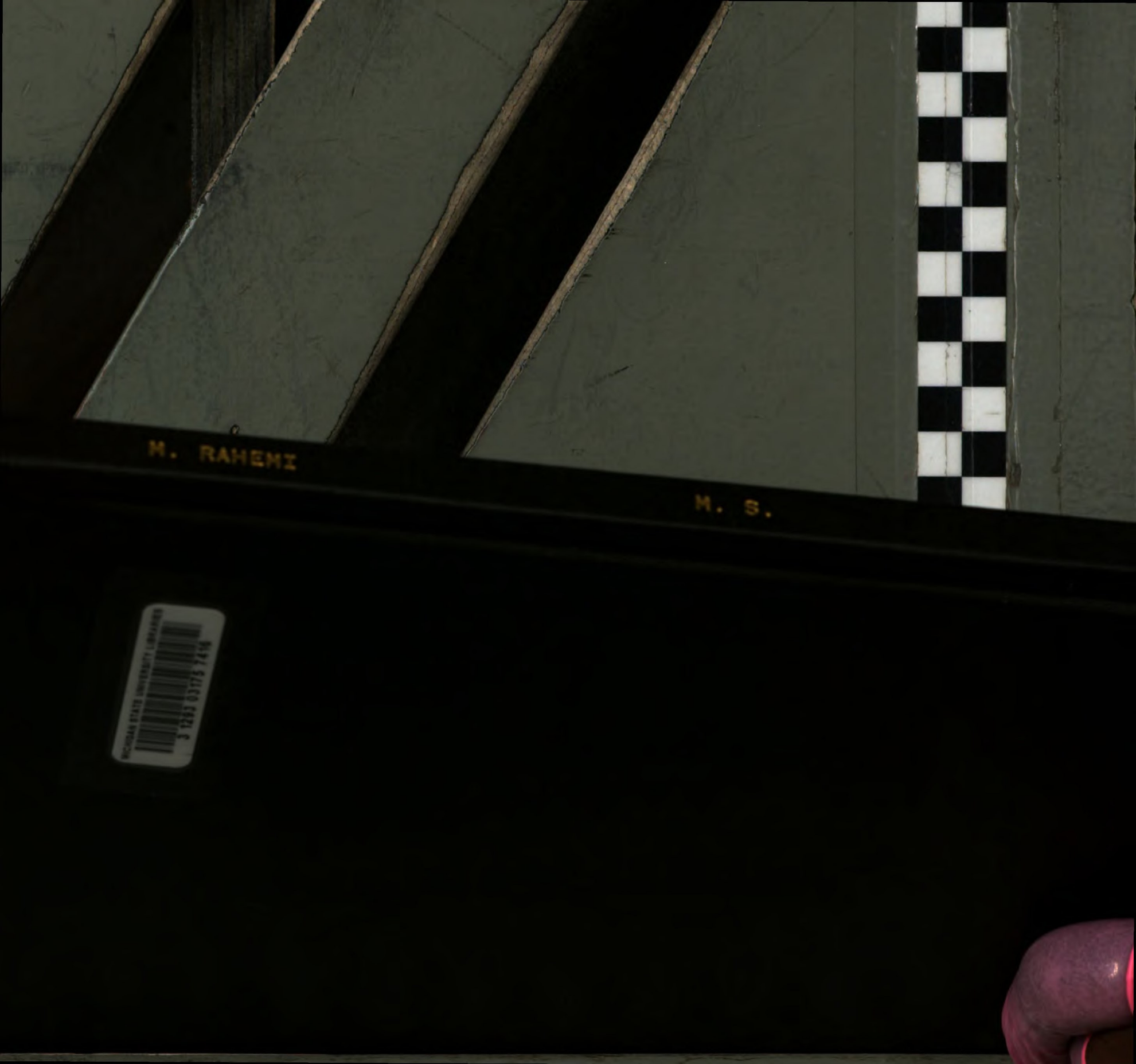
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INOCULATION AND INDEXING PRACTICES FOR USE IN SCREENING SOUR CHERRIES FOR GENETIC RESISTANCE TO SOUR CHERRY YELLOWS DISEASE

By

Majid Rahemi

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ABSTRACT

INOCULATION AND INDEXING PRACTICES FOR USE IN SCREENING SOUR CHERRIES FOR GENETIC RESISTANCE TO SOUR CHERRY YELLOWS DISEASE

By

Majid Rahemi

Virus indexing tests were conducted for necrotic ringspot and prune dwarf viruses on 18 isolates from a wide geographic area of Michigan's commercial sour cherry orchards. In 1976, 10 of 18 isolates showed positive reaction on Cucumis sativus L., a non-differentiating indicator. In 1977, 47 isolates from 10 orchards were indexed on Chenopodium quinoa and Cucurbita maxima var. Buttercup, with 27 isolates having a positive reaction, 19 to necrotic ringspot virus, 8 to prune dwarf virus and 4 to both. Serological agar diffusion tests for the identification of necrotic ringspot virus and prune dwarf virus were inconclusive in 1976 but showed some isolates to have both prune dwarf virus and necrotic ringspot virus in 1977 tests. Methods of inoculation were evaluated to

establish their effect on virus infection and subsequent identification. Budding diseased buds into one-year-old 'Montmorency' on Prunus mahaleb rootstock gave the greatest percent infection and best identification results. It is suggested that this method would be best for inoculation of one-year-old tests that propagates in a breeding program for tolerance to prune dwarf and necrotic ringspot viruses.

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INTRODUCTION

Introduction

In Michigan and other Great Lakes states where sour cherries are grown, sour cherry yellows is a serious disease of 'Montmorency' sour cherry trees. The disease is important to the cherry growers because it causes reduction of growth, premature defoliation, poor spur formation, poor fruit set, and ultimately decreases the profitable life of the orchard. It has been reported that in 13 to 21 year-old trees known to have been diseased for five years or more, the average reduction in yield was approximately 50 to 62 per cent (52).

Initially, sour cherry yellows was thought to be a physiological condition within the tree. Keitt and Clayton (39) demonstrated that SCY was graft transmissible. They reported finding yellows symptoms on leaves in cherries (Prunus cerasus L.) and concluded that a virus caused the disease. In 1948, Moore et al. showed mechanical transmission of a virus from sour cherry to cucumber (Cucumis sativus L.). Subsequent work has shown herbaceous host range and symptom differences among viruses isolated from sour cherry, indicating transmission of more than one virus (22, 32). It was soon recognized that prune dwarf virus PDV was involved in the sour cherry yellows disease (37, 67). The relationship of necrotic ringspot virus NRSV to the sour cherry yellows disease was to remain a problem for 20 years (26).

Necrotic ringspot virus NRSV was known to occur widely among Prunus species (9). It was reported that while NRSV could occur alone, the yellows disease apparently was always associated with it (10), and the yellows symptom usually followed NRSV infection by a year or more (43).

Synergism, interference, and cross protection phenomena have all been reported in research dealing with NRSV and PDV cherry viruses (13, 46). Thus, plant breeding for virus tolerance must take into consideration both viruses.

PDV and NRSV are pollen born viruses and can be easily transmitted by pollen from old orchards to the young trees in the new ones (8, 14, 34, 61, 65) and through seed when infected seedling rootstock are used (6, 31, 58). They are both graft transmissable (39).

The rate of virus spread in young orchards is dependent upon the age of the orchard, the proximity of older diseased trees and the amount of disease within the orchard. Demski and Boyle (20) and Gilmer (32) reported that more than 90% of old orchards were infected by NRSV and PDV. The maximum rate of spread of NRSV can occur at any time after the 4th year while PDV does not spread rapidly until after the 10th year (16).

Since 1950, the use of virus-free budwood has been recommended for the control of sour cherry yellows disease. This practice greatly improved the quality of sour cherry nursery stock in New York State (5). In 1951 virus-free 'Montmorency' budwood became available in Ontario,

Canada and by 1953 most of the nursery stock being offered for sale was propagated from virus-free budwood, but about 4% of the rootstocks used carried virus.

Even though virus indexed, nursery trees are now commonly used throughout the sour cherry industry, the disease has persisted as an economically serious factor. This is due mostly to the infected pollen being transferred from old orchards to the new ones, because of lack of isolation (adequate distance).

No chemical substances (viricides) are available as yet for controlling virus diseases of plants.

