69-16,174

PLUNGE, Al George, 1928-
DEGENERATION OF GRAPEVINE, Vitis labrusca L. 'CONCORD', IN MICHIGAN: DESCRIPTION OF THE DISEASE AND THE ASSOCIATIONS OF VIRUSES AND VECTOR SPECIES.

Michigan State University, Ph.D., 1969
Agriculture, plant pathology

University Microfilms, Inc., Ann Arbor, Michigan

DEGENERATION OF GRAPEVINE, Vitis labrusca L. 'CONCORD', IN MICHIGAN: DESCRIPTION OF THE DISEASE AND THE ASSOCIATIONS OF VIRUSES AND VECTOR SPECIES

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Entomology

1969

ABSTRACT

DEGENERATION OF GRAPEVINE, Vitis labrusca L. 'CONCORD', IN MICHIGAN: DESCRIPTION OF THE DISEASE AND THE ASSOCIATION OF VIRUSES AND VECTOR SPECIES

By

Al Plunge

Several pertinent observations of the grapevine Vitis labrusca L. 'Concord' that displayed symptoms of virus or virus-like diseases in the Paw-Paw and Lawton, Michigan areas are described. These were: (1) delayed bud-break, (2) malformed and discolored leaves, (3) malformed canes and atypical vine growth, (4) berry shelling, and (5) vine decline. Since all of these were observed in association with one another, they were described as (components of a syndrome) herein named grapevine degeneration. A 'Concord' grape vineyard that was approximately 75-years old and severely affected with grapevine degeneration disease was used as the test vineyard in this research.

Mechanical sap-transmissions were attempted from trap plants (Petunia hybrida Vilm.'Pink Magic') that were grown in relation to the grapevine and from affected grapevine leaves. TRSV virus was isolated with ease from trap plants, whereas, it was initially difficult to transmit the virus from the grapevine plants. Further transmission tests with leaves of various ages and several buffers related that sap extracts from leaves soon after bud-break buffered with

series of young herbaceous plants revealed that garden pea Pisum sativum L. 'Midfreezer' was the differential host. No reactions were obtained on 'Midfreezer' plants inoculated with PRMV, however, distinct systemic lines and ringspotting reactions were noted on 'Midfreezer' plants inoculated with TRSV.

Several virus-free, 1 yr-old V. labrusca 'Concord' plants were mechanically-inoculated with a known strain of CMV (since many of the petunia trap plants in the test vineyard became infected with CMV from aerial vectors) to learn if grapevine was a suitable host. It was found that CMV infects the grapevine and becomes systemically established; however, CMV was recovered from the mechanically-inoculated grapevine by adults of the green peach aphid, Myzus persicae (Sulzer), and not by mechanical-sap inoculations.

ACKNOWLEDGEMENTS

I wish to thank the Michigan Grape Grower's Association and their staff, who suggested this project and aided in establishing a research plot.

This thesis is dedicated to my colleagues: to those persons, past and present, who have been associated with me; for to each and every one I owe much, and it is only the splendid cooperative spirit of this group that has made this thesis possible.

Finally, I would once more acknowledge my indebtedness to my wife and parents, past and present, who have given much help and encouragement in carrying the entire program through to completion.

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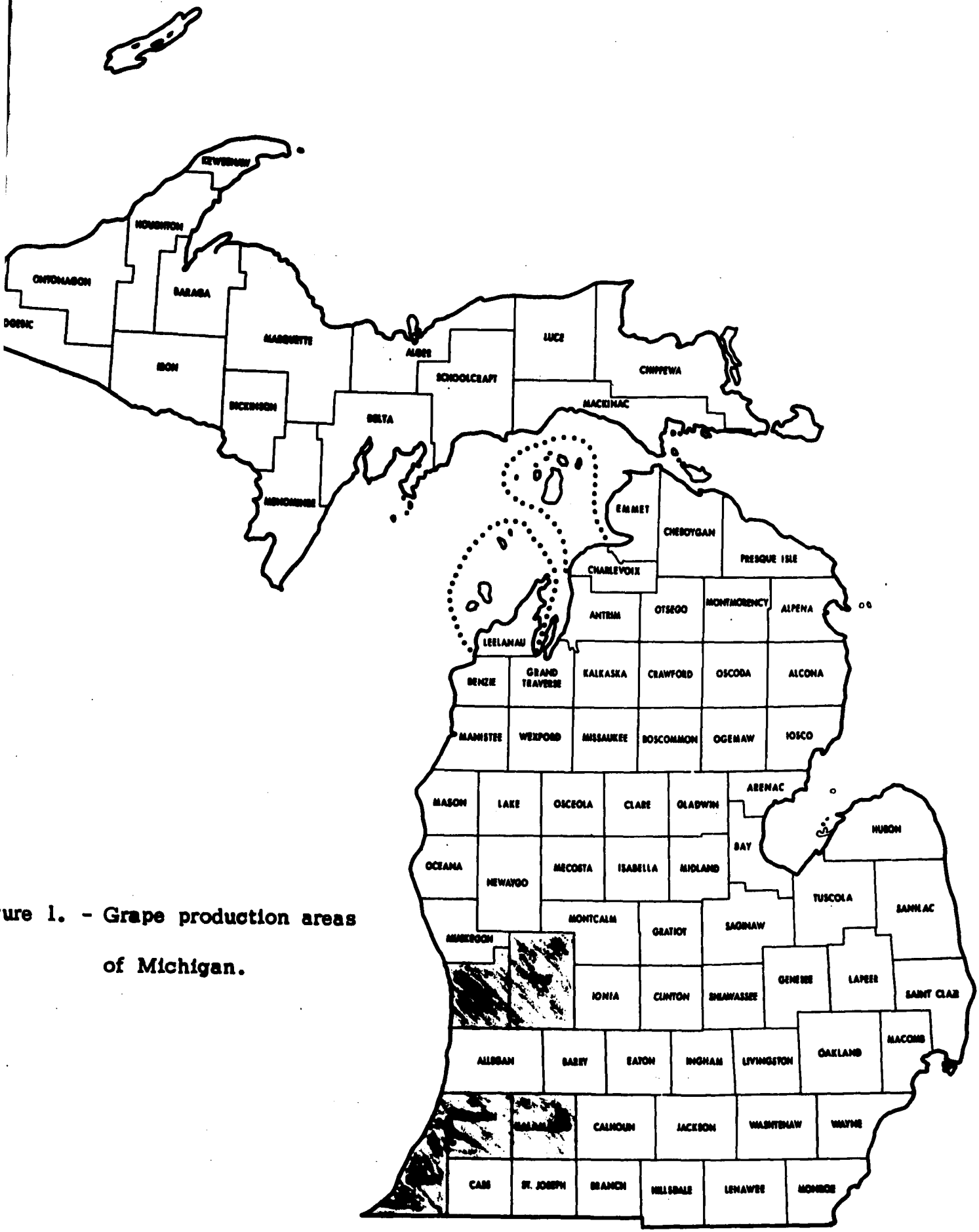
INTRODUCTION

Grapes are an important agricultural commodity in Michigan. The State has ranked high in the Nation in grape production - 3rd in 1965 with a crop of 71,500 ton at a value of $6.5 million and 5th in 1967 with 39,000 ton at $4.6 million (Michigan Department of Agriculture 1966, 1968). All of the grapevines in Michigan are Vitis labrusca L. (predominantly of the Concord variety) and are used primarily for juice and wine. As of 1968, 16,000 acres were in production; 90% of the acreage is in Berrien and Van Buren Counties (in the southwestern corner of the State), with the remainder in Kalamazoo, Ottawa and Kent Counties (Fig. 1).

