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EPIDEMIOLOGICAL PARAMETERS AFFECTING THE SPREAD OF THREE VIRUS DISEASES OF SMALL FRUIT CROPS AND THEIR DETECTION USING REMOTE SENSING.

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By

Adele M. Childress

A DISSERTATION

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ABSTRACT

EPIDEMIOLOGICAL PARAMETERS AFFECTING THE SPREAD
OF THREE VIRUS DISEASES OF SMALL FRUIT CROPS AND
THEIR DETECTION USING REMOTE SENSING

BY

Adele M. Childress

Three virus diseases, two affecting highbush blueberry **Vaccinium corymbosum** L. cv. Jersey and one affecting grape **Vitis labrusca** L. cv. Concord, were investigated to determine their mode of transmission and spread in the field and vineyard. In addition, remote sensing techniques were used to delineate virus-infected blueberry bushes and grapevines from healthy plants under field conditions.

Highbush blueberry cv. Jersey, is susceptible to blueberry leaf mottle virus (BBLMV) and blueberry shoestring virus (BBSSV). Peach rosette mosaic virus (PRMV) incites a disease in grape and is vectored by the nematode **Xiphinema americanum** (Cobb).

The blueberry leaf mottle virus has been classified as a putative member of the nepovirus group. However, previous attempts to demonstrate nematode transmission have been unsuccessful. While pollen transmission is a secondary mode of spread for members of this virus group, few plant virus diseases have been described associating honeybee-mediated transmission of a virus via virus-contaminated pollen. Using serological techniques, e.g., enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA), BBLMV

has been detected on or in pollen obtained from infected bushes in the field. The virus has also been associated with pollen collected from the pollen-baskets of honeybees (**Apis mellifera**) foraging within BBLMV-infected fields. Serological blocking experiments have shown that BBLMV is located both as a contaminant on the outside of the pollen grain and possibly within. Blueberry leaf mottle virus has been successfully rub-inoculated to **Chenopodium quinoa** using virus-infected pollen as inoculum. Blueberry leaf mottle virus was also transmitted in the field to healthy two-year-old cv. Jersey trap-plants surrounding caged BBLMV-infected source plants and containing a small hive of honeybees. Serological assays of blueberry seeds surface disinfected with a NaOCl solution have shown that BBLMV is associated with the seed coat and possibly occurs within the seed. However, only a low percentage of the resulting seedlings was infected. Although the soil beneath a few BBLMV-infected bushes contained a low population of three genera of potential vector nematodes, generally their occurance was not associated with the disease in the field. Blueberry leaf mottle virus was not detected by RIA in **Xiphinema americanum**, **Longidorus** spp. or **Trichodorus** spp. obtained from soil beneath infected bushes or when allowed an acquisition access period (AAP) on the roots of BBLMV-inoculated **C. quinoa** seedlings. Weeds obtained from within infected blueberry fields where potential nematode vectors were present or absent, were negative for BBLMV when tested by ELISA.

Grape seeds from PRMV-infected vines are often returned to vineyards for mulch, following commercial processing of the grapes for juice. The role of infected seed and seedlings in the spread of the disease was investigated. Peach rosette mosaic virus was detected by ELISA in a high percentage of the seed following the processing treatment, but was not found in seedlings that germinated from PRMV-infected seed lots.

Grapevines infected with PRMV exhibited a marked reduction in yield compared to virus-free vines. Although PRMV was detected in only a few of the germinated seedlings, the potential for establishing new infection sites exists. Therefore, it is recommended that pomace not be returned to the vineyard as mulch.

Spectral reflectance of plant canopies was measured using aerial and low-altitude photography, as well as a field-portable spectroradiometer. Ground truth was acquired by serologically testing individual plants in blueberry fields and grape vineyards. While subtle differences were observed on color, color-infrared and black/white infrared film, these were attributed to environmental influences or the difference in growth stages between and within the plants. Generally, reflective differences were observed between virus-infected and healthy plants in the near-infrared region of the electromagnetic spectrum, using the spectroradiometer. However, no consistent pattern of increased or decreased reflectance was observed. This technique appears to have limited usefulness as a tool for rapidly surveying areas of the field or vineyard infected with these virus diseases.

In Dedication to Madeleine

Mother, First Teacher, Friend

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CHAPTER I LITERATURE REVIEW

A. CROPPING PRACTICES

The factors affecting the selection of a host plant by a pathogen, its vector or both are partially controlled genetically and have evolved to optimize this relationship (50,119). However, features of the crop's habitat (cropping practices) such as size, crop spacing and duration of the growing season will influence whether a disease will become established and govern the extent of the damage, often overriding genetic predisposition. Unlike annual crops, where virus infection and symptom expression are rapid (117), infection and disease spread in perennials is slow, often involving a long latent period before symptom expression and virus titer are detectable (220). While the spread of disease is slower in perennial crops, their economic loss may be greater since replacement cost is high and several years are required to return to full production (9,114, 123, 209).

Michigan is one of the leading producers of woody small fruit and tree fruit crops. This is primarily due to the moderate climate produced from the "lake effect". Much of the production is located near the coast of lake Michigan. In 1981, Michigan had over 116,000 acres of land in full production for the major fruit crops, realizing a cash return of 158 million dollars. Depending on the season, Michigan is the fifth largest grape producer, primarily cv. Concord, and the largest highbush blueberry producer in the United States. Blueberries represent 13,000 acres in

production with a cash value of over 40 million dollars (J. Nelson, personal communication) while 14,000 acres are in grape production with a return of 14 million dollars (1.2 % of U.S production) (149). Most of these crops are produced in the southwest district of the state and the counties of blueberry and grape production overlap.

The highbush blueberry, **Vaccinium corymbosum** L.,is the most widely planted commercial species. Approximately 56.2 % of the total acreage of commercial plantings are of the cv. Jersey (Rubel X Grover), introduced in 1928 (47) for its vigor, ease in harvesting and late season. Blueberries are grown on marginal, acid soils (pH 4.2-5.2) and require moist conditions, since they are shallow-rooted (21) and lack root hairs, resulting in a low water absorption capacity (40). The rootlets grow about 1 mm per day and as the plant gets older, the roots may extend into the space between the rows making contact with roots of plants in opposite rows (47). In commercial plantings bushes are usually spaced 4-5 feet apart in the row by 8-10 feet across. As they reach maturity after about 8 years, the close spacing results in an enclosed canopy with branches of adjacent plants overlapping, facilitating insect movement.

The bell-shaped corolla of the flower is inverted, closed and adapted for insect pollination. Bees and other insects are attracted by the blossoms color and the sweet nectar located in glands on the corolla. Coville (40) has found the heavy pollen grains clumped and not easily spread by wind. Pollen released from the stamens does not usually fall onto the stigma but is deflected due to the angled shape of the stigma, thus reducing self-pollination. While blueberries are self-fertile, pollination by bees is favored (40). Blueberry pollen is in a tetrad, containing four cells, but only one germ tube is usually produced per

grain (146). Merrill (146) found that 79 % of the pistils of highbush blueberry are receptive to pollen 4 days after blossoms open. Once the pollen grain germinates it takes three to four days for fertilization to occur. Since only one germ tube is produced per pollen grain, resulting in one seed, many grains are necessary to produce large berries. To maximize yield, recommendations in Michigan include growing varieties in solid blocks with one other cross-pollinating variety and the use of one to three strong colonies of honeybees per acre of crop (120). Highbush blueberry cultivars are susceptible to a number of pests, including fungal and viral pathogens, insects and nematodes. Breeding programs are active in producing resistant cultivars.