The need for the development of methods to control the spread of virus in new orchards is obvious. One method which has been suggested for this purpose is the application of growth regulators to delay flower bud formation in the young orchards. Another approach to virus disease control is resistant (tolerant) varieties which could tolerate infections by the viruses. In order to breed a tolerant variety, a breeder needs to know virology techniques for identification of virus or viruses and how to separate them. Then he needs to have knowledge of genetic variation between the isolates and knowledge of genetic variation between cherry varieties in their symptom expression from virus(es) infections. With the information, he ultimately chooses tolerant parents for hybridization

This study was related to techniques to be used in a virus tolerance breeding program. Its specific purposes were (1) to become familiar

with virology methods needed for virus breeding; (2) to determine whether or not NRSV and PDV were present in old orchards of Michigan and; (3) to determine when and how to best inoculate virus free 'Montmorency' sour cherry (P. cerasus) with buds from infected trees which have both NRSV and PDV.

LITERATURE REVIEW

History and Distribution

The yellow leaf disease of sour cherry was observed by growers in Michigan as early as 1920 (56). It was also reported in New York in 1919 by Stewart (59) and again in 1928 by Gloyer and Glasgow (35). Since sour cherry yellows may be caused by a synergistic effect of PDV and NRSV, it is appropriate to present a brief review of each.

In Ontario, Canada, NRSV was first observed in 1939 and its virus nature was demonstrated in 1940, when typical symptoms were expressed on 'Montmorency' sour cherry as a result of inoculations by budding from infected trees. At about the same time NRSV symptoms on sour cherry were discovered independently in New York, Wisconsin, Pennsylvania and Michigan. NRSV causes some degree of symptom development in many species of Prunus (28). Distribution of this virus occurs world wide in temperate regions (28). Spread of NRSV is rapid in sour cherry in Midwestern and Northeastern states, but relatively slow in sweet cherry (63). Most evidence indicates that the place of origin was (63) the Middle East or Western Asia.

PDV was described as a virus disease by Thomas and Hildebrand (60). It was soon recognized that PDV was involved in the sour cherry yellows disease (37). Chronic effects of PDV infection on the tree habit and fruit production of sour cherry were perhaps observed first in France in (1758) and in England in (1839). The virus may have been introduced into North America early in the 19th Century in 'Large

Montmorency' sour cherry orchards (63).

Effect of NRSV and PDV on Yield and Growth.

Sour cherry yellows appears to be economically the most important known virus disease of sour cherry in the United States and Canada (62).

It is known that the rate of spread of NRSV and PDV is related to the age of orchard and the amount of disease within the orchard (16). Gilmer (32) reported when an orchard was 12 years of age, 94% of the trees had become infected with SCYV or PDV. Demski and Boyle (20) concluded that the rapid increase of disease incidence was in the tenth year, after 25% of the trees had become infected. By the twelfth year, over 91% of all trees in the orchard were infected. Both viruses can spread over a considerable distance. NRSV at least 800 yds and SCYV about 100 yds but most infections of both occur within 50 ft of a known source (16). The effect of these two viruses on symptom expression, yield, growth, and spur formation has been demonstrated.

Cropley et al (12) reported PDV and NRSV under East Malling conditions were synergistic in 'Montmorency' and trees were infected with PDV did not develop yellow symptoms in the absence of NRSV. Before they reported their results, Berkely and Willison (1948) proposed that sour cherry yellows was caused by a complex of PDV and NRSV. In further studies (13) Cropley supported the previous results. He inoculated 'Montmorency' trees on F12/1 rootstocks by double-budding in August 1961 with either NRSV from P. malaleb seedlings. PDV from P. mahaleb seedlings

or with buds from an established orchard tree containing these two viruses. He observed that NRSV alone did not cause sour cherry yellows symptoms, PDV alone occasionally caused sour cherry yellows on only a few trees while the yellows syndrome developed in all trees infected with both viruses. The result of this experiment are shown in the following table:

Effects of NRSV and PDV on 'Montmorency' cherry.

Inoculation 1961	1962	Sour cherry yellows	Stemgirth % of control
-	-	0/3	100
CH_{12}	-	0/3	50
NRSV	-	0/4	83
NRSV	PDV	4/4	56
PDV	-	3/10	72
PDV	NRSV	12/12	55
-	CH_{12}	3/3	55
-	NRSV	0/3	85
-	PDV	0/3	79

Interference between NRSV and PDV was also evident (12). F12/1 cherry (P. avium) trees were inoculated by buds from 'Montmorency' either with NRSV, PDV or both. The plants infected by NRSV showed very severe leaf necrosis; plants infected with PDV showed very small necrotic spots on the leaves while plants infected with both viruses

developed intermediate symptoms. The presence of PDV suppressed symptoms caused by NRSV.

Cross protection is a phenomenon in which plant tissues infected with one strain of a virus are protected from infection by other strains of the same virus (2). The phenomenon has been reported by Marenaud and Bernhard (46) with stone fruit viruses. They observed a cross-protection effect between a mild, but not pure, source of NRSV and a severe and probably pure isolate. They used homozygous and healthy peach seedlings for this experiment. The results of their tests follow:

NRS severe strain (V. 566) alone		112 necrosis for 146 twigs observed
NRS + PD (mild strain)	(S. 1174) +	3 necrosis for 56 twigs observed
NRS severe strain (V. 566)		

Lewis (45) reported that the reduction of yield by NRSV was greatest the first year symptoms appeared and related the severity of the symptoms. Maximum yield reduction by yellows occurred several years after infection (45).

Moore (52) recorded comparative yield of yellows-infected and yellows-free trees in 2 commercial orchards in Door County, Wisconsin for several years. He observed that the rate of reduction in yield was little or moderate in the first or second year following first observation of symptoms and greater reduction occurred in the ensuing 2 or 3 year period. He also reported that in the 13 year-old orchards, for the trees known to have been diseased for 5 years or more, the average

reduction in yield was approximately 50% and in 21 year-old orchards, approximately 62%.

Cain and Parker (S) observed that yellows virus disease caused reduction in yield, percentage of fruit set and spur formation of 'Montmorency' cherries. Spur formation on trees with light infection was nil. Also, both percentage fruit set and number of spurs produced decreased progressively as the severity of yellows increased (8).

In commercial sour cherry varieties, which are self-fertile, a large proportion of the fruits are probably set by self-pollination, therefore, fruit yields of yellows infected trees will be reduced by self-pollination because most of the pollen available is from an infected donor. Some of the infected pollen-tubes may burst during fertilization and reduce fruit set (30).

Way and Gilmer (66) pollinated two branches of a healthy English Morello cherry with healthy and yellows infected 'Montmorency' donors. Pollen from the healthy donor set fruits on 46 of 1057 flowers (4%) but pollen from the infected donor set fruits on only 2 of 685 flowers (<1%). Therefore, they concluded the percentage of fruit set is reduced when pollination achieved with pollen from yellows infected trees.

Klos and Parker (43) reported increased fruit size on yellows trees over fruit on healthy trees in years following initial infection was probably due to less growth of fruit spurs and to less fruit set on diseased trees. They also reported percentage of fruit set was lower on yellows affected trees and ringspot affected trees than on healthy trees.

The reported yellows-affected trees lose a large number of leaves early in the season, which undoubtedly influences fruit bud formation for the next season and probably accounts for poor fruit set (43). They said that the loss of leaves early in the season would reduce accumulation of carbohydrates, which would be needed for good fruit set in the following season. Defoliation of infected trees varies in different years and different locations (56). Cool weather induces yellow leaves to appear in infected trees and subsequent defoliation (45). The maximum defoliation begins in late June and early July and continues for two or three weeks (40). The defoliation typically begins with older leaves and extends towards the younger (40). The size of leaves is dependent on the amount of defoliation. If light defoliation occurs, the tree has normal leaf (normal size), normal spur and shoot growth; but if the trees show heavy defoliation, they bear large leaves, few spurs and long bare spaces occur on the twigs in subsequent years (56).

The effect of NRSV and PDV on the growth of sour cherry trees and the question of which virus has a greater retardation effect on the growth has been studied by Davidson and George (17). NRSV and SCYV both have significant effect on growth of young trees with SCYV having greater retarding effect on growth than NRSV. The literature often refers to SCYV in ways that may in some cases refer to the complex of PDV and NRSV and in others only refers to PDV. In this particular case, Davidson and George must have been referring to PDV. They also reported the growth rate of trees infected with both viruses was very similar to that

for trees with SCYV only and indicated that the predominance of this virus in these combinations. They said when trees were infected with only NRSV at 1, 2, or 4 year-old, there was a 10 to 30% reduction in growth and yield was reduced 36 to 56%. They recommended that growers should try to keep new trees healthy during the first four or five years by isolation and deflowering sprays. The main purpose of deflowering would be to keep young orchardsfrom infection, thus creating a heavy spur system and an increase in vigorous growth of crowns of trees. Post-bloom spray of GA_3 at high concentration in the range of 100 ppm will greatly reduce bloom the following year in young cherry trees. Flower bud initiation does not occur following the GA_3 spray and increases the number of vegetative buds. These vegetative buds develop into the spurs which bear fruit in subsequent seasons. GA_3 directly interferes with bud formation rather than altering the vegetative-reproductive competition on terminal growth (11).