As one would expect, Michigan's grapevines have not been immune to insect and disease attack. The grape berry moth, Paralobesia viteana (Clemens), and the grape leafhopper, Erythroneura comes (Say) have been the most injurious, throughout the industry, of the insect pests while the rose chafer, Macrodactylus subspinosus (F.), the grape flea beetle, Altica chalbea (Illiger) and several species of climbing cutworms in the family Noctuidae have been of a lesser problem. Black rot, a destructive rot of the berry caused by the fungus Guignardia bidwellii (Ellis), and dead arm, a killer of entire vines or single 'arms' caused by the fungus Phomopsis viticola (Sacc.), have been the two most damaging to grapevine in Michigan.



jure 1. - Grape production areas of Michigan.

Powdery mildew, caused by Uncinula necator (Schw.) and downy mildew, caused by Plasmopara viticola, (Berlese and de Toni) are endemic to Michigan vineyards but their economic importance, while recognized, is undefined. No virus diseases have been identified in 'Concord' grapevine in Michigan, but several growers and research personnel have observed a shelling problem (the berries drop off the cluster prematurely) and a delayed bud-break condition since about 1950 and been suspicious that viruses have been the cause.

Numerous unsuccessful attempts have been made at various times by pathologists in the Michigan Agricultural Experiments Station to isolate and identify agents from 'Concord' grapevines that break bud late and/or shell. Failures also have been obtained in attempts to isolate agents from grapevine leaves displaying a fanleaf condition that first occurred about 1950 and was feared as the grapevine fanleaf virus disease of Pacific Coast vineyards.

As best as could be determined, attempts to transmit viruses from Michigan grapevine have involved (1) mechanical inoculations of sap extracts from diseased grapevine to healthy grapevine (or occasionally to potentially-susceptible herbaceous weed hosts), and (2) grafts of buds or scions from diseased grapevine to healthy grapevine. Virus transmission experiments to grapevine are hindered by a very long virus incubation period (2-3 yr.) in the plant; thus, symptoms and test results are long delayed. It appeared reasonable that extensive use of alternate, herbaceous host

plants and/or trap (bait) plants would enable the transmission, isolation and identification of virus incitants if they were present; furthermore, transmission results would be known in a few weeks or less.

Therefore, this research project was undertaken and the following objectives were established at the onset:

1. Describe the array of disorders of unknown cause.
2. Transmit and isolate viruses from diseased vines.
3. Identify those isolated viruses.
4. Identify vector-virus relationships.
5. Determine methods for assay of grapevines and separation of the 'resident' viruses.

Soon after the start of this project, a few leaf samples from shelling 'Concord' grapevines in Lawton, Michigan were tested for virus by a noted grapevine virus researcher, H.F. Dias, at the Vineland Research Station, St. Catherines, Ontario. He isolated from those leaves peach rosette mosaic virus (Cation 1942) - a virus that he and D. Cation (Michigan Agricultural Experiment Station) had successfully grafted from peach to 'Concord' grapevine and recovered through mechanical inoculation methods (personal communication).

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[1]Arden Ewing Farm, Porter Township and Section 8, Road M-119, Lawton, Michigan.

REVIEW OF THE LITERATURE ON VIRUS DISEASES OF GRAPEVINE - THEIR SYMPTOMS, HOST RANGES, DISTRIBUTIONS AND MEANS OF TRANSMISSION

GRAPEVINE FANLEAF

Grapevine fanleaf is the most devastating virus disease of grapevine in the world (Bovey 1958). This disease is caused by the grapevine fanleaf virus and has been also called (1) the fanleaf disease of grape, (2) urticado (Dias 1950 a), (3) court noue (Vuittenez 1956), and (4) component of degenerescence infectieuse (Bovey 1958).

Symptoms. - Grapevine fanleaf virus causes the primary veins of the leaf to grow close together resembling the ribs of a partially closed fan. Furthermore, the sinuses are lobed or deeply-cut, and the leaf blade is asymetrical. The leaves are mottled in different shades of green (Hewitt et al. 1956).

This virus affects the appearance of grapevine canes and causes malformations of varying types - short internodes, double nodes, fasciation, zig-zag growth between nodes, and flat canes (Hewitt and Gifford 1956). The fruit set is poor on fanleaf-diseased vines as the fruit 'shell' profusely and set in very straggly bunches. In many varieties of grapevine, fanleaf causes decline and gradual death of the vines (Dias 1963).

Host range. - Amaranthus retroflexus L.; A. tricolor L. 'Molten Fire'; Phaseolus vulgaris L. 'Bountiful'; Cucumis sativus L.; Gomphrena globosa L.; Nicotiana clevelandii Gray; N. attentuata Torr.; Chenopodium amaranticolor Coste & Reyn.; Chenopodium quinoa Willd.; Erodium macrophyllum Hook & Arn.; Vitis vinifera L.; Vitis rupestris Scheele 'St. George'; and Vitis labrusca L. 'Concord' (Hewitt et al. 1962).

Geographic distribution. - California, New York, Portugal, Spain, France, Switzerland, Italy (including Sicily) and Germany (Dias 1950 a).

Means of transmission. - Hewitt (1956) established the soil-borne nature of the disease in California and a search for the mode of spread led to the discovery of Xiphinema index Thorne and Allen as the vector (Hewitt et al. 1958). Though 2 tests in this report implicated Criconemoides xenoplax Raski, subsequent tests have been negative. Grapevines in California, also are subject to attack by a wide range of other plant-parasitic nematodes (Raski and Lider 1959). Representatives of most of these including another dagger nematode X. americanum Cobb have been tested as possible vectors causing diseases of grapevines, without success. Cadman, Dias, and Harrison (1960) indicate the grapevine fanleaf is serologically related to arabis mosaic virus (AMV).

Cadman, Dias, and Harrison (1960) have shown in serological tests that raspberry yellow dwarf and strawberry yellow crinkle are strains of arabis mosaic virus. Arabis mosaic virus occurs in the continent of

Europe and in widely separated localities in Scotland, England and Wales; and the vector was reported in 1959 as X. diversicaudatum Micoletzky.

The soil-borne viruses that cause fanleaf, yellow mosaic, and vein banding are all mechanically sap-transmitted and produce very similar symptoms in different herbaceous hosts. Thus these viruses can only be separated on the basis of their affects on the grapevine plant. The vectors for all 3 viruses are X. index (Raski et al. 1965).

GRAPEVINE YELLOW MOSAIC

Grapevine yellow mosaic is as vicious a grapevine killer as grapevine fanleaf (Hewitt et al. 1962), in that the vines gradually degenerate. Yellow mosaic virus causes this disease, which has been also called (1) paranchure (Vuittenez 1952); and (2) clorose infecciosa (Dias 1950 b). Grapevine yellow mosaic virus is considered as a strain of grapevine fanleaf virus (Raski and Hewitt 1965), and only the symptoms of the grapevine will be discussed.

Symptoms. - Yellow mosaic causes either a few small irregular yellow spots on some grape leaves, many yellow blotches, or the leaves are completely chlorotic (Hewitt and Delp 1953).

In grape varieties of Vitis vinifera and V. rupestris infected with yellow mosaic virus, the grape clusters are straggly, with small seedless berries, and are smaller than those on non-infected vines.