Ninety percent of the grapes (**Vitis labrusca L.**) grown in Michigan are of the cultivar Concord. It is grown because of its tolerance of humid summers, cold winters and retention of flavor during the pasturization process for juice and jam. Vineyards are established in sandy to sandy-loam soils with vines generally spaced 8 feet between plants within a row and 8-10 feet between rows. Most of the root system is of a spreading type in the upper 2-5 feet of soil, but may penetrate as deep as 6-12 feet. Grapes have a large absorption surface containing numerous root hairs. Although there is controversy as to the type of fertilization (64) process, vines are generally self-pollinating, with fruit set occuring by the end of June and harvest in early to mid-October in Michigan.

Ripeness and the cash value of the crop are determined by the soluble solid or sugar content. During the pasteurization process for juice, grapes are crushed in a hopper containing an enzyme (Klearzyme), to break down the pulp (W. Grevelding, personal communication). The grapes are

heated to a temperature of 140 F (60 C) for 2 hours. Remaining skin and seeds (pomace) are often returned to the vineyards and used as mulch. Although seeds germinate poorly, Flemion (64) and Sechet (189) obtained a high percentage of germination when seeds were stratified outside, under moist conditions at temperatures between 0-10 C. for 3 months.

Both grapevines and blueberry bushes may be started in the field as woody cuttings. Generally 2-year-old plants are used. Clean cultivation is recommended for increased plant growth in both crops. Harvesting of most of the Concord grapes and blueberries is facilitated by mechanical harvesters, but problems may arise due to harvester "shaker" damage, predisposing plants to infection as well as transporting infested soil.

Highbush blueberries and Concord grapevines are susceptible to a number of common fungal, bacterial, viral, and nematode pathogens. Powdery mildew of blueberry (**Microsphaera vaccinii** (Schw) Cooke, and of grape (**Uncinula necator**), cankers, leaf and cane spot diseases (**Phomopsis** spp.), crown gall (**Agrobacterium tumefaciens**) and Botrytis rot (**Botrytis cinerea** Pers. ex Fr.) are pathogens common to both crops. The diseases incited by viruses, peach rosette mosaic virus (PRMV), tomato ringspot virus (TmRSV) and tobacco ringspot virus (TRSV) have been described from both crops and are all vectored by the dagger nematode **Xiphinema americanum** (Cobb). Blueberry leaf mottle virus has been described on blueberry, but has not been proven to be vectored by **X. americanum.** The epidemiology and vector relations of two virus diseases of blueberry, incited by blueberry leaf mottle virus (BBLMV) and blueberry shoestring virus (BBSSV), and one virus disease of Concord grape, incited by peach rosette mosaic virus (PRMV) will be addressed in this dissertation.

In Michigan, fruit orchards are often grown and replanted on the same land for decades, in a continuum of fruit culture. This may have a deleterious effect as a result of the holdover inoculum from infected plant parts or vectors, as well as the potential adaptation to new host species.

B. VIRUS DISEASES

Blueberry Shoestring Virus (BBSSV)

The blueberry shoestring virus (BBSSV) disease symptoms were first described by Varney (221) in New Jersey on cultivated highbush blueberries and by Lockhart and Hall (137) in Nova Scotia on lowbush blueberry (**V. angustifolium** Ait.). Spread to other major blueberry producing areas includes Michigan (177), Washington State (P. Bristow and D. Ramsdell, personal communication), North Carolina (R.Milholland, personal communication) and Nova Scotia (137). The disease has probably been spread via vegetative propagation of infected nursery stock.

Hartmann et al (104) found virus-like particles (VLP) 26-28 nm in diameter and small crystalline arrays primarily to be localized in the epidermal, palisade and the spongy mesophyll cells of highbush blueberry leaves. Using the electron microscope, VLP were found in the xylem of roots but not in other vascular tissues. High starch accumulation was noted in the chloroplasts along with increased vacuolar and tannin production in the leaf palisade layer. Lesney and Ramsdell (131) and Ramsdell (176,177) were able to purify isometric particles from infected blossoms of cv. Jersey and to infect healthy blueberry seedlings, thus proving the disease was incited by the virus. The BBSSV particle measures

27 nm in diameter and is a single component type containing one species of single stranded RNA. Tentative classification of BBSSV in the southern bean mosaic virus group (sobemoviruses) is based on the similarity of physical and chemical properties (177). However, it differs from other group members in that it is serologically unrelated and transmitted by an aphid instead of by beetles. The host range of the virus is limited to **Vaccinium** species and can be transmitted via grafts or by rub inoculation of purified virus. Ramsdell (177) demonstrated the transmission of BBSSV by the blueberry aphid, **Illinoia pepperi** (MacGillivray).

Symptoms of the disease are expressed on many parts of the plant in the field. Reddish streaks are more noticable on the current and 1-year-old stems and suckers in the spring, but dissipate as the wood matures. Infected leaves may be "strap-like", or cresent shaped and develop red-veinbanding or reddish-streaks along the mid-rib. The blossoms and petals may also exhibit these red colorations. Blueberry appears to be the only natural host and a four year latent period is often required before symptom expression occurs. Commercial varieties display a range of susceptibility to BBSSV and tolerance to the virus has been observed in a few cultivars (188).

Giles (82) reported that both **I. pepperi** and **Myzus scammelli** colonized highbush blueberry in commercial plantings. Although they were shown to feed on several plants in various families, they had a preference for plants in the **Compositae** and **Ericaceae** families. The biology of **I. pepperi** has been examined (81,139) and is polymorphic during the growing season. Early colonies have been found on the lowest one-third of the

bush, but become more evenly dispersed as the season progresses (61). Hancock (J. Hancock, personal communication) has demonstrated differences in host preferences of **I. pepperi** on highbush cultivars. Elsner (61) observed aphids feeding on the undersides of young succulent leaves, shoots and bud swellings, with stylets placed close to the vascular tissue. He noted that aphid feeding did not appear to cause host symptoms. Transmission of BBSSV occurred with a 24 hour acquisition access period (AAP) followed by a 1-hour inoculation access period in the laboratory (153). Petersen (165) detected ^{125}I-labeled BBSSV throughout the alimentary canal and ferretin-labeled BBSSV in the salivary glands after being feed on sachets containing purified virus particles. Both alate and apterous aphid populations were highest in the early part of the season (61,153), while only apterous forms were found throughout the season. Morimoto (153) demonstrated transmission of BBSSV to 2-yr-old cv. Jersey healthy trap-plants in the field throughout the season, but transmission occurred most frequently between May and June. **Illinoia pepperi** was shown to overwinter on blueberry bushes caged in the field and an alternate host has not been found (61, 153).

Spread in the field appears to be down the row and movement of viruliferous aphids is facilitated by an overlapping bush canopy (132). Lesney (132) showed an infection rate of 0.269/unit/yr. Based on laboratory transmission experiments, and virus uptake studies using serological and electron microscope techniques, the virus appears to be semi-persistent in its vector. Disease control measures currently emphasize reduction of the vector population and removal of infected source plants. However, due to a four year latent period, detection of symptomless but infected bushes is difficult and considerable disease spread will have occurred before infected bushes are removed.

BBLMV

Blueberry leaf mottle virus incites a disease of highbush blueberry in Michigan (179). It is closely related serologically to a disease of grape, incited by grapevine bulgarian latent virus (GBLV-NY) described by Uyemoto (217) in New York. Blueberry leaf mottle virus is also distantly related serologically to GBLV-Eu, found in grape in Europe (143). The virus has a limited host range but can be sap-transmitted to herbaceous hosts. Blueberry cvs. Rubel and Jersey are particularly susceptible. Infection of healthy cv. Rubel blueberry and cv. Niagara grape seedlings was obtained with the Michigan strain of BBLMV, but only grape was infected with the New York strain (178,181).

Symptoms on highbush blueberry are visible as a mottling and deformation of the leaves. The leaves appear puckered with narrowed leaf blades and are particularly noticable on root sprouts during the growing season. A dieback of the main stems in the canopy is often observed along with a general reduction in plant vigor. Often, infected bushes are cut back by growers but symptoms are noticeable on emergent infected root shoots.