A. Detection of viruses:

The first step in detection of sour cherry yellows is to be familiar with the types of symptoms and factors which induce symptom formation. NRSV and PDV, when inoculated in 'Montmorency' separately, have different symptoms and environmental requirements for expression.

Keitt and Clayton (39) reported yellow symptoms on the leaves of sour cherries (Prunus cerasus L.). They observed chlorotic areas on only part of the leaf lamina. If the leaves persisted long enough

on the tree, some of them became entirely yellow, the leaf symptoms appeared 3 to 4 weeks after petal fall. They did not report the name of the virus or viruses which caused the disease.

In 'Montmorency' sour cherry, the initial symptom of NRSV is delayed foliation of individual limbs or entire trees. Leaves on affected branches are reduced in size and before they unfold, they may show light green spots and dark rings which vary in size from 1 mm or less up to 5 mm in diameter. Infected trees may have smaller leaves than healthy trees (63). The first indication of infection by NRSV is shock (16, 24). "Shock" is manifested as a necrotic symptom produced on the first new growth following infection with some viruses; also called acute symptom (59). Trees in shock due to NRSV soon recover from this symptom and by the end of that season, terminal elongation and lateral shoots are showing no shock (15).
PDV causes a typical yellow leaf symptom during late June followed by the casting of affected leaves (15). Davidson and George (15) reported that the secondary symptoms of sour cherry yellows; "yellow leaf symptoms" appeared 2 years after shock symptoms (initial symptoms of infected trees).

Davidson and George (15) reported the type and distribution of initial symptoms of PDV and NRSV varied with time of inoculation. They reported trees that were inoculated in April developed shock symptoms at bud-break a few weeks later. The time of appearance and progress of symptoms were identical for PDV and NRSV. Inoculation of cherry trees

with PDV during May, June, and July resulted in general shock symptoms at bud-break the following spring. Presumably, this virus moved downwards to the roots and did not become systemic until spring. Trees were inoculated with NRSV during the same period developed secondary etch symptoms the next season without any observable shock or retardation of growth. Presumably, this virus in contact with PDV became systemic before bud-break. August inoculation with PDV also resulted in shock symptoms the following spring, but in some cases adjacent branches were symptomless. They concluded that the virus moved from inoculation point into the surrounding tissue before growth ceased in the fall. Therefore, they concluded that seasonal symptom variation revealed that the rate of movement of the PDV differed from that of the NRSV.

It is known that temperature has a profound effect on the expression of symptoms and leaf casting of sour cherry yellows disease. Moore and Keitt (52) reported that under greenhouse conditions, yellow leaf symptoms on 'Montmorency' cherry could be expressed at 16°C and NRSV symptoms over the range from 16° to 28°C. In another experiment (53) they budded 'Montmorency' trees with buds from yellows infected trees. When a group of budded trees were placed at approximately 16°C, they showed yellows while trees placed at 16°C after exposure to 24°C (1-4 wks after budding) showed no symptoms.

The expression of symptoms and spread of NRSV in sour cherry were delayed at temperature below 20°C and were very poor or lacking at night temperature of 10°C (52).

In Western North America, low night temperature (10 to 16°C) alternating with relatively high day temperature (30 to 35°C) seems to be favorable for leaf casting symptoms. Under favorable conditions for development of symptom, 30 to 60 percent of leaves may drop but leaf dropping gradually decreases as temperature increases (63).

B. Indexing:

PDV and NRSV have been indexed on many herbaceous and woody plant species and show different symptoms.

Cucumber has been used by many investigators to index sour cherry yellows disease. Moore et al. (54) demonstrated that a virus could be transmitted mechanically to cucumber from sour cherries which were infected with necrotic ringspot. At first, identity of the cucumber infecting virus was in doubt because of the inability to transmit the virus from the cucumber to sour cherry. It was then confirmed by other workers that the virus transferred to cucumber was NRSV from sour cherry.

The first transmission by Moore et al. (54) of stone fruit virus to cucumber (Cucumis sativus L.) showed that several viruses could be transferred by sap inoculation from a Prunus host to herbaceous plant species.

Cropley et al. (12) worked on the isolation of necrotic ringspot and prune dwarf viruses in herbaceous indicators. They reported NRSV and PDV can co-exist and multiply together in both Prunus and herbaceous hosts but prior infection of 'Montmorency' trees with one

virus reduced the severity of shock symptom by another (interference effect).

Cucurbita maxima var. Buttercup has been used as a herbaceous indicator. This plant shows a positive reaction to PDV (12, 14, 50, 63). Waterworth and Fulton (64) reported that Cucurbita maxima L. var. Buttercup was systemically infected by all the isolates which reacted with PDV antiserum. Chlorosis as vein banding and mosaic are the most frequently observed reaction (12, 48, 64). Necrotic ringspot virus shows large necrotic lesions on inoculated cotyledons of Buttercup squash but no systemic infection (12). Das and Milbrath (14) reported that squash plants can be infected by pollen from plants infected with stone fruit ringspot virus.

Chenopodium quinoa also has been used to index NRSV (12, 61). NRSV shows necrotic lesions on inoculated leaves and severe distortion rings and lines on systemically infected leaves (12).

Many woody plants have been used to index these two viruses. Pine and Williams (55) used Prunus persica var. Rio Oso Gem peach seedling as an indicator host for NRSV. Two bark chips from suspected trees were placed in each test plant. Symptoms of NRSV appeared within 7-21 days. 'Montmorency' sour cherry (P. cerasus) can be used as a host indicator for SCYV or PDV in regions where the temperature is 65^{o} or below after bloom (32).

Shirofugen flowering cherry (P. serrulata) has been used as an indicator for sour cherry yellows disease by many investigators.

(36, 49, 55) but NRS and PDV cannot be distinguished with this indicator (55). Both of these viruses produce an intially localized necrotic reaction with gumming in Shirofugen. The buds which show negative results on Shirofugen are not a definite indication of virus free buds, because they may contain laten virus quantities below the detection threshold of the Shirofugen indicator (36).

Means of transmission:

1. Pollen transmission: PDV is pollen-born in cherry and infects previously healthy trees, in low percentage, when they are pollinated with infected pollen (28). NRSV is also pollen borne in cherry and infects trees when they are pollinated with virus-carrying pollen (27).

Gilmer (34) pollinated healthy cherry (P. avium L. cv Yellow Glass) using SCYV infected sweet and sour cherry pollen (P. cerasus L. cv 'Montmorency') and obtained tree to tree transmission which takes place by pollen action. Sweet cherries which were infected via pollination demonstrated that SCYV moved from the flowers or young fruit into woody tissue. He also reported that interspecific transmission of PDV is possible between sweet cherry and sour cherry which bloom at the same time.

Pollen transmission also takes place between wild species and cultivated species if bloom dates overlap. Wild Prunus species can transmit infected pollen to healthy cultivated plants. In Michigan,

there are several wild species of cherries from which infected pollen transmission is possible in seasons when the bloom date of cultivated cherries and wild cherries overlap. Bloom development on wild (P. avium) is usually one or two weeks after sweet cherries have bloomed. P. seratina and P. virginiana and the other common wild species usually bloom two weeks later than sweet cherries and plum, and one week later than sour cherries. However, the rate of dissemination of NRSV and PDV in sweet cherry is very much slower than sour cherry. In some years, when a warm period is followed by a cool condition at the beginning of normal blossom season, a rush of late bloom occurs. Under such conditions, pollen transmission between wild and cultivated species is possible or vice-versa (19).

2. Transmission through seed: NRSV and PDV are seed-borne and it can be expected that some wild P. avium should carry one or both of them. Megahed and Moore (58) reported transmission of NRSV through seed resulted in recovery ranging from 1% in Italian Prune seed to 91% from 'Montmorency' sour cherry seed. With English Morello recovery was 50%, with Gold sweet cherry 37%, with P. mahaleb 70% and with P. pensylvanica 36%.