GRAPEVINE VEIN BANDING

Vein banding disease is generally associated in the grapevine with yellow mosaic and fanleaf and appears now that all 3 diseases may be caused by strains of the same virus (Hewitt et al 1962). Vein banding is caused by the grapevine vein banding virus (Goheen and Hewitt 1962).

Symptoms. - Vines affected with vein banding produce less fruit than do normal vines, and the clusters of the fruit are straggly with small, seedless berries. The characteristics of vein banding on the grape leaf are chlorotic bands along the veins, either light green or chrome yellow (Goheen and Hewitt 1962).

GRAPEVINE YELLOW VEIN

Grapevine yellow vein disease is damaging to grapes but has been confined primarily to California with a few scattered-infections in New York of little economic significance (Gilmer et al. 1968). The disease is caused by the yellow vein virus, also known as tomato ringspot virus, and was first reported by Hewitt (1956).

Symptoms. - The leaves have chrome-yellow veins and varying shades of yellow. The grapevines affected with yellow vein are larger than the non-infected vines, and the clusters are straggly with large, seedberries

and small seedless berries (Hewitt 1956).

On occasions, young leaves show a mild chlorotic mottle in an oak leaf line pattern which fades as the leaves age. It is not uncommon for clusters of the grape to drop off after bloom (Gooding 1963).

Host ranges. - Mechanical sap inoculations can be made to Chenopodium amaranticolor Coste and Reyn, and Gomphrena globosa L.; Vitis vinifera L.; Vitis labrusca L.; Vitis rupestris Scheele; and in many woody plants such as elderberry, currant and raspberry (Gilmer and Kelts 1968).

Geographic distribution. - California and New York (Gilmer and Kelts 1968).

Means of transmission. - Breece and Hart (1959) established the soil-borne nature of the disease yellow bud mosaic strain and found the vector to be X. americanum. Harrison (1960) has shown by serological tests, yellow bud mosaic is a strain of tomato ringspot virus and yellow vein virus. Fulton (1962) first demonstrated the transmission of tobacco ringspot virus (TRSV) by X. americanum. The ability of transmitting grape yellow vein virus by X. americanum was established by Teliz et al. (1966).

PIERCE'S DISEASE

Pierce's disease kills most varieties of grape Vitis vinifera and V. labrusca (Hewitt 1953). It is the only virus disease affecting grapevines that is known to be spread by air-borne vectors. Pierce's disease is caused by lucerne dwarf virus, also referred to as alfalfa dwarf virus.

Symptoms. - The leaf symptomatology of Pierce's disease is characterized by scalds and burns. The leaf-burning moves in concentric zones, and moves from the margins to the petiole of the leaf.

Fruits affected by this virus color prematurely before drying or withering. Canes mature irregularly and the leaves drop, with petioles still attached to the canes (Hewitt et al. 1962). Death of roots usually follows closely the decline of the top (Hewitt 1953).

Host ranges. - Alfalfa, Medicago sativa L.; white sweet clover, Trifolium incarnatum L.; crimson clover, T. pratense L.; Vitis vinifera L.; and V. labrusca L. (Smith 1957).

Geographic distribution. - Gulf coast states, California, Mexico and Argentina (Hewitt et al. 1958).

Means of transmission. - The virus is not sap-transmissible but can be transmitted by grafting. The insect vectors are all Cicadellid leafhoppers belonging to the subfamily Amblycephalinae; the following species have been identified as vectors: (1) Draeculacephala minerva (Ball); (2) Carneocephala fulgida (Nott.); (3) C. triguttata (Nott.); (4) Helochara delta (Oman); (5) Neokolla circellata (Baker); (6) N. gothica (Sign.); (7) N. confluens (Uhler); (8) N. hieroglyphica (Say); and (9) Cuerna occidentalis (Oman and Beamer) (Smith 1957).

Freitag and Frazier (1954) have conducted tests to determine the percentage of leafhopper vectors that were carrying the virus under natural conditions in a number of different habitats. In these tests particular emphasis was placed on 3 economically important vectors:

(1) the green sharp-shooter, Draeculacephala minerva (Ball); (2) the red-headed sharp-shooter, Carneocephala fulgida (Nott.) and (3) the blue green sharp-shooter, Neokolla circellata (Baker). The results suggest that generally the virus occurs naturally wherever the 3 important vectors are found (Smith 1957).

GRAPEVINE LEAFROLL

Grapevine leafroll affects vineyards primarily in California and Europe with the greatest severity being in Germany (Scheu 1936). Scheu estimated that over 80 per cent of all grapevines planted in Germany were infected; some varieties were so completely diseased that the varietal descriptions were based on symptoms of leafroll-affected plants. Grapevine leafroll virus is responsible for this disease, which has been also called (1) Rollkrankheit (Scheu 1936); (2) white Emperor (Harmon and Snyder 1946); and (3) grapevine leafroll (Harmon et al. 1956).

Symptoms. - The first symptoms appear during the middle of the growing season on the older leaves at the base of the canes. Varieties bearing white or green fruit affected with the leafroll virus have light green leaves and the fruit does not color properly. Burning of the leaves is common on diseased vines in the hot interior valley of California (Goheen, Harmon and Weinberger 1958). The characteristic inward rolling of the leaf margins toward the back of the lamina gives the disease its name.

Production is decreased with vines affected by leafroll and the sugar content is lowered from 1-4 degrees Balling. The full color of the fruit does not develop.

Host ranges. - Vitis vinifera L. ; symptomless carrier Vitis rupestris Scheele 'St. George' (Hewitt, Goheen, Raski and Gooding 1962).

Geographic distribution. - California, New York, Portugal, Spain, France, Switzerland, Italy, Bulgaria, Czechoslovakia and South Africa (Gilmer et al. 1968).

Means of transmission. - Hewitt (1962), indicates the mode of transmission of leafroll has been undetermined. Leafroll is not sap-transmitted to herbaceous hosts.

GRAPEVINE ENATION

Grapevine enation disease only occurs in southern Italy and very little is known about the disease other than the symptoms produced on the grapevine Vitis vinifera L. (Graniti 1959).

Symptoms. - Leaves with enation are generally misshapen, dwarfed, and rough. The veins appear to be larger than normal and are prominent on the lower surface (Hewitt 1954).

Host range. - Vitis vinifera L.

Geographic distribution. - Italy (Graniti 1959).

Means of transmission. - Grapevine enation disease has no known vector, but can be graft-transmitted (Hewitt 1954).

GRAPEVINE CORKY BARK

Grapevine corky bark disease only has been described at Davis, California as graft-transmitted V. rupestris 'LN 33' from Vitis vinifera 'Carignane', 'French Colombard', and 'Grenache' vines that did not

show symptoms. The disease, caused by the corky bark virus, is not important (Hewitt et al. 1962).

Symptoms. - Since grapevine corky bark disease was graft-transmitted to 'LN 33' from vines that revealed no symptoms, very little is known about this virus other than the symptoms that developed on 'LN 33'. In canes of 'LN 33', the bark was thick, soft and spongy and often split into longitudinal cracks with heal-forming fissures (Hewitt et al. 1962).

Host ranges. - Vitis vinifera L.; and Vitis rupestris Scheele 'LN 33' (Hewitt et al. 1962).

Geographic distribution. - Graft-transmitted in California to 'LN 33'.

Means of transmission. - Spread undetermined for the disease grapevine corky bark.

AN UNDESCRIBED DISEASE

This undescribed disease is caused by tobacco mosaic virus which produces no visual symptoms in the grapevine and has no economic importance in grape (Gilmer and Kelts 1968). Tobacco mosaic virus only has been detected in the grapevine by mechanical transmission to host plants and confirmed by serological testing (Gilmer and Kelts 1965).