Purified virus particles separate into three components in sucrose density gradients. The middle and bottom components measure 28 nm in diameter and the top component appears as empty shells when stained with uranyl acetate. The protein coat subunits have a molecular weight of about 57,000 daltons. Sedimentation coefficients of the T, M, and B components are 53, 120 and 128 S, respectively. These physical and chemical properties are characteristic of the nepovirus group (102). Two species

of single stranded RNA were observed and both are required for optimum infection.

Blueberry leaf mottle virus is placed as a member of the nepovirus group (179,181). It is distantly related serologically only to GBLV (143,179,181,217), and it has similar physical and chemical properties. The pattern of spread in the field is uncharacteristic of a nepovirus and attempts at transmission by nematodes have been unsuccessful (T. Vrain and D. Ramsdell; J. McGuire and D. Ramsdell, unpublished data). Alternate sources of spread are characteristic for members in the nepovirus group (100) and there may be selection for one of these other methods in the field.

PRMV

In 1958, Fanleaf virus was the only soil-borne virus disease known on grape (**Vitis sp.**) in the United States (109). To date some 53 viruses have been reported infecting grape world-wide (110). The following viruses infect grapevines in the United States: grapevine fanleaf virus (GFV), arabis mosaic virus (AMV), tomato ringspot virus (TmRSV), tobacco ringspot virus (TRSV) and peach rosette mosaic virus (PRMV). Peach rosette mosaic virus was first reported inciting a disease of peaches in Michigan (28,29). Symptoms observed on infected grapevines consisted of delayed bud break, shortened internodes, crooked canes and a condition called "berry cluster shelling". Abnormal development of the main sinus of affected leaves may result in a downward cupped-like appearance. Symptoms are expressed in the field usually after a latent period of several years.

Dias and Cation (54) were able to transmit the disease by bud grafts to healthy peach, plum and cherry. Steam or chlordane treatment of the soil prevented transmission (70), therefore the disease was thought to be soil-transmitted. Klos et al (126) were able to transmit PRMV by planting healthy peach seedlings in soil infested with **Xiphinema americanum** (29 % transmission) and possibly by **Cricinemoides** spp. Experimental transmission was achieved by grafting infected peach buds to grape (126), but the disease known as "grape decline" was only later reported in the field in Ontario (52) and Michigan (174). Isolates obtained from infected peach and grapevines in Michigan were shown to contain the same viral agent, inciting the peach rosette and grape decline diseases (54). Peach rosette mosaic virus has only been reported in grape in Michigan, but has recently been found in peach orchards in Ontario, Canada (4).

The virus particles of PRMV were distinguished by centrifugation in sucrose density gradients; the top component(T) consisted of empty shells, while the middle (M) and bottom (B) components contained single stranded RNA of two different molecular weights. The molecular weight of the middle component (RNA-2) is between 1.3 to 2.4 x 10^6, while the molecular weight of the bottom component (RNA-1) is approximately 2.6 x 10^6. The virions measure 28 nm in diameter, the protein coat has a molecular weight of 57,000 and both RNA species are infectious (54). Although PRMV is not serologically related to other members of the nepovirus group, it is similar in physical-chemical properties, seed and nematode transmission. PRMV can be sap inoculated to a limited number of herbaceous hosts (53,54) and has been isolated from three weed species growing in infected vineyards (175). It is seed borne in **Chenopodium quinoa** (54), dandelion

(**Taraxacum officinale** Weber) and concord grape (175), but it is not pollen borne in grape (175).

The spread of PRMV in the field is slow and in an elliptical pattern (175), similar to other nepoviruses (216,217). **Xiphinema americanum** has been found between 5 to 7 feet deep in the soil below PRMV-infected vines in Michigan vineyards (175), making conventional fumigation techniques ineffective. Superimposed shallow and deep fumigation methods have eliminated the population of **X. americanum** over a six year period, resulting in no virus reinfection of replanted vines (180).

C. PLANT VIRUS TRANSMISSION-VECTORS

Transmission and spread of virus diseases may occur resulting from the activities of nematodes, insects, arachnids and man or passively through seed and pollen of commercial or naturally occuring wild hosts, with various combinations of multiple modes of dispersal. Dispersal of viruses through seed and pollen facilitates the establishment of new infection foci which serve as secondary reservoirs during low vector populations or adverse environmental conditions. Insect vectors provide protection for the virus while outside the host, facilitate long and short distance dispersal and may actually predispose or create entry sites in the host from feeding wounds.

1. APHID BIOLOGY

Aphids may become economic pests of crop plants not only from injury incurred by feeding, but also as the result of vectoring plant viruses. Twenty-six percent (226) of the 640 known plant virus diseases are aphid

transmitted and over half of these species belong to the subfamily **Aphidinae.** Aphids are often efficient virus vectors due to their high reproductive rate, dimorphism and readiness to colonize different plants. Populations are greatly influenced by environmental and cultural conditions.

Aphid transmitted viruses are included in thirteen of the seventeen plant virus groups, most of which have single stranded RNA genomes. These viruses are further characterized by the mode of transmission, based on how long the virus is retained between acquisition and transmission and the location of the virus within its vector (95). Several theories have been proposed as to the effects of preacquisition fasting, probing behavior, and host site selection on virus transmission efficiency (95,232) of aphid vectors.

2. NEMATODE BIOLOGY

Two of the sixteen groups of plant viruses are transmitted primarily by nematodes and secondarily by seed and or pollen (98). Nepoviruses (nematode transmitted polyhedral viruses) are transmitted by species of the genera **Longidorus** (62,150) and **Xiphinema** (33). Members of the tobravirus group are rod-shaped and transmitted primarily by species of the genera **Trichodorus** and in a few cases by **Paratrichodorus** (113). Of the four genera, **Longidorus, Xiphinema** and **Trichodorus** are found in commercial fields in Michigan, particularly in blueberry fields and grape vineyards.

All plant parasitic nematodes described so far are classified in the order **Dorylaimida,** class **Adenophora.** They are characterized by a two-part esophagus without the median valve and with an odontostylet connected to a

stylet extension called the odontophore, which may contain basal swellings. Members of the family **Longidoridae, Longidorus** species (needle nematodes) and **Xiphinema** species (dagger nematodes) are characterized further by their long, slender bodies (2 to 12 mm) and stylet. They differ in that **Longidorus** species usually have a long odontophore with the guide ring located near the anterior part of the odontostyle. The flanged odontostyle of **Xiphinema** species is forked at the odontophore junction with the guide sheath containing two guide rings, the posterior of which is most obvious (113). **Trichodorid** species (stubby root Nematode) have short (2 mm), thick bodies and a short stylet called an onchiostyle that curves ventrally.

Most species of **Longidorus** and **Xiphinema** are root ectoparasites, feeding on the outside of plant roots. **Longidorus** spp. have a preference for feeding at the root tips of many herbaceous plants, producing galls. **Xiphinema** spp. may feed at other sites but usually produce small galls at the root tips of woody plants (34,38,63). Due to the stylet composition, species of these two genera can penetrate into the stele and vascular tissue of small feeder roots (168), releasing virus. Cohn (34), attributed the long feeding time at one site to this deep penetration; feeding may vary from 15 minutes to several days depending on the host. Feeding begins with short test probes, followed by full extension of the stylet into host cells and the initiation of a parastaltic-like action of the esophageal bulb during ingestion (38,196). The capability of deep cellular penetration enables species of **Longidorus** and **Xiphinema** to acquire virus particles and inoculate them into any root tissue i.e. epidermal, root hairs or cortical cells (230).