Inoculation from cotyledons of sour cherry and Italian prune seedlings showed chlorotic symptoms and golden mottle on squash. With Mahaleb cherry, cotyledon and seed coat infected both cucumber and squash. Also, they reported that transmission occurred with both mature and immature seeds of 'Montmorency'.

Cation (6) took Mahaleb and 'Montmorency' seeds from infected trees and grew them to seedling stage and then indexed them on seedling peach in the field. He reported that at least 10% of Mahaleb seeds transmitted NRSV and at least 8.7% transmitted sour cherry yellows. SCYV was not transmitted through the seeds of 'Montmorency' but 30% of seeds carried NRSV. In 1952, he did another experiment wherein he used seedlings from an infected Mahaleb tree with PDV and NRSV and grew them in the greenhouse to seedling stage. These seedlings were indexed on peach in the field and he found 2 to 1 ratio of PDV to NRSV. 'Montmorency' seedlings showed 1 to 4. He indicated that viruses were not uniformly distributed through Mahaleb buds but some buds were apparently virus free, however, it can be uniformly distributed through Mazzard buds.

George and Davidson (31) reported that when SCYV symptoms predominated as indicated by consistently strong yellows symptoms with only mild etch, 80% of seed were infected. But when NRSV symptoms were predominant, 99% of seeds carried virus. About one-third of the seeds from trees with sour cherry yellows symptoms were aborted. Trees with necrotic ringspot produce fruit with fewer aborted seeds as compared with sour cherry yellows SCY. The results of their experiment showed virus infected trees, especially with SCYV, tended to increase the abortion rate of 'Montmorency' seed.

3. Transmission by vector: No insect vector is known for

PDV and NRSV (26, 27).

George and Davidson (31) introduced insects into growth chambers, containing diseased and healthy trees. In just one of the chambers, transmission took place by Thripidae or bees. When they introduced bees alone in the chamber, the bees caused more transmission of the virus than the Thripidae. This indicates rapid spread of NRSV is probably related to an easy dissemination by an Arthropod vector.

Genetic tolerance to virus infection in woody plants:

Although health or vigor of host plants confers no resistance on immunity to virus disease, breeding plants for hereditary resistance to virus is of great importance and many plant varieties resistant to certain virus diseases have already been produced (2).

Study on the rootstock and scion varieties and rootstock-scion combination for resistance to the virus disease of fruit has been done by many investigators. For example, during the past 25 years, virus diseases have become a critical factor in the citrus industry throughout the world. The most damaging citrus viruses include tristeza, psorosis and exocortis, and sometimes cachexia. Tristeza and exocortis act primarily on specific rootstock-scion combinations, while psorosis affects many scion varieties, irrespective of rootstock. One of the widely injurious virus reactions known had been the destruction of sweet orange on sour orange rootstock by tristeza. The trifoliate orange was found as a tolerant rootstock to tristeza.

Studies on the reaction of cherry rootstocks and scion varieties and rootstocks-scion combinations to either PDV, NRSV or both for specific selection of parents with greater innate resistance is necessary to improve the sour cherry and sweet cherry to the sour cherry yellows disease.

Prunus species have different genetic make-up because they differ in number of chromosomes (38). They have shown different reactions to either PDV, NRSV or both viruses (13), and different reaction of Prunus species to either PDV, NRSV or both. He reported that with combination of both viruses, a synergistic effect on growth and symptom expression occurred on 'Montmorency' (P. cerasus L.) and an interference effect on symptom expression of F12/1 cherry (P. avium).

MATERIALS AND METHODS

MATERIALS AND METHODS

Commercial 'Montmorency' sour cherry orchards in the western part of Michigan were used as a source of virus isolates. The ages of the orchards were 15 to 53 years. They represented a broad geographic sample of the entire 'Montmorency' sour cherry orchard industry.

I. Identification: Eighteen samples were taken from old orchards before bloom time in March, 1976 and were placed in water and forced to bloom under greenhouse conditions. The samples were composed of a few branches per tree per orchard. Several new leaves and blossoms were taken from each sample, were put in a sterile mortar which contained 5 mls of phosphate buffer solution pH_8, and ground with a sterile pestle to a soupy consistency. The crude juice was applied to the cotyledons of cucumbers (Cucumis sativus L. var. Lemon and Ohio MR17) by rubbing with cheese cloth. The cucumbers were dusted by carborundum before inoculation to facilitate inoculation. Inoculated cucumber pots were covered with wet paper towels in order to supply moisture for better penetration of virus or viruses. After inoculation, they were transferred to an isolated greenhouse where they were kept under high light intensity and high humidity conditions for a few days for symptom evaluation. The humidity was provided by misting every ten minutes for ten seconds in order to prevent collapsing of tissues of the inoculated cucumbers.

In April 1977, 5 orchards in Southwestern and West Central

and 5 orchards in Northwestern Michigan were sampled. One sample was taken from each of three adjacent trees in each orchard. The samples were collected from swollen flower buds and new leaves. For a more comprehensive study of a single orchard, the (F.P.) orchard in Southwestern Michigan was used and 20 individual tree samples were taken from two adjacent rows. The samples were kept in cool condition during transport to East Lansing where indexing was accomplished.

Cucurbita maxima var. Buttercup and Chenopodium quinoa were used as herbaceous plant indicators for this part of the experiment. They were grown in four-inch pots in the greenhouse. A few flower buds and new leaves from each sample were inoculated using the same techniques as in 1976. Both squash and Chenopodium quinoa plants were kept under fluorescent lighting for 15 hours and below 80°F during the experiment, but no intermittent misting was used in 1977.

II. Serology test: In 1976, tests were made to identify virus entities present in field isolates. Flat bottom petri-dishes were coated with 12 ml of agar solution. Agar solution was made by the following method .85 g NaCl added to 100 ml 0.01 M tris-solution pH_7 after sodium chloride was completely dissolved 0.75 grams Inoagar was added and heated on the steam bath in order to dissolve the Inoagar in the solution. The agar solution was allowed to cool to about 60°C, then 2 mls sodium azid was added to inhibit growth of fungi and bacteria. Then 12 mls of warm agar solution was poured into each

petri dish and allowed to solidify. They were kept in moist, cool condition for a few days (3).

After the isolates showed symptoms on cucumber cotyledons, they were transferred for a few times on cucumbers to increase the titer of virus. Then 10 - 12 infected cucumber cotyledons were ground with 2 ml phosphate buffer pH_8. The sap material was extracted and centrifuged at 10^4 rpm for 15 minutes, then centrifuged at 35 x 10^3 rpm for two hours. When the virus or viruses were purified and concentrated, 10 drops of the buffer, pH_8, was added and put on the shaker in about $40^{\circ}F$ cool room overnight (29).

Patterns of three wells surrounding a central well, each well being 3 mm in diameter and spaced 7 mm apart were prepared. They were punched by sharpened brass tubing. The central wells were filled by one drop of PDV and/or NRSV antisera (1:50) and the outside wells were filled up by concentrated virus or viruses for identification. The petri dishes were placed in a moist chamber and covered with a plastic bag to prevent the agar from drying and incubated for 24 hours when the zones were visible.

In spring 1977, three samples from (H.B.) orchard, which was the source of diseased budwood for inoculation experiment, were indexed on cucumber (Cucumis sativus L. var. Lemon). After the symptoms appeared, crude extracts of infected cotyledons were tested against antiserum of PDV and NRSV diluted at 1:50, 1:25 and undiluted antisera.

Ten to 12 infected cotyledons were ground with phosphate buffer solution, pH_8. The crude extract was prepared by squeezing through cheese cloths. The rest of the test was followed as the method in 1976 except the crude extract was not concentrated by centrifugation.

III. Inoculation of sour cherry by budding: In this part of the study, the experiment had three treatments. One-year-old Prunus mahaleb L. rootstock seedlings were obtained from virus certified stocks of Hilltop Orchards and Nurseries, Inc. These were grown during the summer of 1976 in soil placed in 30 pound frozen food containers to be used as rootstocks for each treatment. Virus certified 'Montmorency' scion wood obtained from the "Dowd Orchards" was used for all grafting to create trees for each treatment.

Diseased buds were taken from (H.B.) orchard that showed positive reaction to PDV antiserum, yellow symptom and leaf dropping in June, 1976.

Treatment No. 1: P. mahaleb rootstocks were T-budded with diseased budwood of 'Montmorency' on August 3, 1976 and then they were T-budded with virus certified budwood of 'Montmorency' on September 3, 1976.