Host ranges. - Vitis vinifera L.; Vitis labrusca L. 'Concord'; Chenopodium quinoa, Willd.; Nicotiana glutinosa L.; N. tabacum L.; Phaseolus vulgaris L.; and many more too numerous to mention (Smith 1957).

Geographic distribution. - France, and New York (Gilmer and Kelts 1968).

Means of transmission. - The virus is sap transmissible and is one of the most infectious of the plant viruses (Gilmer and Kelts 1968).

GRAPEVINE ASTEROID MOSAIC

Grapevine asteroid mosaic is a disease confined primarily to California and is not important (Hewitt and Goheen 1959). The disease is caused by the grapevine asteroid mosaic virus and has no other known names.

Symptoms. - The leaf symptoms are represented by small, lucid, third-order veins which often coalesce to centers to give the appearance of star-like spots (Hewitt and Goheen 1959). The affected vines produce little or no fruit and are stunted.

Host ranges. - Vitis vinifera L.; and V. rupestris Scheele 'St. George' (Hewitt et al. 1962).

Means of transmission. - The spread of grapevine asteroid mosaic virus is undetermined.

OBSERVATIONS

Every grape vineyard surveyed (50 or more) in the Paw Paw and Lawton, Michigan areas contained vines that displayed symptoms of virus or virus-like diseases. In addition to the fanleaf, shelling, and delayed bud-break symptoms of disease that 'triggered' the start of this project, other virus or virus-like disease symptoms were uncovered. These were the following: (1) malformed and discolored leaves without the typical vein anastomosis of the fanleaf condition, (2) malformed canes and atypical vine growth, and (3) vine decline.

Most of the surveyed-vineyards appeared nearly healthy, except for a widespread occurrence of the fanleaf condition in early August. This ailment is manifested in leaves with an open ventral sinus, serrated margins, and vein anastomosis; the latter is most diagnostic and varies from slight to severe to give the effect of an open to closed fan, respectively, as the leaf narrows with severity (Fig. 2). Since this malady was found in every vineyard surveyed whether the other observed disease symptoms were evident or not, it was judged as a disease unrelated to the others.

Two vineyards[1] were closely observed as they contained vines

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[1]Arden Ewing Farm, Porter Township and Section 8, Road M-119, Lawton, Michigan

[1]Harold Wilder Farm, Porter Township and Section 7, Valley Road, Lawton, Michigan

Figure 2. - 'Fanleaf' condition on a 'Concord' grapevine leaf.

displaying all of the disease symptoms that were recorded throughout the survey and healthy vines as well. The remainder of the vineyards were only scarcely diseased in relation to these two vineyards. Both of the vineyards were characterized by patches of dead or declining vines in some areas of the vineyards and apparently healthy vines in other areas; fanleaf occurred on nearly every vine by September in 1966, 1967 and 1968.

Since all of the observed symptoms occurred on these two farms, it appeared likely that all of the symptoms (except the widespread fanleaf) were part of a syndrome that could be best described as grapevine degeneration.

The grapevine degeneration syndrome appears to be as follows:

Delayed bud-break. - The dormant buds expand until a few millimeters in length, but then remain at a standstill for 10-20 days instead of 'breaking'. Vines affected with these maladies develop slower than normal vines and fruit blooms also 'break' late.

Malformed and discolored leaves. - The leaves on young stems early in the season are mottled with blotches of dark and light green tissue; mottle fades out as leaf ages. Leaves acquire a shape much like that described for the fanleaf condition, but serration of the leaf margin and anastomosis of veins does not occur (Fig. 3). The marginal sinuses on some leaves are deeply-cut and the apical tip is obliterated. Affected leaves usually fall earlier than unaffected leaves; leaf drop begins at the base of the shoots.

Malformed canes and atypical vine growth. - Some canes are atypical in structure and development. These canes are flat rather than round (in cross-section) and often contain short internodes. Laterals from these canes grow in a zig-zag manner as they elongate. Maturity of these canes (when the outer tissue hardens) occurs about 2-3 weeks later than normal.

Berry shelling. - Soon after bloom several of the berries in a cluster drop; some berries remain attached to the pedicel but do not enlarge (shot-berries). Ripening of the remaining fruit is delayed several days and the fruit do not attain full size. This ailment leaves vines with straggly berry clusters of less than one-half the normal number of fruit or with clusters of no fruit (Fig. 4 and 5).

Vine decline. - Shoots become dwarfed and contain malformed, discolored and small leaves; growth is very sparse. When growth is reduced to only a few weak shoots, the vine dies during or soon after its next dormancy.

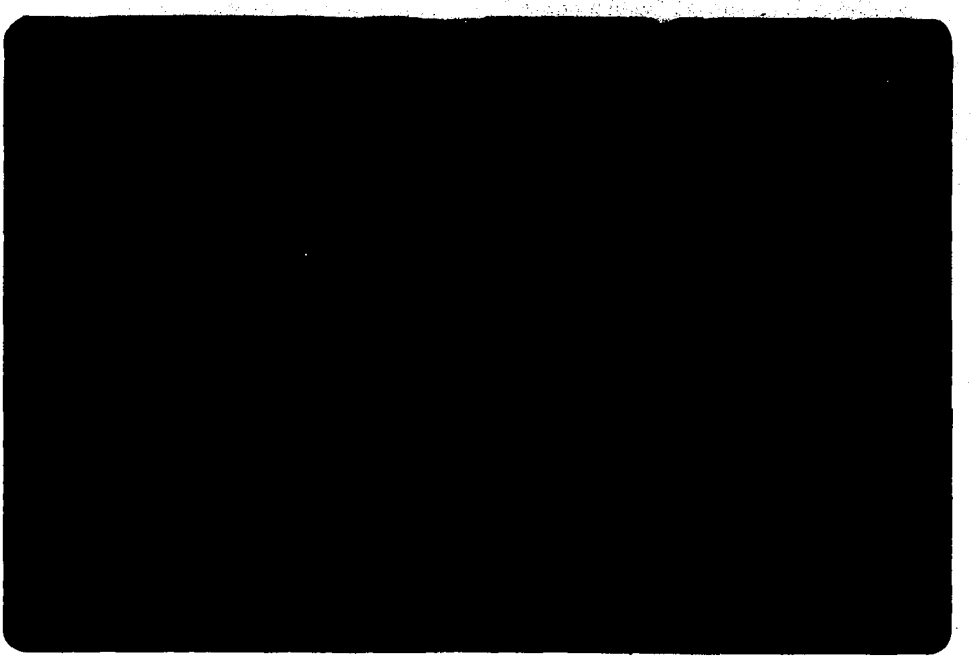

Figure 4. - 'Shelling' condition on 'Concord' grapevines.

Figure 5. - Normal 'Concord' grape bunches.

EXPERIMENTATION

GENERAL METHODS AND MATERIALS. - Test plants, viruses, and vectors. - To achieve a uniform crop of test plants, the seeds were sown in a pan of sterilized soil. Seedlings were transplanted 8-10 days later into 4 inch clay pots in sterilized loam soil and used in experiments within 2 weeks after they were transplanted. These plants were grown in a special room well isolated from other rooms utilized in this research greenhouse.

