starking's	++++	17	89	49.9	3.5
"	Red Deli-	--	17	100	49.4	3.4
M. M. 104	cious	++++	10	100	-	4.7
"	"	--	10	100	-	4.3
E. M. XVI	Winter	+++	10	100	50.2	3.5
"	Banana	--	10	100	52.1	4.6
Seedling	Yellow	++++	10	80	64.5	5.2
"	Delicious	+	10	100	64.7	5.4
"	"	--	10	90	61.6	4.8

budded. Seventy four percent of the buds that failed were from sources testing either severe or very severe on Hopa.

Eight of 12 comparisons of average height and stem caliper showed significantly greater growth measurements for trees grown from virus free budwood. The trees grown from budwood testing severe or very severe on Hopa were smaller both in height and stem caliper. In instances where mildly reacting budwood was used for propagating trees the average height and stem caliper measurements for these trees were not significant. A part of the experiment included trees grown from negative buds propagated on M. M. 104 rootstocks. The layering bed from which these rootstocks were taken was indexed and found diseased after the nursery experiment had been started. Growth evaluations for stem caliper showed larger average measurements for trees grown from infected budwood than for trees grown from negative budwood. Infection in the rootstocks induced poor performance of negative indexed budwood in this experiment.

DISCUSSION

Oregon State University and the Oregon Department of Agriculture have, through a nursery improvement program, supplied nurserymen with virus free foundation stock for many of the stone fruit varieties. A similar program for apples has not been feasible because (1) growers and buyers of apple nursery stock have not been aware of virus problems and (2) virus free apple propagation stock was not available. For the most part only latent viruses have been studied in the past and their importance to apple production has not been demonstrated. The work reported in this thesis was an attempt to investigate some of these problems.

Indexing of the apple variety scionwood trees and apple rootstocks used in Oregon nurseries has shown that a quick and reliable indicator host, Hopa crab, proposed for use by J. E. Reynolds and J. A. Milbrath (17), is valuable as an experimental tool in latent virus determination. Whether Hopa detects all latent viruses of apples or only a single virus is not known. Hopa indexing data have shown, however, that 83 percent of the scionwood sources and 65 percent of the rootstock layering bed sources in Oregon are infected with one or more viruses. The stock that did not show a virus reaction will undergo additional indexing as new techniques for detecting

other viruses are developed. In the meantime, apple varieties shown to be virus negative by this preliminary indexing will be furnished to interested nurserymen.

Nurserymen have not been aware of virus infection in the Malling series of rootstocks commonly used to dwarf or adapt apple trees to specific conditions. The indexing experiments with these stocks have helped to demonstrate the importance of maintaining identity and isolation of clonal lines. Spread of infection has been noted in the case of "nurse rooting". Variation in results of indexing East Malling I beds shows that the source of material may determine virus infection. E. M. I beds established from plants obtained at Oregon State University during 1950 showed reactions but a bed having its origin from Oregon State University in 1957 indexed negative. Most of the varieties which were Hopa negative were on rootstock sources now known to be free of virus. Of 63 uninoculated Hopa crab index trees used as controls during experiments I and II, none reacted. This indicates that seedlings used as rootstocks for these trees were not infected with latent virus. The negative varieties found on Malling rootstocks were on E. M. XVI and M. M. 106. When layering beds of these clones were indexed on Hopa, a single sample of E. M. XVI reacted. All samples of M. M. 106 were negative for virus.

Data in Table VII show a close correlation between the severity ratings for varieties grown on East Malling clonal rootstocks and the ratings for the layering beds indexed independently. Very close correlation is noted with E. M. II, VII, and IX. Although data for E. M. IV were not as significant there was a comparison trend toward a milder rather than a more severe rating. Samples of E. M. IV were from one layering bed, the only remaining one in Oregon. This small sample size may account for the variation in percent. All of the indexing data suggest that the degree of latent virus severity in rootstocks used for propagation very decidedly influences the severity in the varietal portion of any apple tree grown on such rootstocks.

Table VII. Comparison of Hopa Crab Reactions of East Malling Layered Apple Clones with Apple Varieties Grown on East Malling Clones.

Source	Percent negative* or mildly reacting sources	Percent severely** reacting sources
Scion varieties on E. M. I clones	15	85
E. M. I clones	37	63
Scion varieties on E. M. II clones	33	67
E. M. II clones	36	64
Scion varieties on E. M. IV clones	51	49
E. M. IV clones	83	17
Scion varieties on E. M. VII clones	40	60
E. M. VII	40	60
Scion varieties on E. M. IX clones	22	88
E. M. IX clones	21	89

* includes moderately reaction sources.

** includes very severly reaction sources.

Nurserymen have mentioned, from time to time, the inconsistencies in bud stand noted with some varieties during certain years. They believed that seasonal weather or horticultural factors have influenced successful or unsuccessful budstands. Very likely virus was the cause of poor budstand. Data from experiment III support this view. Ninety percent of the bud failures in one year old nursery trees were associated with infected bud sources, primarily the ones rated severe or very severe.

Preliminary experiments have demonstrated that average height and caliper comparisons were greater for apple trees propagated from negatively reacting sources. Although the sample sizes were small, 8 of 12 comparisons showed superior growth in favor of negative buds propogated on negative rootstocks. Measurements of trees grown from negative buds on infected M. M. 104 rootstocks were inconsistent. Latent virus has an important influence on other nursery crops and when larger experimentation is employed with apple stocks more conclusive information may be available for apples.

SUMMARY

1. In order to investigate the importance of growing virus free apple trees surveys were made to determine the incidence and severity of latent viruses in Oregon propagation stock.
2. Experiments were performed to determine if non-infected apple trees grew better than infected apple trees in the nursery row.
3. Hopa crab apple, a variety sensitive to strains of latent viruses, was used as the indicator for all virus determinations.
4. Two hundred and forty of 290 scionwood trees and 244 of 373 Malling rootstock sources produced virus symptoms in Hopa. Reactions on Hopa varied in degree and were rated in five categories from negative to very severe.
5. Scionwood trees on seedling rootstocks produced a mild Hopa reaction more frequently than scionwood trees on Malling rootstocks.
6. Rootstocks definitely influenced the presence and severity of latent virus infection in the scion varieties tested. Scion trees with E. M. I, II, IV, VII and IX rootstocks caused a Hopa reaction very similar to the reaction given by clonal sources of these rootstocks. Varieties indexed as virus free were on seedling rootstocks, E. M. XVI and M. M. 106 and samples from the

original source of these rootstocks also indexed free from virus.

7. Scion varieties popular in Oregon were over 70 percent infected. Jonathan showed the largest percent negative reacting scions.

8. Many layering beds of Merton Malling 104, 106 and 111 tested negative for virus but M. M. 109 was uniformly infected. Some sources of East Malling clones I, II, VIIa and XVI tested virus free and all sources of E. M. XXVI were virus free. Other E. M. clones were uniformly infected. East Malling I layering beds established from divergent sources during different years did not react consistently. Infection was associated with one source and not another.

9. None of the many control trees used in the experiments developed virus symptoms. This supported the other reports that latent viruses are not seed transmitted.

10. Nursery experiments demonstrated that virus free budwood propagated on virus free rootstocks produced superior trees in the nursery row.

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