Treatment No. 2: P. mahaleb virus free rootstock were T-budded by virus certified and diseased budwood of 'Montmorency' sour cherry at the same time on September 3 and 4, 1976.

Treatment No. 3: Prunus mahaleb L. rootstock seedlings were planted in cans in May 1976 in the greenhouse. They were chip-budded in May with virus certified 'Montmorency' budwood. They were transferred to the Horticulture Research Center (HRC) in East Lansing in early summer and were bud inoculated with diseased 'Montmorency, HB strain' budwood on September 3, 1976.

Budded trees of the three treatments were grown at the (HRC) during fall, 1976 and winter, 1977. Then on March 3, 1977, 40 trees from each treatment which appeared to have live buds were moved to the greenhouse. These 120 trees were chosen on the basis of visual inspection of the graft union knit. The trees were arranged in four blocks. Each block contained three treatments with 10 plants per treatment. After the buds which were initially clean started to grow, (approximately 25 cm) new leaves were taken from individual trees from each treatment and indexed on squash (Cucurbita maxima L. var Buttercup) and Chenopodium quinoa for PDV and NRSV using the techniques previously described. The symptoms appeared after 6 to 8 days.

IV. Indexing on Shirofugen: The buds were taken from new growth of buds which were initially clean, T-budded into Shirofugen flowering cherry (P. serrulata) at the Michigan Department of Agriculture "airport repository". The results were recorded after 3 weeks on the basis of gumming or non-gumming visual classifications.

RESULTS

RESULTS

I. Herbaceous indicator:

Cucumber: In 1976, the samples from the old orchards, Table 1, were indexed on cucumber (Cucumis sativus L. var. Lemon and Ohio MR17). Both cucumber varieties showed two types of symptoms 3 to 7 days after inoculation. First, local lesions appeared on cotyledons followed by systemic symptoms (mosaic) on new leaves after a few days, Table 2. These symptoms were not used to differentiate between PDV and NRSV. When the titer of virus or viruses was sufficiently high, the inoculated cucumber seedlings collapsed immediately after local lesions became visible on the cotyledons.

Separation of viruses: Table 3 shows the 10 orchards from the southwest and northwest which were used for sampling in the spring of 1977 to determine the distribution of virus or viruses between and within orchards.

Squash: The isolates were indexed on squash (Cucurbita maxima L. var. Buttercup). Two types of symptoms developed after inoculation. Some isolates induced necrotic lesions on their cotyledons but no systemic infection, others caused necrotic lesions followed by yellow areas and vein clearing on systematically infected leaves. Necrotic lesions on cotyledons were probably induced by strains of NRSV which the systemic infection were probably due to PDV, and were similar to those reported

Table 1. The identification and location of orchards in Michigan which were sampled in the spring of 1976.

Date	Name of orchard		City and location		Age
3/23/76	F. Pugsley	(F.P.)	Paw-Paw	(S.W.)	15 yrs
3/23/76	D. Friday	(D.F.)	Hartford	(S.W.)	31 yrs
3/23/76	R. Kinney	(R.K.)	Eau Claire	(S.W.)	21 "
3/23/76	K. Weber	(K.W.)	Coloma	(S.W.)	30 "
3/23/76	H. Overhiser	(H.O.)	South Haven	(S.W.)	26 "
3/23/76	R. Thomas	(R.T.)	Bangor	(S.W.)	21 "
3/25/76	H. Brother	(H.B.)	Casnovia	(W.C.)	25 "
3/25/76	Ch. Hill	(C.H.)	Bailey	(W.C.)	22 "
3/25/76	R. Bull	(R.B.)	Fremont	(W.C.)	25 "
3/25/76	G. Lewis	(G.L.)	New Era	(W.C.)	25 "
3/25/76	F. Fox	(F.F.)	Shelby	(W.C.)	36 "
3/25/76	V. Bull	(V.B.)	Shelby	(W.C.)	25 "
3/25/76	A. Lister	(A.L.)	Ludington	(W.C.)	25 "
3/29/76	W. Cox	(W.C.)	Williamsburg	(N.W.)	53 "
3/29/76	B. McLachlan	(B.M.C.)	Kewadin	(N.W.)	43 "
3/29/76	J. Galleager	(J.G.)	Traverse City	(N.W.)	25 "
3/29/76	B. Deering	(B.D.)	Northport	(N.W.)	30 "
3/29/76	B. Underwood	(B.U.)	Traverse City	(N.W.)	35 "
3/29/76	A. Carroll	(A.C.)	Traverse City	(N.W.)	30 "

W.C. = West Central
S.W. = Southwest
N.W. = Northwest

Table 2. The type of symptoms lesions (L) or mosaic (M) on cucumber vars. Ohio MR17 and Lemon which were inoculated with isolates from old orchards in the spring of 1976.

Name of isolate	Ohio MR17		Lemon	
	L	M	L	M
H.B.	+	+		+
J.G.		+	+	+
D.F.	+			+
B.M.C.$_2$		+	+	
B.U.		+	+	
R.T.		+		+
V.B.	+		+	
B.M.C.			+	
K.W.			+	
F.F.			+	

by the following investigators (12, 14, 48, 49, 62).

Chenopodium quinoa: The isolates were indexed on Chenopodium quinoa for NRSV. The new leaves of C. quinoa were inoculated by crude sap of each isolate and necrotic lesions appeared on the inoculated leaves. Some of the inoculated C. quinoa seedlings showed systemic infection but they were not the result of the inoculum. The systemic infection also developed on the healthy seedlings. Systemic symptoms did not appear at early stages of growth but developed when flowers started to appear. The local lesion symptoms in this experiment were similar to those reported in previous investigations (12, 61).

The results of indexing of isolates for NRSV and PDV on squash and Chenopodiums have been tabulated in Table 4.

II. Serology test:

The results of reaction of purified virus or viruses to antisera (PDV strain B) and (NRS Strain G) are shown in Table 5. None of the samples in 1976 showed positive reaction to both antisera.

In the spring of 1977, the H.B. orchard isolate was indexed on cucumber. Crude extracts of infected cotyledons were tested against the antisera diluted at 1:50, 1:25 and non-diluted, Table 5. The non-diluted antisera showed, in some cases, reaction to both healthy and infected cucumber cotyledon extracts. Diluted antisera 1:25 and 1:50 showed only reaction to the isolates. HB_1 showed positive reaction to PDV antiserum in 1976 and positive reaction to NRSV antiserum in 1977 (Table 5). HB_2 showed positive reaction only to non-diluted PDV and

Table 3. The name and location of orchards were used for sampling in the spring of 1977.

Date	Name of orchard	City and location		Age
4/23/77	R.K.	Eau Clare	(S.W.)	21 yrs
4/23/77	K.W.	Coloma	(S.W.)	30 yrs
4/23/77	H.O.	South Haven	(S.W.)	26 yrs
4/23/77	H.B.	Casnovia	(W.C.)	25 yrs
4/23/77	F.P.	Paw Paw	(S.W.)	15 yrs
5/4/77	W.C.	Williamsburg	(N.W.)	53 yrs
5/4/77	B.U.	Traverse City	(N.W.)	35 yrs
5/4/77	B.M.C.	Kewadin	(N.W.)	9 yrs
5/4/77	A.C.	Traverse City	(N.W.)	30 yrs

Table 4. The results of indexing of samples on squash and Chenopodium in the spring of 1977.