Black cowpea Vigna sinensis (Torner) 'Black RS', resistant to tobacco ringspot (TRSV) and susceptible to cucumber mosaic virus (CMV); black cowpea V. sinensis (Torner) 'Black SR', susceptible to TRSV and resistant to CMV (De Zeeuw and Crum, 1963); Chenopodium quinoa Willd.; Nicotiana tabacum L. 'Xanthi-nc'; Petunia hybrida Vilm. 'Pink Magic'; Phaseolus vulgaris L.; Cucumis sativus L.; Pisum sativum L. 'Mid-freezer'; and Vitis labrusca L. 'Concord'; were used exclusively as test plants.

The plants used in this study were either grown in or transferred to the greenhouse and were held at a temperature of about 22 C., relative humidity 60% and photoperiod 12 hr. All rooms used for plant-growing were fumigated each week with nicotine or parathion smoke bombs.

The growth chambers utilized in this study were adjusted to a temperature of 22 C, 12 hr photoperiod and 50% relative humidity.

Green peach aphids, Myzus persicae (Sulzer), used in these experiments to remove the CMV from the mechanically inoculated 'Concord' grape leaves, were maintained in the greenhouse. The aphids were allowed to feed on test plants prior to a 2-hr starvation feeding in order to be sure no viruses were present in the green peach aphids used in this study.

The virus-free nematodes were obtained from an area where white pine trees were growing and known as the Sandford Wood-Lot at Michigan State University. This wood lot was never utilized for agricultural crop production, thereby eliminating the presence of grape diseases. Large numbers of virus-free nematodes were obtained by processing many 1-pint samples of soil through the use of the technique developed by Christie and Perry (1951).

Test farm. - Petunia plants were planted adjacent to the grapevine, affected with grapevine degeneration at the farm of Mr. Arden Ewing, Lawton, Michigan. Fig. 6 shows the results of the grapevine degeneration disease - a drastic decline is quite evident.

Mr. Ewing's vineyard was approximately 75-years old and was replanted with a new 'Concord' grapevine wherever old vines were rogued out. The grapevines were trained and pruned each year prior to bud break, and fertilized with a complete fertilizer. A green-manure or cover crop was planted in the fall to check grapevine growth, thus giving the vine time to mature its wood properly.

Figure 6. - A 'Concord' grape vineyard in southwestern Michigan showing drastic vine decline.

No herbicides have ever been used in this test vineyard, however, a cultivation program of keeping the grape vineyard free from weeds was practiced. In general, the grape spray program for control of diseases and insects was conducted as outlined by the extension service.

Twelve rows of approximately 50 grapevines per row were present in the test vineyard. The vines were planted in a sandy-loam soil, located on a gradual slope. The grape rows ran north and south and on the south end of the field was a wood-lot while on the north and east ends of the field were more grapevines. On the west side of the test vineyard was a productive asparagus field. This test vineyard was an excellent illustration of grapevine degeneration.

Mechanical inoculations. - In the mechanical inoculations, other than grape leaf tissue extracts, disodium phosphate .07 molar was used as a buffer. Only enough buffer was added to liquify the tissue extract and plants were inoculated after 2 to 3 weeks of growth using carborundum on the test plants as an abrasive and washing the leaves with water immediately after the plant sap was applied to the leaves by finger.

Those inoculations where young grape leaf tissues were taken from grape plants believed to be infected with grapevine degeneration were thoroughly triturated in 2-3 ml of Kirkpatrick-Lindner buffer 3% nicotine 0.05 M K_2HPO_4 - 0.005 M cysteine hydrochloride (1963).

Two to three leaves of the test plants were lightly dusted with 400-mesh Carborundum and inoculated with the grape leaf triturated material. The plant sap was applied to the leaves by finger. Surgical

gloves were used and washed after each inoculation. The inoculated leaves were rinsed 2 minutes after inoculation. This prevented the nicotine from entering the test plants and causing phytotoxicity. The test plants were removed to an insect-proof greenhouse room to await symptom development. To guard against contaminating insects from entering the room, a fumigation program was initiated at the greenhouse as explained earlier. The test plants were undisturbed for 2 or 3 weeks after inoculation before symptom readings were made. After the readings were taken, the plants were mechanically back-inoculated to their respective plants.

Ouchterlony Double-Diffusion Agar Test. - Tobacco ringspot antiserum was obtained from two sources. One source was from R. W. Fulton, Department of Plant Pathology, University of Wisconsin and the other source was from H. A. Scott, Department of Plant Pathology, University of Arkansas. Both antisera were pre-tested with a known tobacco ringspot virus to make sure the antiserums were properly reconstituted.

A 1% Bacto-Agar solution was prepared and properly melted in a water bath. After the solution was melted a 0.02% sodium azide preparation was added to the Bacto-Agar in order to inhibit the growth of micro-organisms.

To each petri dish that was used for the Ouchterlony tests, 20 ml. of Bacto-Agar...02% sodium azide solution was added and allowed to solidify. A plastic pattern of seven, 7-mm rings in 3 rows was used

to prepare the wells for antigen-antiserum reactions. The edges of each ring in the pattern were so spaced that the 2 outer rows of 2 were 1 cm from each adjacent ring.

The petri dish of solidified agar was then placed over the pattern and a flamed cork borer was used to punch wells in the agar representing circles of the pattern. The agar in the wells were removed by use of an aspirator. Later a transfer capillary pipette with melted agar was used to place a droplet of agar in each well. This sealed the bottom of the solidified agar dish.

Into the center well, designated D, of the agar plate was added enough tobacco ringspot virus antiserum (1:8) to fill the well. Then to the top 2 wells designated A and B and the bottom 2 wells designated F and G were added petunia sap (diluted 1:8 with disodium phosphate ph 8.0) from the grapevine degeneration plants. To the remaining 2 center wells labelled C and E were added known tobacco ringspot petunia sap (diluted 1 to 8 with disodium phosphate ph 8.0).

These agar gel plates were held at 22 C and 60% humidity properly, marked, and allowed to incubate for 10 days with the tops on the petri dishes (to allow for a moist atmosphere). After incubation the samples were read and properly recorded.

EXPERIMENTS AND RESULTS

Isolation of virus through the use of a trap plant. - Two factors encouraged the use of a trap plant in the isolation of virus(es) from grapevine: (1) the pattern of degeneration in the test vineyard indicated the likelihood of a soil-borne incitant, and (2) grapevine was known to be a poor source plant from which to directly isolate viruses through mechanical means (Gilmer and Kelts, 1967).

Petunia hybrida Vilm. was selected as the trap plant species as it is susceptible to a wide range of viruses, including several of grapevine. Young petunia seedlings were planted adjacent to grapevine plants throughout the test vineyard in May of 1967 and 1968; the two plantings were identical, except the plants in the latter planting were covered with saran-screened cages to exclude aerial insect contamination. Both plantings encompassed grapevines varying in appearance from normal to severely declined. In early fall of each year, petunia plants that showed any indication of virus symptoms were transplanted to large pots or tubs and transferred to the greenhouse. Then the plants were assayed for viruses by mechanical inoculations (in .07 M disodium phosphate buffer) to several herbaceous test plants.

TABLE 1. Response of various plant species when inoculated with sap extracts of trap petunia plants, that were grown adjacent to grapevines affected with grapevine degeneration[a]

Test Plants	Transmissions[b] and symptoms[c] 1967	1968
Chenopodium quinoa Willd.	-	8/13 RN
Nicotiana tabacum L. 'Xanthi-nc'	18/68 LP 14/68 CM	8/13 LP
Vigna sinensis (Torner) 'Black SR'	18/68 NLL	8/13 NLL
V. sinensis (Torner) 'Black RS'	14/68 TMM	0/13 0

[a]Trap plants in 1967 were not protected from aerial insects; plants in 1968 were protected.