3. TRANSMISSION OF NEPOVIRUSES

Transmission of plant viruses can be effected by both the adult and juvenile stages of all vector nematodes (97,118,198,205). The virus does not circulate within the nematode vector (230), nor is it passed through a molt (96,199) or transovarially (96,199). The first report of virus transmission was the transmission of the soil-borne fanleaf virus of grapevines by **X. index** (109). While experimental transmission of certain viruses by non-vector nematodes has been achieved, the relationship between the virus particle and its true vector is usually highly specific. Taylor and Robertson (200) demonstrated that this specificity is primarily controlled by the association and adsorption of the protein subunits of the virus particle to the cuticular lining of the nematode esophagus. Nepoviruses have two distinct single-stranded RNA species contained in different particles. While the molecular weight of the RNA-1 (2.6×10^6) is similar for all members, that of the RNA-2 varies from 1.3 to 2.4 x 10^6) (102). The RNA-1 of raspberry ringspot virus (RRV) determines gene replication, while RNA-2 determines the coat protein and nematode specificity (99,101). As the vector feeds, virus particles are adsorbed to sites of retention as plant sap passes to the intestines, during ingestion (200). Taylor et al (199) found arabis mosaic virus particles that are not vectored by **L. elongatus** to be passed to the intestines, but not adsorbed. Specificity may be controlled by mechanisms governing disassociation of the virus particles. Both the English and Scottish strains of RRV were adsorbed by **L. macrosoma**, but only the English strain disassociated (203). The mechanism of disassociation may be the result of a pH change, enzymatic activity or the ionic strength of the nematode saliva (203). Efficient transmission requires adsorption of the virus

from an infected source plant, retention, followed by disassociation into a susceptible host. Several factors affect this process, i.e. environment (5,218), host phenology (20), extraction methods (205) and virus-vector association (199).

Xiphinema americanum transmitted tomato ringspot virus (TmRSV) to a few cucumber plants after 1 hour, but 100 % became infected after a four hour acquisition access feed (204). TRSV was transmitted by **X. americanum** after 49 weeks of starvation (12), but longer periods resulted in reduced transmission.

Many species of **Longidorus** and **Xiphinema** have a long life cycle, wide host range, multiply rapidly on woody perennials and can survive on a number of plants without multiplying. In vineyards, **Longidorus** and **Xiphinema** species are found 25-50 cm deep in the soil, depending on soil moisture,temperature and texture (90,103,173). **Xiphinema americanum** adults and larvae do not survive in frozen soil. Primarily the eggs and a few larvae overwinter (89,90). They are more sensitive to soil moisture than temperature but live in a wide range of soil types. They are not easily cultured under laboratory or greenhouse conditions (1,169).

4. TRANSMISSION THROUGH SEED AND POLLEN

Transmission of virus through the seed of either cultivated or wild plants occurs in a number of different plant virus groups, but especially the nepovirus group (6,11,71). However, transmission through virus infected seed and pollen occurs less often and is considered to be of only secondary importance with respect to other methods of transmission (10). The frequency of these types of transmission depends on the host-virus relationship and is affected by temperature, host phenology and stage of

seed maturation at time of infection (43,44). Hanada et al (93) demonstrated the variable transmission of four nepoviruses through chickweed (**Stellaria media**) at different temperatures. Usually virus infected weeds do not exhibit symptoms in the field (135).

In many cases, virus transmission through seed or pollen depends on whether infection occurs and whether the virus becomes systemic prior to or after anthesis (39,45). Generally, true seed-borne viruses are found in both maternal and embryonic tissue, while non-seed-borne viruses usually occur in the seed coat only (11,44). Grapevine fanleaf virus infects the seed coat and endosperm, but not the embryo (44,109) and is not transmited through grape seed, its only natural host (71,191). However, PRMV is transmitted through grape seed (175). For seed transmission to occur, the embryo or pollen must be invaded early in its development by virus from either maternal tissue or from virus-infected pollen. Cheo detected a high percentage of southern bean mosaic virions in the embryos of immature, but only in the seed coat of mature bean seeds (30).

The factors governing transmission of virus through seed and its frequency are unclear and many methods have been proposed. Duggar (59) proposed that the inactivation of tobacco mosaic virus (TMV) in tobacco seeds was due to an inactivating substance in the seed. While Crowley (43) detected potential inhibitors in seed parts, none were demonstrated to inactivate the virus. Caldwell (22) proposed disruption of the protoplasmic bridges between the developing embryo and maternal tissue. Since embryos are active metabolically and considered meristematic in nature, virus infection may be inhibited due to an inability to compete with the host for available biochemical components (23,152). Lister and

Murant (135) suggested that the high efficiency of seed transmission of many nepoviruses was due to their ability to overcome the plant's defenses and infect the meristematic tissue. This is supported by the ability of infected plants to recover after the initial infection and symptom expression. Non-embryonic transmission of virus through seed was demonstrated by Broadbent (17) to occur from wounds created during seedling emergence from the seed coat.

The percentage of virus transmission to the seed was observed by Medina and Grogan to depend on whether virus is transmitted via the male or female gametophyte and is highest when both are infected (145). However, transmission is usually lowest when pollen is the virus source (11). Infection of healthy ovules and mother plants from virus infected pollen, has been demonstrated for a number of nepoviruses as well as apple chlorotic leafspot virus, black raspberry latent virus, necrotic ringspot virus and prune dwarf virus. Das and Milbrath (48) reported 10 % transmission of prunus necrotic ringspot virus through the flower via infected pollen. Infection of healthy stonefruits from movement of virus-containing pollen by honeybees has been demonstrated for prunus necrotic ringspot virus (77) and prune dwarf virus (85) in Montmorency cherry. Infection was not detected until three years after the initial inoculation. Whether virus is found on the inside of pollen grains (26,231) or outside (91) may determine the mode and frequency of infection via pollen. Cole et al demonstrated surface contamination of prunus necrotic ringspot virus on pollen from infected almond and cherry orchards (35).

D. PLANT VIRUS EPIDEMIOLOGY

Plant virus epidemiology or ecology can generally be defined as the study of those complex interactions of host-vector-virus and environment that influence disease establishment and spread. Since plant viruses cannot exist outside their host or vector, a unique relationship has evolved to maximize spread not only within the field, but during dispersal to new hosts or during adverse conditions. The onset and establishment of an epidemic requires that these interactions be in synchrony, the monitoring and understanding of which requires a multidisiplinary approach. This involves observing the behavior of vectors, how the disease is spread in the host and then being able to predict the factors favorable for infection.

1. Vector Behavior and Patterns of Spread

The establishment of virus infection and spread within or outside of a field depends on many factors as previously described, but also on the strategies of "invasion" utilized by the virus and vector (27). Different strategies must be adopted by both the virus and vector to be successful in a continuous cropping system or perennial crop than in a short-seasoned annual crop. Crosse (42) has termed this success "epidemiological competence". Harper's (94) approach to studying these strategies of disease establishment in the field, made direct comparisons to MacArthur's and Wilson's (138) theory on "discrete island habitats", where this efficiency or rate of infection "r" depends on the carrying capacity "K" of the host and the available sources of inoculum. Thresh (210,212) has discussed at length the advantages of dual modes of dispersal adopted by

both the vector and virus. Much of the existing literature discusses the dispersal mechanism and patterns of arthropod vectors and particularly aphids (18,119,183,210). Thresh (213) described active vectors, especially those that are polyphageous, polymorphic and migratory, such as aphids, as "r" strategists. Gradients of virus spread by these "r" strategists are initially shallow and infected plants may appear randomly in the field (211). As areas of infection "foci" become established, further spread is facilitated by less active forms developing on or near the host. Methods of insect movement and conditions for dispersal have been investigated (46,66). The pattern or rate of spread is also influenced by the mode of transmission (persistent or nonpersistent), the source of inoculum (wild or cultivated hosts) and the population characteristics of the vector (16,57,58). Cucumber mosaic virus (CMV) is nonpersistent in its aphid vector, and short frequent probes on plants may result in a fast rate of virus spread in the field (232). This type of disease spread mechanism requires that virus infected plants be available locally. The pattern of spread may appear as several infected plants in an area of "infection foci", with fewer infected plants or a steep gradient of disease extending outside the field. Some other plant virus-vector relationships have a bimodal method of transmission. Cauliflower mosaic virus (CaMV) is acquired and transmitted both in a nonpersistent and persistent manner depending on the feeding behavior of its vector, the cabbage aphid, **Brevicoryne brassicae** L. (134). This type of transmission facilitates both rapid local spread and a potential for long distant spread outside the field.