Region	Isolate	Squash var Buttercup		Chenopodium quinoa	
S.W.	R.K.$_1$	Local lesions		local lesions	
" "	R.K.$_2$	-		-	
" "	R.K.$_3$	local lesions		-	systemic
" "	H.O$_1$		systemic		systemic
" "	H.O$_2$	local lesion		local lesion	
" "	H.O.$_3$	-		-	systemic
W.C.	H.B.$_1$	local lesion	systemic		
W.C.	H.B.$_2$	local lesion	-		
W.C.	H.B.$_3$	local lesion	systemic	local lesion	
S.W.	K.W.$_1$	local lesion			
" "	K.W.$_2$				
" "	K.W.$_3$	local lesion			
S.W.	F.P.$_1$	local lesion		local lesion	
" "	F.P.$_2$	-	-	-	systemic
" "	F.P.$_3$	-	-	local lesion	systemic
" "	F.P.$_4$	-	-		
" "	F.P.$_5$	-	-		systemic
" "	F.P.$_6$	-	systemic		-
" "	F.P.$_7$	-		-	-
" "	F.P.$_8$	local lesion		-	systemic
" "	F.P.$_9$	-	-	-	-
" "	F.P.$_{10}$	local lesion		-	
" "	F.P.$_{11}$	local lesion	systemic	-	-
" "	F.P.$_{12}$	-	-	-	systemic
" "	F.P.$_{13}$	-	-	local lesion	systemic
" "	F.P.$_{14}$	local lesion	systemic	local lesion	
" "	F.P.$_{15}$	local lesion		local lesion	
"	F.P.$_{16}$	local lesion		local lesion	systemic
" "	F.P.$_{17}$	local lesion	systemic	local lesion	-
" "	F.P.$_{18}$	local lesion	-	local lesion	-
" "	F.P.$_{19}$	local lesion	-	local lesion	-
" "	F.P.$_{20}$	local lesion		local lesion	systemic

Table 4. The results of indexing of samples on squash and Chenopodium in the spring of 1977. (continued)

Region	Isolate	Squash var Buttercup		Chenopodium quinoa
N.W.	W.C.$_1$	Necrotic lesions	systemic	local lesion
"	W.C.$_2$	Necrotic lesion	-	local lesion
" "	W.C.$_3$		-	local lesion
N.W.	J-G$_1$	-	-	
" "	J-G$_2$	-	-	local lesion
" "	J-G$_3$	-	-	local lesion
N.W.	A.C.$_1$	-	-	-
" "	A.C.$_2$	-	-	-
" "	A.C.$_3$	-	-	-
N.W.	B.M.C.$_1$	-	-	local lesion
" "	B.M.C.$_2$	-	-	-
" "	B.M.C.$_3$	local lesion	-	
N.W.	B.U.$_1$	-	-	-
" "	B.U.$_2$	-	-	-
" "	B.U.$_3$	-	-	-

Table 5. Serological reaction of samples which showed symptoms on cucumber in the spring of 1976.

Isolate	Antiserum (PDV) 1:50	Antiserum (NRSV) 1:50
H.B.$_1$	+	-
V.B.^{1}	-	+
B.M.C.$_2$	-	+
B.U.	+	-
D.F.	-	+
J.G.	+	-
B.M.C.$_1$	-	+
K.W.	+	-
F.F.	+	-
R.T.	-	-

Table 6. Serological reactions of samples from H.B. orchard in 1977 which was used as a source of diseased budwood for inoculation experiments.

Antiserum	HB_1	HB_2	HB_3
PDV 1:25	-	-	-
PDV 1:50	-	-	-
PDV (nondiluted)	-	+	+
NRSV 1:25	+	-	+
NRSV 1:50	+	-	+
NRSV (non-diluted)	+	+	+

NRSV antisera. HB_3 showed positive reaction to NRSV antiserum diluted at 1:25 and 1:50 and non-diluted NRSV and PDV antisera.

III. Inoculation of sour cherry by budding:

Budded trees of the three treatments were forced in greenhouse in spring 1977. After the new growth had developed from the buds which were initially clean, new leaves of individual trees were used to index on squash for PDV and Chenopodium quinoa for NRSV. The types of symptoms on squash and Chenopodium were the same as samples which were taken from the old orchards in 1977.

The amount of transmission of NRSV and PDV or both viruses, from diseased buds to the buds which were initially clean were tested using two way contingency tables.

Data tabled in (8) yielded X^2 estimates which were calculated to learn the difference between treatments for amount of transmission of PDV from diseased buds to the new shoots which came from clean buds. Calculated X^2 was significant at 5% and 1% levels indicating treatment differences existed.

Table 7. The observed and expected values of trees per each treatment which showed positive and negative reaction when they were indexed for PDV in the spring of 1977.

Treatment	PDV	NRSV	Total
	+	-	
T_1	1 8.07	26 18.92	27 $n_{3.}$
T_2	9 11.96	31	40 $n_{2.}$
T_3	22 11.96	18 28.03	40 $n_{1.}$
	32 $n._1$	75 $n._2$	107 $n..$

Cal. $X^2_{(2)}$ = 21.84** Tab. $X^2_{.05\ (2)}$ = 5.99 Tab. $X^2_{.01\ (2)}$ = 9.21

Expected values and X^2 were calculated by the following formula.

$$E_{ij} = \frac{n_{i.} \times n.j.}{n..} \qquad X^2 = \sum \frac{(O_{ij} - E_{ij})^2}{E_{ij}} \qquad (r-1)(c-1)$$

r = No. of rows

c = No. of columns

From Table 8, X^2 was calculated to see if treatment differences were present for transmission of NRSV from diseased buds to the new shoots that came from healthy buds. Calculated X^2 was significant at 5% and 1% level.

From Table 9, X^2 was calculated to see the differences between treatments for transmission of both PDV and NRSV from infected to the new shoots which came from clean buds. Calculated X^2 was significant at 5% and 1% level.

Table 8. The observed and expected values of trees per each treatment which showed positive and negative reaction when they were indexed for NRSV in spring of 1977.

Treatment	NRSV		Total
	+	-	
T_1	3 9.58	24 27.41	27
T_2	7 14.20	33 25.79	40
T_3	28 14.20	12 25.79	40
	38	69	107

Cal. $X^2_{(2)}$ = 33.44** Tab. $X^2_{.05\ (2)}$ = 5.99 Tab. $X^2_{.01\ (2)}$ = 9.21

Table 9. The observed and expected values of budding time treatments which showed positive and negative reaction when they were indexed for PDV and NRSV in the spring of 1977.

Treatment	PDV AND NRSV		No. of trees p34 treatment
	+	-	
T_1	0 5.04	27 21.95	27
T_2	4 7.47	36 32.52	40
T_3	16 7.47	24 32.52	40
	20	87	107

Cal. $X^2_{(2)}$ = 20.14** Tab. $X^2_{.05\ (2)}$ = 5.99 Tab. $X^2_{.01\ (2)}$ = 9.21

IV. Indexing on Shirofugen:

In late spring 1977, buds from graft inoculated trees of each treatment were T-budded on Shirofugen flowering cherry tree (P. serrulata). Infected buds showed localized necrotic reaction with gumming in Shirofugen 21 - 23 days after budding. The results of such indexing are shown in Table 10.

From Table 10, X^2 values were calculated to learn if treatment differences were present for transmission of virus(es) from diseased buds to the new shoots which came from healthy buds. Calculated X^2 was not significant at 5% and 1% level. There were no significant differences between treatments.

The results of indexing of individual trees in each treatment on squash, Chenopodium, and Shirofugen were compared to see which of them had shown positive reaction to at least one of 3 indexing tests. The result of this comparison are shown in Table 11. From Table 11, X^2 values were calculated to see if treatment differences were present for transmission of virus(es) from diseased bud to the new shoots which came from initially clean buds. Significant X^2 at 5% level was obtained. There were significant differences between treatments with regard to the time of inoculation.

Table 10. The observed and expected values of indexing on Shirofugen flowering cherry in the early summer of 1977.

Treatment	Shirofugen (gumming)		No. of trees per treatment
	+	-	
T_1	18	8	26
	19.1	6.9	
T_2	27	13	40
	29.33	10.67	
T_3	32	7	39
	28.6	10.4	
	77	28	105

Cal. $X^2_{(2)}$ = 2.42 n.s. Tab. $X^2_{.05\ (2)}$ = 5.99 Tab. $X^2_{.01\ (2)}$ = 9.21

Table 11. The observed and expected values of trees in each treatment which showed reaction to at least one of the 3 indexing tests in spring of 1977.

Treatment	Three indexing tests		No. of trees per treatment
	+	-	
T_1	20 21.95	7 5.05	27
T_2	29 35.52	11 7.48	40
T_3	38 32.52	2 7.48	40
	87	20	107

Cal. $X^2_{(2)} = 7.89^*$ Tab. $X^2_{.05\ (2)} = 5.99$ Tab. $X^2_{.01\ (2)} = 9.21$

Table 12. Cumulative results of trees per each treatment that showed shock and yellow leaf symptom under greenhouse conditions in spring 1977.

Symptoms	Date	No. of Plt/ $treatment_1$	No. of Plt/ $treatment_2$	No. of Plt/ $treatment_3$
Shock	4/15/77	0/27	0/40	11/40
Yellow leaf	4/15/77	0/27	0/40	1/40
Shock	5/26/77	1/27	2/40	8/40*
Yellow leaf	5/26/77	0/27	0/40	12/40
Shock	6/2/77	1/27	3/40	4/40*
Yellow leaf	6/2/77	8/27	20/40	33/40

*Leaf loss due to abscission accounts for gradual reduction of numbers during spring observations.