[b]Numerator=number of infections; denominator=number of trials. Each trap petunia was assayed once on the range of test plants.

[c]CM=chlorotic mottle; LP=line and oak leaf patterns; NLL=necrotic local lesions which are reddish-brown; 0=no symptoms; RN=rosetting and necrotic lesions; TMM=trifoliate leaves mottled and mosaic; -=test plants not utilized for assay.

Four different types of virus reactions were obtained (Table 1.) The positive response of V. sinensis 'Black RS' which is resistant to tobacco ringspot virus and susceptible to cucumber mosaic virus to the affected petunia sap suggested in the petunia plants were infected with CMV in the vineyard.

Another virus reaction was the development of an oak cluster on the 'Xanthi-nc' plant. This response is typical of a TRSV reaction.

A TRSV-susceptible reaction was also obtained on Vigna sinensis 'Black SR'. Primary lesions of necrotic spots appeared on the cotyledons and within several days the virus became systemic.

In essence, two viruses were found in the petunia plants interplanted during the 1967 season in the affected vineyard...CMV and TRSV.

From studies made in 1967, Table 1 demonstrates that the TRSV was soil-borne and present in the affected vineyard during the 1968 growing season and possibly in 1967 as well.

Isolation of virus directly from grapevine. - Several hundred attempts were made to isolate virus(es) from grape leaves, stems, roots and fruit between June and September, 1967 with the use of 2.5% nicotine sulfate as a buffer and C. quinoa as an assay plant. Two extracts made from the stem and terminal leaves of a degenerate grapevine on June 4, 1967, during an initial isolation attempt, were infectious and caused initially a mosaic and finally a severe terminal stunt and chlorosis reaction on C. quinoa. None of the other extracts were infectious in 1967.

The numerous failures obtained in 1967 led one to suspect that (1) another buffer system might more suitably stabilize the inoculum than nicotine sulfate, and (2) the relative titres of virus and inhibitor might be optimum for mechanical virus transmission at, or soon after, bud-break. Thus, extractions in 1968 were begun at bud-break and made once a month thereafter. A late frost in May set back most of the grapevines approximately 3 weeks, therefore bud-break in 1968 did not occur until mid-June. Also, at the suggestion of R. M. Gilmer, Department of Plant Pathology, Cornell University, sap extracts were triturated in 2-3 ml of Kirkpatrick-Lindner buffer (Kirkpatrick et al., 1963).

TABLE 2. Responses of various plants species when mechanically inoculated with extracts from leaves of grapevine, Vitis labrusca L. 'Concord', affected with grapevine degenerations

Plants tested	Transmissions[a] and symptoms[b] 22 June	6 Aug.	6 Sept.
Chenopodium quinoa Willd.	21 RN	0	0
Nicotiana tabacum L. 'Xanthi-nc'	14 LP 7 EM	8 EM	0
Vigna sinensis (Torner) 'Black SR'	13 NLL	7 NLL	0
V. sinensis (Torner) 'Black RS'	5 TMM	0 TMM	0

[a]Number of transmissions per 22 grape plants tested; the same plants were assayed on each date.

[b]EM=epinasty and mosaic; LP=line and oak leaf patterns; NLL=necrotic local lesions which are reddish-brown; 0=no symptoms; RN=rosetting and necrotic lesions; TMM=trifoliate leaves have a mosaic pattern.

Identification of tobacco ringspot virus. - Twelve plates of ion-agar were prepared to ascertain the presence of TRSV in the caged petunia plants interplanted in the grapevine degeneration vineyard during May, 1968, using the technique known as the Ouchterlony agar double-diffusion test. Known phosphate-buffered laboratory induced TRSV petunia extracts and phosphate-buffered extracts from the caged trap petunias were placed in wells in a gel diffusion plate, and the TRSV antiserum was placed in an adjoining well. Both the 'caged petunia' extracts and the known laboratory induced TRSV petunia extracts reacted with the antiserum, and the preciptin zones coalesced in a reaction of identity. Definite precipitation zones were observed with 8 out of the 12 antigen-antibody reactions. This experiment further proves that TRSV was present in the degenerate grapevine test vineyard.

Nematodes associated with a degenerate vineyard. - The likelihood of a soil-borne incitant in the test grape vineyard suggested the hypothesis that nematodes were the vectors. Additionally, some growers were adamant that the incitant was grapevine fanleaf, which is transmitted only by Xiphinema index Thorne and Allen in California (Hewitt et al. 1958); this nematode is only known to occur as far north as southern Indiana (J. Ferris, personal communication). Thus, soil samples were taken throughout the root zone of the grape plant to a depth of 12 inches and within 2 ft. of the base of the plant with a soil probe to ascertain the nematode composition in the test vineyard. These soil samples were taken from the degenerate vineyard in May of 1966, 1967, and 1968.

The Jenkin's (1964) sugar flotation technique was employed to extract the plant parasitic nematodes from the soil samples. The sugar flotation technique was used rather than the Christie-Perry (1951) technique because some nematodes, such as, Criconemoides sp., are unable to wiggle through the cloth filter in the Baermann funnels utilized in the latter technique.

Soil samples were processed in 50 ml quantities and all nematodes genera known or suspected to be parasitic to grapevine were sought in sample readings; only X. americanum Cobb and X. index Thorne and Allen were determined to the species level.

TABLE 3. Plant parasitic nematodes found in the root zone soil area of grapevine, Vitis labrusca L. 'Concord', affected with grapevine degeneration

Nematodes sought	The number of nematodes[a] per 50 cc of soil sampled in May of specified years		
	1966	1967	1968
Aphelenchus sp.	3	0	0
Criconemoides sp.	65	10	40
Hoplolaimus sp.	1	8	0
Longidorus sp.	0	2	0
Pratylenchus sp.	3	3	5
Tricodorus sp.	2	0	0
Tylenchus sp.	12	4	2
Xiphinema americanum	15	22	8
Xiphinema index	0	0	0

[a]Nematodes were extracted by the Jenkins (1964) sugar flotation technique.

Representatives of the 3 nematode genera (Longidorus, Trichodorus and Xiphinema) known to transmit plant viruses were extracted from the test vineyard (Table 3). However, only 2 individuals of Longidorus and Trichodorus were obtained and on a single occasion. Xiphinema americanum individuals were obtained in relative abundance in each sample. As might be expected no X. index individuals were uncovered.

Transmission of TRSV by Xiphinema americanum extracted from the root zone of degenerate grapevines. - Since TRSV was isolated from bait petunia plants that were grown in the test vineyard, it was assumed that nematodes were the vector organisms. This assumption was tested through transmission trials involving individuals of Xiphinema americanum that were extracted from soil collected from the root zone of degenerate grapevines; samples were taken only from around vines that were free of weeds.

Large numbers of nematodes were obtained by processing many 1-pt. samples of soil through the use of the technique developed by Christie and Perry (1951). Individual X. americanum were hand-picked into small vials of water in lots of 30-50 nematodes. These nematode lots were introduced to the healthy test plants (petunia), which were growing singly in 150 ml. beakers in a sterilized media of 1 part loam soil to 1 part Turface, a granular calcined pyrophyllite (Fig. 7). An inoculation period of 14 days was allotted in a controlled environment chamber; then, all petunia plants were indexed by mechanical inoculations of expressed sap to V. sinensis 'Black SR' and N. tabacum 'Xanthi-nc '

Figure 7. - Seedlings in sterilized soil and granular calcined pyrophyllite in 150-ml beakers. The system used for acquisition of viruses by hand picked nematodes which were introduced into beakers.

test plants.