Disease spread by nematodes is slow, with steep gradients of infection that persist for many years (211). They are described as having

a "K" strategy of survival. Due to the slow movement of the nematode through the soil, virus diseases are spread at a slow rate of 2-3 feet per year (96,109). Generally, nematodes are considered poor vectors since the virus persists in the nematode for only 8 weeks to 11 months allowing for transmission from one season to the next but not during long fallow periods. Long distance spread may be by infected propagative material with or without the vector (142). The most classical case is the transmission and establishment of grapevine fanleaf virus and its vector **X. index**, introduced from Europe to North America (173). Short range spread may be obtained by planting infected material in fields with an established vector, through seed or established weed species. The importance of wild and cultivated plants in the spread of virus was demonstrated by Murant (155), where weed hosts of the virus and vector serve as reservoirs for survival and as a source of inoculum. Unlike tobraviruses (eg. tobacco rattle virus, spread by **Trichodorus** spp.), nepoviruses can have a high level of virus-seed transmission, up to 100% (135). Virus diseases whose movement is restricted by low vector mobility or do not have a wide host range, may develop additional means of dispersal (103). Spread by seeds or pollen may both facilitate long distance virus transmission, and the pattern of spread may be representative of several types of transmission strategies. For example, the susceptible time period of a crop for pollen transmission may be a few hours to a few days (191). The pollen-borne virus, prunus necrotic ringspot cannot be spread until flowering occurs (77,151). Taylor et al (197) found that the roots and cotyledons of seedlings from infected seed contain virus, but the seedlings were not infected unless wounded. He suggested that transplanting of seedlings was responsible for wounding.

Two out of 2500 seedlings grown became infected with TMV. He concluded that embryonic transmission occured rarely, but seed-borne transmission may be important as a source of inoculum for initial infection. Pollen spread may be unimportant when the vector is present, but becomes important in establishing disease where vector populations are low or not present.

2. Quantitative Methods Of Measuring The Spread Of Plant Viruses

Unlike fungal and bacterial diseases where the rate of spread can be followed by measuring the amount of inoculum or infected tissue present, monitoring the spread of virus diseases requires determining the number of infected plants per area. Thresh has discussed the restrictions of assessing the spread of virus diseases in perennial crops (211). Until recently, little quantitative research has been done on the incidence of viruses within commercial crops, weeds and wild species. Infectivity assays on indicator hosts require much time and space, making large virus screening programs impractical. Other problems often encountered are the symptomless state of infected plants or confusion with other pathogens, pests and cultural practices. The development of sensitive serological techniques such as the enzyme-linked immunosorbent assay (ELISA) (32,223), radioimmunoassay (RIA) (79,86), and immunosorbent electron microscopy (ISEM) (83) have facilitated advances in plant virus epidemiology. These techniques have been used to study virus-vector relationships and their influence on spread. Virus-nematode associations, such as site of virus retention, mechanisms of movement and transmission in the host have been widely studied (200,201,202).

Diagnosis of virus infection in seed lots for certification programs has been possible using serological assays (14,136). Bowers and Goodman

(13) detected soybean mosaic virus (SMV) in the testa, cotyledons and embryos of soybean varieties using ELISA. Ghabrial (79) found RIA more sensitive than ELISA in detecting low levels of lettuce mosaic virus (LMV). Cole et al used serological blocking techniques to detect PDV and PNRSV in cherry pollen (35).

Various mathematical approaches have been presented to determine trends and predict patterns of virus spread in the field. Van der Plank (219) has proposed the use of the doublet test to investigate patterns of spread and monitor disease progress of fungal diseases. Gregory and Read (88) defined disease gradients by dispersal units from a point source using trap plants as detectors. Watson et al (226) used environmental data in conjunction with vector behavior in predicting the severity of sugarbeet yellowing virus in sugarbeet crops in England. Gibbs (80) and Madden et al (140), used computer modeling and simulations to define disease progress. Frazer (66) used computer models to study the effects of aphid populations on the spread of alfalfa mosaic virus.

The advent of recent advancements in vector behavior studies, serological techniques for virus detection and host-vector relationships has, however slowly, encouraged more research in plant virus epidemiology. The indexing of infected plants in the field along with the determination of rates and patterns of spread, is still costly and time consuming. The applications of remote sensing techniques affords one more tool to better understand virus related epidemics and may provide a rapid assessment of disease incidence.

E. REMOTE SENSING

Remote sensing can be defined as the detection of a subject's spectral properties from a distance or location by reflected or emitted electromagnetic radiation. It is usually measured in narrow bands of wavelengths between 300 and 1500 nanometers (nm) or the visible through near infrared (N-IR) spectrum. Applications for remote sensing techniques have been realized not only for military interests, surveilance and reconnaissance during World War II, but also for civilian interests. Varying success has been achieved in the areas of yield and biomass estimation (161,186), forestry (133,159), pollution (69,108), water and land utilization (167,227,228), insect and disease damage (31,36,124,125,184). Much of the previous success of remote sensing techniques has been with fungal diseases of field crops incited by leaf rusts and blights (36,115,124,125,184). Colwell made extensive comparisons of leaf reflectance using different photographic parameters of film types, scale, filters, angle and solar information (time of day, season) in the detection and differentiation of cereal diseases of oats, wheat, barley and rye (36). Detection of diseases of annual crops with a viral etiology have only recently been studied (7,8,112,185,214,215) and even fewer with perennial crops. While success has been achieved in detection of diseases exhibiting obvious symptoms of leaf spots, wilts, and insect damage, there has been little success with previsual detection of plant diseases (19,107,141,147). Manzer et al (141) were able to detect late blight of potato (**Phytophthora infestans** deBary) by aerial photography 1-3 days before obvious symptom expression. Burns and coworkers (19) could detect tobacco ringspot virus in snapdragon (**Antirrhinum majus** L.) a day before symptoms were visible. This

variability in success is brought about by many factors e.g. inherent reflectance variability of plant tissue, different plant growth stages, field conditions, and variable responses to disease and equipment sensitivity. However, to initiate an effective disease control program, detection must be made well before symptoms are detected visually. At best, detection once disease is established could be utilized to rapidly survey the areas of infection and observe conditions favorable for spread.

To obtain an understanding of the spectral reflectance changes brought about by disease, one must be aware of the factors contributing to light incident on the plant as well as the inherent optical properties of a plant. Radiant energy incident on a plant or leaf is a compilation not only of direct solar radiation but that reflected from the plant's surroundings, scattered skylight, atmosphere, clouds, soil and other leaves or plants. Several mathematical equations have been developed to describe the effects of these types of radiation on the plants energy budget and reflectance (37,72,229). Depending on the wavelength of energy striking the leaf canopy, a portion of this energy will be emitted, absorbed, reflected and/or transmitted. Substances contained in the atmosphere such as carbon dioxide, water, and ozone will influence the absorption, diffusion and emittance of solar radiation. This is illustrated by the particular troughs and peaks on a solar scan and particularly in the 400 to 1400 nm range. Most major and minor carbon dioxide and water absorption bands are beyond the wavelength region of current interest for plant stress surveys, except for two minor water absorption bands, one at 940 nm and the other at 1100 nm (128). Below 100 meters, target to radiometer distance, the effects of the atmosphere are not usually considered influential (130).