V. Symptoms:

The individual trees were checked for yellow leaf and "shock" during the experiment. The leaf symptoms described below developed after transmission by budding. The yellow leaf and "shock" symptoms, were observed on some of the trees of each treatment in the greenhouse in the spring of 1977. The results are shown in Table 12. According to Table 12, the maximum number of trees which showed shock and yellow leaf symptoms were obtained from Treatment No. 3 and least from Treatment No. 1. Most shock symptoms appeared by early spring. The maximum amount of yellow leaf symptoms occurred in early June when the temperature was around 70°F (21°C). Chlorotic areas of light green appeared on the leaf lamina, then chlorotic areas progressed in area of leaves showing this symptom and developed to advance stage of yellow color of whole lamina.

DISCUSSION

DISCUSSION

Herbaceous indicator:

The result of indexing of isolates from the old orchards in 1976 on cucumber are shown in Table 2. From the 18 orchards that were used for sampling, 10 showed positive reaction on cucumber. Field observation on amount of bloom growth condition, extent of yellow leaf symptoms, and leaf dropping supported the hypothesis that a virus or viruses were present in our samples, but for some reason some did not transfer to cucumber.

One explanation for the failure of transmission of virus(es) might be that too low of a concentration of virus existed in some of the samples. There is a possibility that there was non-equal distribution of virus(es) within the trees.

The residue of plant material which was used for inoculation was not washed from the surface of inoculated cucumber cotyledons after inoculation. Maybe the residue of plant material reduced transmission of virus or viruses. Fulton and de Zoeten (29) reported the residue of infected plant material on inoculated leaves may cause damage to epidermal tissue and decrease infection.

At the time of inoculation, bud scales were not removed from the field isolate plant material. They could have reduced the rate of transmission. Davidson and Rundans (18) found the presence of bud scales slightly reduced the percentage of transmission. A virus inhibitor in the bud scales can inactivate virus(es) and such inactivation can occur in the interval between grinding the tissue and applying the extract as inoculum (25).

Milbrath (47) says petals are favorable material for mechanical transmission of virus in woody plants. Fulton (25) said viruses could be transmitted mechanically from woody plants mostly by preparing inoculum from growing new leaves. Both believe that these plant materials have fewer virus inactivators than other older tissues.

The lower than expected transmission of virus(es) to cucumber could have been caused by using old buffer solution. The fresh buffer solution contains 2-mercaptoethanol which gradually breaks down after a few weeks. Mercaptoethanol can prevent oxidation of phenolic compounds when extracts are exposed to air (25).

The results of indexing of isolates from the S.W. and W.C. Michigan (spring 1977) on squash and Chenopodium were better than with isolates from northwest Michigan (spring 1977). Fresh phosphate buffer was used for inoculation of all isolates and the inoculated squash and Chenopodium were kept in the greenhouse in which the temperature was kept under $80^{O}F$. Therefore, the virus or viruses probably did not inactivate during inoculation because of using fresh buffer solution and cool temperature in the greenhouse. The inoculum was composed of the new leaves, petals, and pollen grains.

Failure to inoculate squash and Chenopodium by isolates from N.W. may have been due to various causes like low virus concentration at the time of sampling, action of temperature during sampling, and non-susceptibility of squash and Chenopodium.

Squash seedlings were good indicators for PDV and they were not susceptible to other viruses. They grew very fast and a few

days after planting, the seedlings were ready for inoculation. Chenopodium seedlings were not a good indicator because they were susceptible to a disorder which was suspected to be another virus. This disorder resulted in symptoms appearing on non-inoculated leaves of inoculated and uninoculated control plants. This suggests the systemic symptoms may have been transmitted through seeds or by vectors such as aphids.

Serology test:

Serological techniques are very helpful in plant virus work for several different purposes. Since serology reactions are highly specific, they may be used to identify viruses and to determine relationships between them. Serology is a rapid method for detection of specific virus or viruses in woody plants. Many factors interfere with such tests such as low concentration of virus and denaturation of virus by inhibitors during extracting of infected tissue (25). Fresh phosphate buffer solution can prevent inactivation of virus(es) during extracting. The buffer solution which was used for mechanical transmission and serology may not have been fresh enough in 1976 when poor results were obtained. Buffer should be changed every week to have enough 2-mercaptoethanol to prevent inactivation of virus(es) (25).

It was hypothesized that both viruses were present in the old orchards and could be detected by serology tests. None of the 10 isolates in 1976 showed the presence of both viruses. Probably one of two viruses which had less concentration was inactivated during extracting

because of using old phosphate buffer solution. In order to test for the presence of both viruses, crude extracts of the infected cotyledons were centrifuged to purify the virus(es). The centrifugation was done by two steps at different speeds. By each centrifugation, we may have lost some percentage of virus(es) and ultimately the virus with the low titer may not have shown a reaction in diffusion agar tests.

Another attempt was made to increase the titer of virus(es) by transfers in cucumbers. After initial symptoms appeared on cucumbers, the isolates were transferred to other cucumbers for 5 to 6 times. It is possible that during inoculation, one of the viruses could have been lost by inactivation, non-susceptibility of cucumber, or the particles of virus not coming in contact with a susceptible site during inoculation.

In 1977, by changing the techniques, both viruses were detected in some of the samples from the H.B. orchard using serology tests. After symptoms became visible on inoculated cucumber cotyledons, virus(es) were transferred two times and fresh buffer was used for inoculation. The crude extract was used instead of centrifuged extract of infected cotyledons.

No explanation is apparent for by HB1 showed positive PDV antiserum reaction in 1976 and negative in 1977. Leaf drop symptoms occurred in the field on HB_1 in 1976 and yellow leaves resulted from grafting experiments so it does have sour cherry yellows.

Inoculation of sour cherry by budding;

Early reports by Keitt and Clayton (40) strongly suggested the

disease was bud transmissible and another experiment which was done by Davidson and Rundans (18), supported this idea that the PDV and NRSV were present in dormant buds. The results of the present experiment support the above results. Viruses can be transmitted from diseased bud to the new shoots which come from clean buds. Tables 7, 8, and 9.

The trees of Treatment No. 3 (subsequent inoculation) grew very uniform with high vigor in spring 1977 after breaking dormancy. The results of indexing of the trees on squash and Chenopodium for transmission of PDV and NRSV from diseased buds to the clean buds, Tables 7 and 8, showed that the trees had high concentration of viruses. In contrast to Treatment No. 3, Treatments No. 1 and 2 grew nonuniformily with less vigor in the spring after breaking dormancy in the greenhouse. The results of indexing trees of Treatments No. 1 and 2 on squash and Chenopodium showed lower transmission rate than Treatment No. 3. This suggested there may have been a lower titer of viruses in Treatment No. 1 and No. 2.

The different patterns of growth for the three treatments were not related to the kind of rootstock (because they had the same rootstocks (P. mahaleb)), but were probably due to the time of budding or the horticultural condition of the rootstocks at the time of budding. Since they were budded at different times, they could not be kept identical at time of budding.

Calculated X^2 from the results of indexing on Shirofugen, Table 10, did not show significant differences between treatments. This suggests that the poor transmission of viruses from trees of Treatment No. 1 and No. 2 may be caused by difficulties with techniques of testing

of the presence of viruses. It was necessary to use older leaves from some of the trees of Treatments No. 1 and No. 2 at the time of sampling for indexing, probably the viruses were inactivated by inhibitors. Fulton (24) said inhibitors such as phenolic compounds inactivate PDV and NRSV. New leaves have less phenolic compounds and high concentrations of viruses (25).

In another statistical test, the results of indexing each tree on squash, Chenopodium and Shirofugen were compared to see how many trees of each treatment showed positive reaction to at least one of the indicators. The reason for doing this comparison was to see how many trees were completely virus free because some of the trees that showed no reaction on Shirofugen (gumming) had shown reaction on squash, Chenopodium or both. From Table 11, calculated X^2 was significant. This result supported the calculated X^2 values from Tables 7, 8, and 9 that there were significant differences between treatments. This suggested that viruses were transmitted from diseased buds to the clean buds but the titer of viruses was different in each treatment.