Sixty percent (12 out of 20 nematode lots) transmitted TRSV to petunia bait plants, as indicated by typical TRSV reactions on the 2 test plants. This confirms that TRSV is present in the root zone of the affected test vineyard and that X. americanum are carriers of the virus.

Separation of TRSV and PRMV on the basis of differential host reactions. - With at least 2 viruses present in 'Concord' grapevine in the test vineyard, surveys will need to be made to determine the distribution and extent of both viruses, together and/or individually. An assay technique which would permit virus separation on the basis of mechanical inoculations of plant sap to differential herbaceous host plants would be most beneficial; thus, trials were made to uncover such host plants.

Sap extracts of TRSV-infected tobacco and PRMV-infected peach were prepared from macerated leaves, triturated in 0.07 M disodium phosphate buffer, and mechanically inoculated to a series of young herbaceous plants (Table 4).

Chenopodium quinoa did not reveal a good differential host reaction for the two viruses tested. Nicotiana tabacum 'Xanthi-nc', Phaseolus vulgaris, Pisum sativum 'Midfreezer' and Vigna sinensis 'Black SR' and 'Black RS' revealed specific differential host reactions (Table 4). However, Pisum sativum 'Midfreezer' illustrated a very distinct systemic line and ringspotting reaction (Figures 8, 9 and 10) to

TABLE 4. Response of several herbaceous plants to tobacco ringspot virus (TRSV) and peach rosette mosaic virus (PRMV)

Plants tested	Plant response[a] to: TRSV	PRMV
Chenopodium quinoa Willd.	DE	DE
Nicotiana tabacum L. 'Xanthi-nc'	LP	E
Phaseolus vulgaris L.	LP	CM
Pisum sativum L. 'Midfreezer'	LP	0
Vigna sinensis (Torner) 'Black SR'	NLL	TMM
V. sinensis (Torner) 'Black RS'	0	TMM

[a]DE=dwarfing and epinasty; E=epinasty; LP=line and oak leaf pattern; CM=chlorotic mottle; NLL=necrotic local lesions; 0=no symptoms; TMM=trifoliate leaves mottled and mosaic.

Figure 8. - BEGINNING stages of ringspotting on the leaves of Pisum sativum L. This plant was mechanically inoculated with tobacco ringspot virus...growth of this virus was systemic.

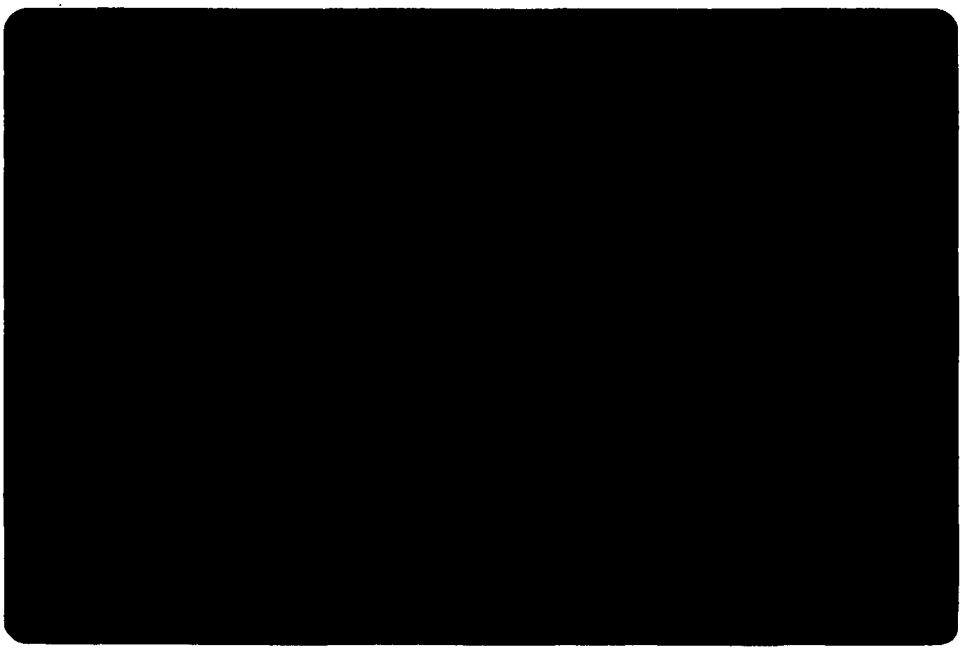

Figure 9. - INTERMEDIATE stages or ringspotting on the terminal leaves of Pisum sativum L. This plant was mechanically inoculated with tobacco ringspot virus...growth of this virus was systemic.

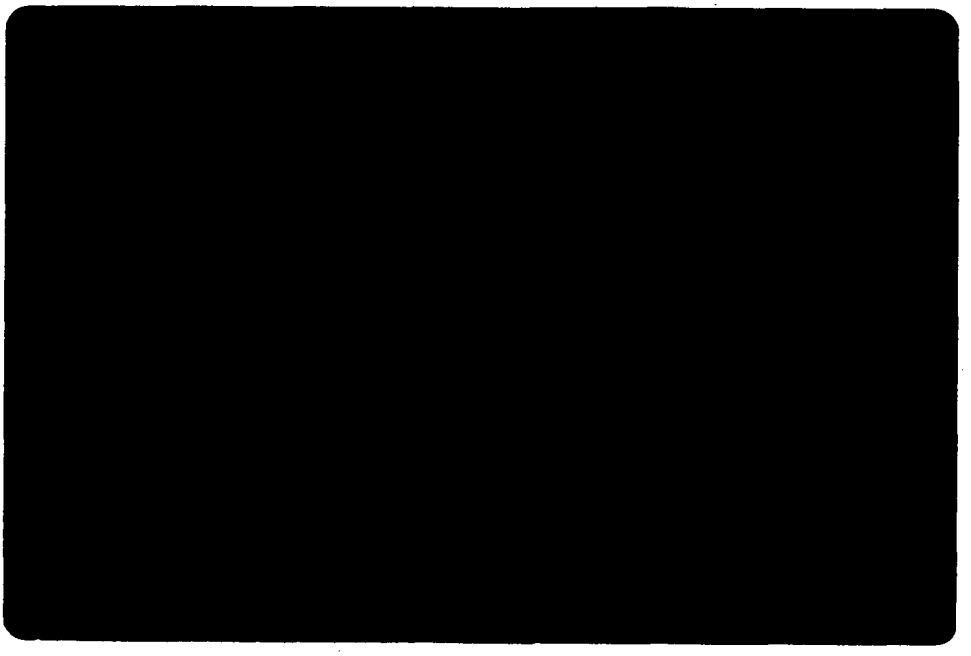

Figure 10. - The FINAL stages of ringspotting on the terminal leaves of Pisum sativum L. This plant was mechanically inoculated with tobacco ringspot virus...growth of this virus was systemic.

Transmission of peach rosette mosaic virus from mechanically-inoculated petunia plants. - The occurrence of peach rosette mosaic virus (PRMV) in the field-grown petunia trap plants in this study and the reported relationships of PRMV to 'Concord' grapevine (Klos et al., 1967 and H. F. Dias, personal communication), prompted experimentation to determine, under controlled conditions, whether X. americanum is indeed a vector of the virus. The tests conducted by Klos et al (1967) were largely field trials and the test plants could have been contaminated by organisms other than nematodes.