Leaves contribute most of the reflectance from vegetation. Sinclair and coworkers (192) modified the theories proposed by Willstatter and Stoll in 1918, that solar radiation through the leaf is reflected primarily by the spongy mesophyll cell wall-air interfaces but also other cellular organelles. Allen (3) compared various methods of detecting changes in cellular leaf reflectance and transmission due to the size and properties of air spaces in the leaf mesophyll layer of corn and cotton. While the primary effector of reflectance is the air spaces in the leaf mesophyll, Gausman (74) demonstrated that various leaf components e.g. crystals, chloroplasts, cell walls and even nuclei may contribute to near infrared reflectance (750-1350 nm). Extensive research has been conducted as to the physiological and morphological factors that affect solar reflectance including, leaf water content (206), phylotaxis (2,160), nutrients (106,207,208). pigments (172) and leaf maturation (25,127). Gates (73) and Moss (154) demonstrated that leaves from different plant species will have similar spectral characteristics, but are affected by the levels of pubescence, wax and leaf thickness.

Several models have been developed and tested in the field to detect reflectance changes of leaves and plant canopies brought about by morphological changes or plant stress (185,194,195). Safir et al (184) used a field portable spectrophotometer at various angles to distinguish between healthy and blighted (**Helminthosporium maydis** Tatum) corn plants. Using models of normal plants, they were able to detect moderately severe infection and to distinguish between severe blight and moisture stress. They found increased reflectance of diseased over healthy plants in the 500-700 nm visible range and the water absorption regions 1450-1950 nm.

Plant responses to pest damage or stress may be expressed either morphologically, physiologically or by an interaction of the two. A morphological reaction can be brought about by a change in plant shape or outline, defoliation, growth reduction, dieback and/or leaf orientation. Physiological damage results in a change in the normal function of the plant or leaf exhibited by decreased photosynthesis, chloroplast degeneration, and/or interuption of translocation of solutes (water and minerals). Often it is difficult to isolate one response from the other, since cellular collapse brought on by disease would result in both physiological and morphological alterations. To understand some of the physiological alterations on leaf reflectance, researchers have infiltrated various substances into the leaf mesophyll. Gausman et al (74) demonstrated the role of internal plant cell structures and substances on leaf reflectance, absorptance and transmittance. Leaves infiltrated with water and various concentrations of cotton seed oil and hexane, exhibited a decrease in reflectance compared to normal noninfiltrated leaves between the 500-2500 nm wavelengths. The breakdown of cells from pathogen invasion and release of alcohols and phenolic compounds would affect both morphological and physiological properties of the target host. Cardenas et al (25) induced cellular breakdown by ammonia infiltration into cotton leaves and observed decreased reflectance between 750-900 nm. They compared these spectral changes to those exhibited by a symptomlessly infected or stressed plant.

The effect of disease on spectral reflectance has been studied in comparison to the normal reflectance of a green leaf (111). Only about 8-10 percent reflectance occurs in the blue (400-500 nm) and red (600-700 nm) regions of the spectrum. These are the wavelengths where chlorophylls

"a" and "b" and to a lesser extent the carotinoid pigments absorb light energy to be converted into chemical energy during photosynthesis (187). Ten to 20 % of the green region of the electromagnetic spectrum (500-600 nm) is reflected, but most (30-70%) of leaf reflectance occurs in the infrared region (700-1100 nm). The transmittance spectrum of a leaf is similar in shape to reflected light over various wavelengths.

Murtha (157) has described the changes in spectral reflectance that occurs as a leaf progresses from the healthy state through the onset of disease. Physiological damage may occur initially without symptom expression, followed by visible symptoms on the plant (e.g. chlorosis), and finally complete loss of function or necrosis. As infection occurs and the pathogen begins to interfere with normal plant functions, but before symptoms occur, a decrease is observed in the near infrared region. This is often observed as a rounding of the plateau, brought about by a decrease in reflectance around 700 nm and 1000 nm (157). Some diseases cause chlorophyll production to decrease, usually due to chloroplast degeneration and chlorosis. A decrease in reflectance in the green band results, or a shift to increased reflectance in the red is observed. This may coincide with a further decrease in N-IR reflectance. Increased reflectance may also be noted in the blue and red regions, due to unmasking of carotenoid pigments. With the onset of necrosis and leaf death, the spectral curve flattens out from further reductions in green and N-IR reflectance and an increase in the red region. Further changes in N-IR reflectance may be due to environmental factors such as dry, wet, or dead foliage. Water is a poor absorber of visible light and a poor reflector of N-IR. Morphological alterations may result in the exposure of more leaf surface in the plant canopy or as a change in the number of

shadows produced. Reflectance may also be altered by a change in the plant's basic form, texture or boundary pattern.

1. Methods Of Spectral Data Collection

Several methods have been employed for the collection of spectral data: satellites, aerial or high-altitude [above ground level, (AGL)] photography with cameras mounted on airplanes, helicopters or balloons; low-altitude photographic imagery or hand-held portable spectroradiometers mounted in towers, located 6-15 feet above the target canopy. Correlations of observable differences on film or density changes are often made using some form of ground truth. Ground truth gives the observer some idea of the extent of deviation from the normal and may be measured by leaf area index, yield estimation, disease rating schemes, inoculum estimation or serological indexing of plant diseases. Maps indicating the status of plants in the field are usually constructed. Correlation analysis of observable ground data and reflective differences on the film are used to make disease predictions.

2. Detection On Film

Interpretation of disease severity requires that spectral changes be discernable on the finished photograph. These differences exhibited on film are a result of the particular absorption capacity of the film dye layer for a specific wavelength of energy. Regular or positive color film has three dye layers; yellow, magenta and red, sensitive to different wavelengths of energy. While the yellow dye layer, made up of red and green color components is sensitive to reflected blue light (400 to 500

nm), the magenta dye layer (red plus blue) is sensitive to green light and cyan (blue plus green) is sensitive to red light. As described by Murtha (157) and Murtha and Hamilton (158), a normal plant leaf reflecting mostly in the green wavelengths would affect more of the green sensitive or magenta forming dye layer but very little of the yellow or cyan dye layers. After processing, white light passed through the transparency, will result in most of the green light being absorbed by the thick magenta dye layer while more red and blue light is transmitted, so the green foliage appears green (red plus blue) on the photograph. Regular color film is insensitive to infrared radiation and only reflective modifications occuring in the visible light region can be detected.

Color infrared (CIR) film or false color film also has three light sensitive dye layers. Since all of the three dye layers are sensitive to blue, a yellow filter (Wratten No. 12) is used to subtract blue light. The yellow dye layer (red plus green) is sensitive to green light (400-500 nm), while the magenta dye layer (red plus blue) is sensitive to reflected red light (500-600 nm), and the cyan dye layer (blue plus green) is sensitive to near-infrared radiation (700-900 nm). As described previously, a normal leaf reflects little in the red and blue region, more in the green, but mostly the near-IR region of the spectrum. Since development of CIR film is by a reversal process, the amount of dye formed on the film is inversely proportional to the exposure or amount of reflected light. Therefore, after film development, normal green foliage will appear red to reddish-magenta due to more of the yellow and magenta dye layers being formed. The darkness of the red color will depend on the amount of green light reflected by the leaf.

A deviation from the normal reflectance pattern would result in a change in the dye layer formed and ultimately the wavelength of light transmitted through the transparency. For example, a decrease in reflectance in the N-IR wavelengths would result in a darker magenta color, more of the cyan dye layer is formed with less red light passing through the transparency. As the tissue becomes more chlorotic, there is less absorbance in the magenta forming layer, more green transmitted along with a further decrease in the red and blue region. When the leaf turns brown and dies, it would appear as more yellow or white on the film. Murtha and Hamilton (158) found little visual change brought about by damage to conifers, but noted a small density change due to decreased N-IR reflectance. They concluded that a change in the visible spectrum along with decreased N-IR reflectance is necessary for a color change on the film.