The trees of Treatment No. 3 probably had a higher titer of viruses because one-year-old 'Montmorency' sour cherry trees were graft inoculated in September and the viruses were able to move in the tree and may have been localized in the dormant buds until spring. In Treatment No. 2, P. mahaleb seedling rootstocks were budded by clean buds and diseased buds at the same time. The transmission of viruses from diseased buds to the clean buds should have taken place through

P. mahaleb tissue. It is possible that diseased buds may have had less titer of viruses by chance selection of the inoculating buds but poorer transmission of viruses in rootstocks than in 'Montmorency' also could have occurred. This might also have been true in Treatment No. 1. The differences between the titer of viruses in each treatment may be due to the different rate of inactivation of viruses by inhibitors which may have differed in P. mahaleb and 'Montmorency'. Different levels and types of phenolic compounds in P. mahaleb and 'Montmorency' may have inactivated viruses more in P. mahaleb than 'Montmorency'. Different cherry varieties have different phenolic compounds. Yu and Carlson (41) reported Mazzard (P. avium L.) and Mahaleb (P. mahaleb L.) were different in three phenolic groups: phenolic acids, coumarins, and flavonoids. In another experiment (42), they found two rootstocks were different in phenolic and coumarin components in the leaf, stems and roots.

In Treatment 1, P. mahaleb rootstock seedlings were budded with diseased buds in August and clean buds in September. In August, the bark of P. mahaleb was not slipping enough for optimum budding success so it may be that the diseased buds did not take well, and dried out because of non-slipping bark and high temperature. Therefore, the diseased buds may not have been present long enough to inoculate the clean buds.

Alternatives to virus tolerance breeding:

According to the greenhouse and field evaluation of NRSV and PDV in the old orchards in this study, it appears that sour cherry yellows disease is a serious problem in Michigan. The field symptoms of NRSV was observed in many young orchards and yellows disease appeared in most old orchards. Therefore, it appears either a virus tolerance breeding program or some other horticultural system may be essential to the practice of keeping tart cherry orchards yielding well.

The literature indicates that sour cherry orchards of ages 10 to 12 are most vulnerable to PDV even though NRSV and PDV can both occur naturally in the field at any time after flowering commences (18). Thus, the 10 to 12 year-old age may for some reason be the key time in the life of a 'Montmorency' tart cherry tree when it is most susceptible to PDV. Research is needed to understand why PDV spreads rapidly so much later than NRSV. The literature also indicates that when both viruses are present in the same tree, they often have a synergistic effect on growth and yield.

Based on the rate of spread of both viruses in orchards, and their synergistic effects on growth and yield of cherry orchards, we should attempt to optimize productivity of sour cherry orchards between 4 to 12 years of age before yellows symptoms become wide spread. If success can be gained in this practice, it might be possible to

escape most of the effect of the yellows and disease by discontinuing sour cherry orchards after they reach about 10 to 12 years of age.

There are a few horticultural practices which might help bring the cherry orchard to the high fruit production between 4 to 12 years of age. One set of such practices is presently under research in Michigan. It uses standard rootstocks, high fertility, optimum soil moisture, gibberrellic acid sprays to prevent premature flowering, close planting, and summer and dormant season pruning to maintain optimum fruiting wood surface in orchards.

Another approach to this problem would be to use smaller trees and induce precocity of such trees with dwarfing rootstocks or interstems. This has been done in sweet cherry by using 'Montmorency' sour cherry interstock between P. mahaleb rootstock and the scion cultivar (38). Very close planting would then be necessary to maximize early production. Other horticultural practices, such as branch bending, scoring, ringing and limiting fertilizer to induce flowering and early fruit set, also deserve research in this type of system.

Foliar application of gibberrelic acid has been effective in stimulating growth of virus suppressed auxillary buds in sour cherry disease which results in increased fruit production (2). This practice may be useful in prolonging the life of close planted orchards.

All of these practices may induce greater productivity of orchards for a short period of time. However, high cost of orchard

establishment will probably cause growers to want to retain orchards as long as possible. They will desire keeping them past age 10 to 12 years, so a need would still exist to breed trees for tolerance to yellows disease.

Cross protection is an alternative approach to breeding for scion variety tolerance to control the virus diseases. It may warrant research consideration in sour cherry production. The phenomenon has been tested on many crops. Salaman (60) observed this phenomenon between mild strain and severe strain of potato virus X. Kunkel (44) for the first time found a cross protection effect between two strains of yellows virus, ordinary aster yellow strains and the California aster yellow strain when transmitted by their leafhopper. Aster yellows has recently discovered that it was caused by mycoplasma not virus.

Cross protection also has been found between NRSV in Prunus species. Cochran (10) found that mild strains of ringspot protected peach trees against some severe isolates from the same virus. Marenaud and Bernhard (46) observed cross-protection effects between mild and severe strains of NRSV. They also observed a transmissible effect of cross-protection on growth of peach.

According to the above information, research still is necessary to study the cross-protection effect between strains of NRSV and PDV in sour cherries. "Virus breeding" may provide an opportunity to make

mild strains of both viruses available in all trees in order to protect the new orchard from infection of the severe strains of isolates. It has the desirable aspect that it would then not be necessary to change scions and rootstock cultivars as would be required in a virus tolerance breeding program.

The literature indicates sour cherry yellows spreads slower in sweet than in sour cherries. Research is necessary to study this to be true. By crossing sour and sweet varieties, it may be possible to establish a cultivar which reduces the rate of spread of sour cherry yellows in young orchards.

SUMMARY AND CONCLUSIONS

Eighteen samples were taken in March, 1976 from commercial sour cherry orchards in Western Michigan to investigate the presence of NRSV and PDV on a cucumber indicator. Ten of the eighteen isolates showed positive reaction. Field evaluation of growth characteristics, yellow leaf symptoms, and leaf dropping supported the idea that virus or viruses was present in samples; but for some reason, they were not always transferred to cucumber. Failure of transmission of virus(es) might be because a low concentration of virus(es) existed in some of the samples, or inactivation of viruses during transferring and inoculations.

Crude extracts of the isolates which developed symptoms on cucumber were centrifuged and they were tested against PDV and NRSV antisera diluted at (1:50). None of them showed positive reaction to both antisera. Probably one of the viruses was inactivated during extracting, centrifugation or during serial transfers to new cucumbers.

In the spring of 1977, 47 isolates were taken from sour cherry orchards in Western Michigan. They were indexed on squash and Chenopodium quinoa with 33 having a positive reaction. Twenty isolates showed local lesions, 10 showed systemic symptoms on squash, and 24 isolates showed local lesions on C. quinoa. The results of indexing of isolates on herbaceous indicators shows that PDV and NRSV are

widespread within most bearing orchards in Western Michigan.

Three treatments were applied to test the time and method of inoculation and subsequent identification.

Teatment No. 1 (prior inoculation) showed very poor results of indexing on squash and Chenopodium and fairly good indexing results on Shirofugen.

Treatment No. 2 (simultaneous inoculation) showed better results of indexing on squash and Chenopodium than Treatment No. 1 but poorer than Treatment No. 3. The trees of Treatment No. 2 showed good results of indexing on Shirofugen.

The poor indexing results of Treatments No. 1 and 2 for (PDV and NRSV may be because of inactivation of viruses by inhibitors during indexing or in the P. mahaleb rootstock seedlings.

Treatment No. 3 worked better than other treatments. The trees in Treatment No. 3 showed better results of indexing on squash, Chenopodium and Shirofugen. Probably the trees in Treatment No. 3 might have had higher concentrations of viruses than other treatments because disease buds were budded directly into one-year-old Montmorency on P. mahaleb rootstock. Probably different levels and types of phenolic compounds (inhibitors) in P. mahaleb and Montmorency may have inactivated viruses more in P. mahaleb than Montmorency.

Another approach to higher rate of transmission of viruses from trees of Treatment No. 3 to herbaceous indicators may be related

to less inactivation of viruses during indexing because new growing tips were more available for indexing than other treatments.

Shock and yellow leaf symptoms were evaluated during the spring of 1977. Shock symptoms appeared at the early stages of growth, but yellows leaf symptoms developed rapidly almost 2 months after shock symptoms following a period of cool weather in early June in the greenhouse.

In breeding programs, there is a need to index trees for presence of virus. Many indicators can be used to evaluate the presence of virus in the tree.

Herbaceous indicators can be used for indexing when new leaves or petal and pollen grains are available. After bloom, when the leaves become old, rate of transmission will decrease due to the presence of high levels of inhibitors in old leaves.

Shirofugen indicator trees can be used at any time of the growing season because any age tissue can be grafted into it for observation of gumming reaction. Shirofugen test does not distinguish clearly between PDV and NRSV.

Peach seedlings, Montmorency, and Italian Prune can be used as specific plant indicators to detect the presence of PDV and NRSV in the suspicious trees.

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