A PRMV isolate from peach was mechanically inoculated with extract buffered with disodium phosphate to young petunia plants that were grown in the beaker system of 1 part loam soil to 1 part Turface, described earlier. About 14 days later when plants were showing symptoms of virus infection, 50-60 hand-picked, virus-free, X. americanum were introduced into the source plant root zones. A 10-day acquisition period was allotted in a controlled environment chamber.

The affected petunia plants were removed from the beakers and new virus-free petunia plants were replanted in the same beakers. The petunia plants remained in the beakers for 14 days and were then indexed to test plants using 0.07 M disodium phosphate as a buffer.

Eighty percent (8 out of 10 nematode lots) transmitted PRMV to petunia bait plants, as indicated by typical PRMV reactions on the test plants.

This experiment revealed that X. americanum was an excellent vector for this virus.

Figure 11. - Cucumber mosaic virus mechanically inoculated to 'Concord' grape leaf exemplifying progression of cucumber mosaic virus from the leaf tip to the leaf center.

Figure 12. - The latter stage of mechanically inoculated cucumber mosaic virus affected 'Concord' grape leaf causing leaf death.

aphids were removed, the plants were sprayed with malathion, and 3 weeks were allotted before symptom readings were made. The experiment was conducted on 2 occasions within one month. After the readings were made, the plants were mechanically inoculated to virus-free test plants of the same variety and the results of these inoculations confirmed the findings.

Green peach aphids were very efficient in the transmission of CMV from grape leaves to cucumber (Table 5). In both trials, all of the aphids tested at a 23-min acquisition period became infectious whereas no transmissions resulted from periods shorter than 10 min. These insects also proved that the virus became systemically-established in the grapevine as they recovered virus from a non-inoculated leaf.

Chemical analysis of grapevine leaves affected with severe vein anastomosis. - The widespread occurrence of the leaf vein anastomosis or 'fanleaf' condition in the grape production areas of Michigan suggested that perhaps the incitant was the herbicide 2,4-dichlorophenoxyacetic acid. However, this was considered unlikely as 2,4-D had not been used in the involved counties for over a decade. Additionally, no viruses have been isolated from 'fan-leaves'.

To test the hypothesis that 2,4-D was the incitant of fanleaf, an analysis was made of the 2,4-D content of severely affected grapevine leaves by electron capture gas chromatography (Erickson and Hield 1962). Grape leaf tissue from the test vineyard contained trace amounts (0.001 p.p.m.) of 2,4-E which probably could not cause the leaf vein anastomosis (Gilmer, personal communication). Thus, the incitant for the leaf vein anastomosis or 'fanleaf' condition in the grape production areas of Michigan was not likely to be the herbicide 2,4-D.

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

From available evidence presented both herein and by H. F. Dias (personal communication), and Klos et al. (1967), it is apparent that vines in the test vineyard in various stages of degeneration are infected with peach rosette mosaic virus (PRMV), and tobacco ring-spot virus (TRSV).

In this study, the sap extracts from grapevine that produced positive TRSV reactions on differential herbaceous hosts were not tested serologically. But sap extracts from petunia plants growing in the same root environment gave identical TRSV reactions on the assay plants, and these were serologically-positive for TRSV. Some of these same trap petunia plants, also yielded sap which tested serologically-positive for PRMV; thus, the original premise that two or more viruses (PRMV and TRSV) were present in the test vineyard was substantiated.

The differential hosts uncovered for TRSV and PRMV separation will facilitate surveys of many vineyards in the Paw Paw and Lawton, Michigan areas to learn the distribution in vineyards of severe, moderate and mild degeneration. Surveys could be made throughout the growing-season through the use of sample cuttings that are subsequently rooted and predisposed with a 5-min per hr photoperiod regime for 2 weeks prior to sap extraction.

Further testing of the 'Concord' grape plants revealed that after mechanically inoculating healthy grape leaves with CMV, the virus infected the grapevine and became systemically established. Since CMV was recovered by the green peach aphid, it is possible that this cosmopolitan aphid, also inoculated field-grown 'Concord' vines with CMV and that CMV is a resident virus in grapevine; however, CMV has not been extracted from field-grown grapevine. Experiments designed to remove CMV from the field-grown grapevine by mechanical means should be investigated.

Attempts should now be made to determine 'cause and effect' relationships between the viruses and specific aspects of the degeneration syndrome. Of paramount importance is a test of the hypothesis presented here that PRMV and/or TRSV can cause berry shelling and premature berry drop. A young 'Concord' transplant probably will not fruit until it is 4 yr old; thus, a long delay would occur before infections of TRSV or PRMV (created through mechanical or nematode inoculation) could be related to fruit set. Perhaps it would be more feasible to graft PRMV - or TRSV - infected buds into virus-free 'Concord' vines that are within 1-2 yr of maturity and anticipate that infections would affect fruit set within 1-2 yr of the grafting operation.

Klos et al. (1967) indicated a causal relationship of PRMV to delayed bud-break and shelling, but only 4 plants were involved in the experiment; only 1 'shelled' of the 13 inoculated seedlings and it occurred 4 yr after inoculation. No mention was made of the condition of the plant or whether the grafted plants became infected. During this

thesis work, several healthy plants have shelled in and out of the greenhouse and suspicion was cast on soil fertility during pot-confinement as the probable cause.

It is likely that any factor that affects grapevine nutrition will affect berry set and maturity. Nearly all of the known grapevine viruses have been shown to affect fruit set, rate of maturity and fruit size. However, fertilization is known to be highly critical in grape production and any shortages of zinc are known to affect the fruiting stage (Shoemaker, 1955); thus, stresses on the nutrition of the plant caused by virus infections could possibly affect fruit development. Any of the viruses isolated are suspect causes. Likewise, it is reasonable that bud-break is dealyed in a plant of poor nutrition as might be caused by a resident virus.

Mechanical methods of virus transmission from grape leaves were difficult. Proper timing of extractions and the use of appropriate buffer systems were shown to be requisite for virus transmission. Only when leaf samples from degenerate 'Concord' grapevines were taken 14 days after bud-break and the Kirkpatrick-Lindner buffer was used were excellent virus transmissions obtained.

Further study should be conducted on the relative titres of the viruses in the grapevine and the elimination of the inhibitors during mechanical inoculation of the grape leaf. Varying the buffer concentration and the chemical composition of the buffer systems may eliminate the problems encountered during mechanical inoculations. Also,

the photoperiod for grape cuttings should be investigated as to optimum and minimum amounts for proper mechanical inoculations to be made with the grapevine viruses (TRSV and PRMV). H. F. Dias (personal communication) has found that a 5-min per hr photoperiod for 2 weeks predisposes the grapevine for optimum success in virus extraction.

X. americanum is capable of transmitting PRMV and TRSV and since one or both of these viruses is a likely contributor to the degeneration disease it is reasonable, even at this early stage of study, to recommend that all new grapevine plantings be fumigated and that all propagations be made only from certified virus-free mother plants. Embargoes should be placed on plants propagated from non-indexed plants.

For those vineyards that are presently infected with grapevine degeneration, a sanitary program for disinfecting pruning shears and cultivation should be developed by the extension service. Where individual grapevine replacement is necessary, a simple hand-gun fumigator or jiffy applicator with plastic covering and fumigant can be employed. Again virus-free replacement plants are a necessity. Furthermore, a virus-free grapevine replacement program should be instigated by the state regulatory department.

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