While advancements have been made in the detection of environmental and pest damage to crops, there are many difficulties remaining. Some of the most difficult problems encountered in this type of disease detection is the lack of equipment sensitive enough to detect small spectral changes, the establishment of a characteristic spectral signature for a normal healthy crop over time, and the association of a deviation from the normal with a specific disease or condition.

F. OBJECTIVES

This dissertation is divided into four chapters. Chapter one dealt with a review of the literature. Chapter two addresses the mode of transmission of BBLMV in highbush blueberry fields by determination of

possible vectors and alternate mechanisms of virus spread. Several serological assay procedures were employed for the detection of virus infection. In chapter three, the factors influencing the spread of PRMV within and outside of cv. Concord grape vineyards were investigated. In chapter four, various remote sensing techniques were evaluated for the detection of spectral changes brought about in the host by viral infection.

Chapter II. Transmission and Epidemiology of Blueberry Leaf Mottle Virus (BBLMV) in Highbush Blueberry, **Vaccinium corymbosum** L. cv. Jersey

A. INTRODUCTION

Blueberry leaf mottle virus, incites a disease of highbush blueberry (**Vaccinium corymbosum** L.), and has recently become a problem in Southwestern Michigan. The disease was first described by Ramsdell and Stace-Smith (179) on the cultivar Rubel. It was later described in cv. Jersey (181). Systemically infected plants exhibit deformed, mottled leaves and a general dieback of the main stems, associated with reduced vigor. Physical and chemical properties of the virus have been determined (181) and BBLMV is placed tentatively as a member of the nepovirus group. The Michigan strain of the virus is closely related to a New York State strain, GBLV-NY, described by Uyemoto et al (217) in cv. Concord vineyards. Both strains are distantly related serologically to grapevine Bulgarian latent virus (GBLV) described by Martelli et al (143). While the sedimentation coefficients of the three virus particles of BBLMV, 53, 120, 128 S., are characteristic of a nepovirus, transmission with nematodes has not been demonstrated. **Xiphinema americanum** did not transmitt BBLMV to **Chenopodium quinoa** or **Nicotiana clevelandii** seedlings whether nematodes were obtained from Michigan (T. Vrain and D. Ramsdell, unpublished data) or Arkansas (J. McGuire and D. Ramsdell, unpublished data). Uyemoto et al (217) were able to isolate **X. americanum** (4/100 cm3 of soil) from only one soil sample taken from beneath virus-free and or GBLV-N.Y.-infected grapevines. **Chenopodium quinoa** and cucumber (**Cucumis**

sativus) seedlings did not become infected when placed as bait plants in these soil samples.

The virus has a limited natural host range, but is sap transmitted to a number of experimental herbaceous hosts. The Michigan strain of BBLMV was able to infect a low percentage of grape seedlings (**Vitis labrusca**) cv. Niagara (178), but the N.Y. strain does not infect blueberry cultivars (179). Uyemoto et al (217) demonstrated a low percentage of transmission of the N.Y. strain through the seed to seedlings of **V. labrusca** (5 %) and **C. quinoa** (12%). The presence of virus particles in pollen, or transmission to seed through pollen, was not determined for the N.Y. strain.

Blueberry bushes infected with BBLMV occur in a random manner in the field and disease spread is not characteristic of a nematode transmitted virus. The objectives of this research were to determine the factors contributing to the establishment and spread of BBLMV within highbush blueberry fields. In particular, field indexing to determine the characteristics and mode of disease spread, survey of possible insect vectors and transmission studies. Transmission of virus through seed and pollen is characteristic of several members of the nepovirus group is the transmission of virus through seed and pollen. Experiments were conducted to observe the presence of virus in blueberry pollen and seed and to determine its role in the further spread of the disease. Although this mode of dispersal is usually considered of only secondary importance, for some virus diseases this appears to be the primary mode of spread (151). Cole et al (35) demonstrated the role of honeybees in spreading prune dwarf virus (PDV) and prunus necrotic ringspot virus (PNRSV) from infected California orchards to cherry and plum orchards in Washington state, via

hives contaminated with virus-laden pollen. Prunus necrotic ringspot virus was detected as a contaminant on the outside of cherry pollen (151).

MATERIALS AND METHODS

1. Virus Purification and Assay Techniques

Procedures for virus purification and antibody preparation were those described by Ramsdell et al (179). **Chenopodium quinoa** plants were used as the propagation host for BBLMV. One gm of infected leaf tissue was homogenized in 2 ml of 0.05 M boric acid-borax buffer (pH 7.0), containing 0.1 % (w/v) each of sodium thioglycolate (TGA) and sodium diethyldithiocarbamate (DIECA). The virus-containing sap, expressed through cheesecloth was frozen overnight to coagulate plant debris, and then slowly thawed at 4 C. Following a low speed centrifugation (10,000 g, 15 minutes), 10 % each (v/v) of chloroform and n-butanol were added to the supernatant and stirred for 15 minutes. The virus-extract was further clarified by a second low speed centrifugation. To the supernatant, 8 % (w/v) polyethylene glycol (PEG, Mol. Wt. 6000) and 1.0 % (w/v) NaCl was added for 30 minutes with stirring, and then given a low speed centrifugation. The pellet was resuspended in 10 % of the starting volume with 0.05 M tris-HCl buffer and allowed to stand for several hours. After a third low speed centrifugation, the supernatant was saved. Virus particles were pelleted by a high speed centrifugation at 38,000 rpm for 90 minutes in a Beckman No. 40 rotor. The pellet was resuspended overnight in a 2-3 ml 0.05 M tris-HCl buffer, pH 7.4. The virus was further purified by centrifugation through 0-30 % linear-log sucrose density gradients in a Beckman SW-41 rotor at 38,000 rpm for 90 minutes at

4 C. The gradients were fractionated at 254 nm using an ISCO fractionator (Instrumentation Specialties Co., Lincoln,

NE. 68504). Virus concentration was determined using $E^{0.1\ \%}_{260nm} = 10$.

A. Antiserum Production

Anti-BBLMV serum was obtained by injecting a female New Zealand white rabbit with purified virus and collecting the blood serum. The rabbit was given an initial intramuscular injection of 1 ml containing 0.5-1.0 mg purified BBLMV emulsified with an equal volume of Freund's complete adjuvant (Difco Products Co., Detroit, MI. 48232). Two additional intramuscular injections were administered at 7-day intervals with 1 ml containing 0.5-1.0 mg purified virus emulsified (1:1 v/v) with Freund's incomplete adjuvant. The rabbit was bled from the marginal ear vein at weekly intervals, 7 to 10 days following the last injection. Serum was separated from coagulated blood cells by incubation for 2 hours in a 37 C water bath, followed by storage at 4 C overnight and finally the clear serum was pipetted off. The serum was lyophyllized and stored at -20 C.

Ouchterlony gel double diffusion tests were used to titer anti-BBLMV-serum against BBLMV-infected **C. quinoa** and healthy plant sap. Fifteen ml of an 8 % agarose solution (w/v) (Sigma Type I, Sigma Chemical Co., St. Louis, Mo. 63178) containing 0.85 % sodium chloride (w/v) and 0.15 % sodium azide (w/v) was pipetted into plastic petri dishes. A circular 6-well pattern, surrounding the center well, was punched (Grafar Co., Detroit, MI. 48238) into the agar. Each well was 4 mm in diameter and spaced 5 mm from each other. Antiserum serial two-fold dilutions from 1:2 to 1:1024 were prepared in distilled water containing 0.85 % (w/v) NaCl.

B. Preparation of Anti-BBLMV-Gamma Globulin

Ten ml of saturated ammonium sulfate was added drop-wise to a 1:10 dilution (v/v) of serum in distilled water and mechanically stirred for 30-60 minutes. After centrifugation at 6,000 rpm (Beckman No. 40 rotor) for 5 minutes, the precipitate was collected and resuspended in 2 ml (v/v) half-strength PBS (0.01 M sodium-potassium phosphate buffer, pH 7.4 containing 0.8 % sodium chloride (w/v) and 0.01 % sodium azide (w/v). This globulin fraction was dialyzed three times against 500 ml half-strength PBS and passed through a 5 cm column of DEAE-cellulose in a 10 ml glass pipette. Half-strength PBS was used to elute the gamma globulin fraction from the column. Two ml protein fractions were collected by monitoring spectrophotometrically at the A_{280nm} wavelength. The first A_{280nm} absorbing peak was collected and spectrophotometrically adjusted to a 1 mg/ml concentration.

C. Conjugation of BBLMV Gamma Globulin and Assay Techniques

Purified anti-BBLMV-gamma globulin was conjugated with either alkaline phosphatase or ^{125}I, depending on the application and desired sensitivity of the assay procedure.

1. Enzyme-Linked Immunosorbent Assay Technique (ELISA)

This technique involves the chemical bonding of an enzyme to the surface of the anti-BBLMV gamma globulin molecule. Detection of virus particles is facilitated by a colorimetric change following the addition of an enzyme substrate specific for the enzyme. The enzyme, alkaline phosphatase (Type V11-S, Sigma Chemical Co., St. Louis, MO 63178), was bound to gamma globulin by methods developed by Clark and Adams (32) and

Voller et al (223). A one ml aliquot of alkaline phosphatase was centrifuged at 6,000 rpm (Beckman No.40 rotor) for 5 minutes. To this pellet, one ml of purified anti-BBLMV-gamma globulin was added, followed by dialysis three times against PBS, pH 7.4. Conjugation of enzyme to the IgG fraction was facilitated by the addition of a 0.05 % (v/v), final concentration, glutaraldehyde (electron microscope grade, Sigma Chemical Co., St. Louis MO. 63178) for 4 hours at 20 C. Glutaraldehyde was removed by dialysis against PBS, three times, and the conjugated-anti-BBLMV-IgG stored with 1.0 % bovine serum albumin (BSA, w/v) at 4 C.

ELISA Protocol

Detection of virus particles was performed using the ELISA double antibody sandwich method, which involved four basic steps (32,223): 1) Anti-BBLMV IgG was diluted in coating buffer (0.05 M sodium carbonate-bicarbonate buffer pH 9.6) to a concentration of 1 µg/ml . A 200 ul volume was adsorbed to flat bottom wells of a polystyrene microtiter plate (Dynatech Laboratories, Alexandria, VA 22314) and incubated for 3 hr at 37 C. 2) Infected and healthy plant sap was homogenized (Tekmar Co., Cincinnati, OH 45222) in a 1:10 (w/v) ratio of tissue to extraction buffer composed of (0.01 M sodium potassium phosphate buffer, pH 7.4, containing 0.02 % sodium azide (w/v), 0.8 % sodium chloride (w/v), 0.5 % Tween-20 (v/v), 2.0 % polyvinyl pyrrolidone (mw 40,000, w/v) (Sigma Chemical Co., St. Louis, MO 63178). A 200 µl quantity of test sample was added to each well and incubated 12-16 hrs at 4 C., 3) Enzyme-conjugate, diluted 1:800 (v/v), in extraction buffer was added to rinsed plates and incubated 3-6 hrs at 37 C. Plates were washed throughly (3-4 times) with PBS containing

5 % Tween-20 (PBS-Tween) between each step to remove unbound reactants that could cause non-specific reactions., 4) If virus particles are present in the plant sap and enzyme-conjugate bound, the addition of 1 mg/ml enzyme substrate, (p-nitrophenyl phosphate, Sigma Chemical Co., St. Louis, MO 63178) dissolved in substrate buffer [10% diethanolamine, pH 9.8 in distilled water containing 0.02 % sodium azide (w/v)], will result in a color change after 1 hr or less at room temperature. ELISA wells were read spectrophotometrically at A_{405nm} with a microELISA Minireader^{R} (Dynatech Laboratories, Alexandria, VA 22314). Test samples were considered positive for virus, if the A_{405nm} value of a sample well was greater than the mean A_{405nm} value plus three standard deviations of the healthy control samples.

2. Radioimmunoassay (RIA) Technique

Iodination (^{125}I) of purified anti-BBLMV gamma globulin followed the chloramine-T method described by Greenwood et al (86). Radioactive iodine ^{125}I is incorporated into the tyrosine site of the IgG molecule by chloramine-T-mediated oxidation of Na ^{125}I. Fifty microliters of anti-BBLMV globulin was added to 5 µl of chloramine-T plus 1 millicurie (mCi) of Na^{125}I. After incubation on ice for 15 minutes, the reaction was stopped by the addition of 5 µl sodium metabisulfite (5 mg/ml in water). Two carriers, 25 µl of sodium iodide (20 mg/ml in PBS) and 100 µl of a BSA-PBS buffer (0.5 % bovine serum albumin (w/v) in PBS), were bound to residual ^{125}I and ^{125}I-labeled gamma globulin respectively. Labeled gamma globulin was passed through a Sephadex G-50 column (10 cm X 1 cm) to separate biologically bound and unbound radioactive iodine. The column was eluted with PBS. One ml fractions were collected. Small aliquots

(10-20 µl) were measured for radioactivity in a Beckman Biogamma II gamma counter. The ^{125}I-IgG fraction was dialysed three times against PBS.

RIA Assay of Plant and Insect Samples

A modification of the ELISA double antibody sandwich assay method was used, with the substitution of ^{125}I-labeled IgG for the enzyme-labeled IgG at step three. Coating IgG (5 µg/ml) in coating buffer was added to flexible polyvinyl "V" bottom microtitre plates (Dynatech Laboratories, Alexandria, VA 22314) and incubated 3 hr at 37 C. A 100 µl aliquot of prepared plant tissue or insect sample was added and incubated 14-16 hrs at 4 C. To each well, 100 µl of ^{125}I-IgG [55,000 counts per minute (cpm)] diluted in a BSA-PBS buffer containing 0.5-1.0 % bovine serum albumin (w/v) in PBS was added. Plates were rinsed repeatedly three times with a BSA-PBS or BSA-PBS-Tween-20 buffer to remove unbound reagents.

Individual wells were obtained by cutting the wells from the plates and placing one well-cup each into plastic scintillation vials. Each vial was counted for radioactivity in a gamma counter for 1 minute. As previously described, positive samples contained radioactive counts greater than the mean of healthy controls plus three standard deviations.

3. Immunosorbent Electron Microscopy (ISEM) Assay Technique

The immunosorbent electron microscopy (ISEM) or serologically specific electron microscopy (SSEM) assay technique was a modification of the method described by Derrick (51). Copper (200 mesh), electron microscope grids were first coated with parlodion, followed by carbon coating. Prepared grids were placed coated side down onto a 30 µl drop of a 1:500 (v/v) dilution of anti-BBLMV-IgG in Sorensen's ISEM buffer (0.06 M

sodium-potassium phosphate buffer, pH 7.0). Following a 3 hr incubation at 37 C, anti-virus-globulin coated grids were rinsed twice for 10 minutes each, by placing the coated side of the grids onto drops of ISEM buffer, with constant agitation. After excess buffer was removed, grids were placed coated side down onto 30 µl drops of a prepared test sample. Covered grids were incubated 20-24 hrs at 4 C. Once the virions contained in the test samples were bound to anti-virus-globulin-coated grids, the virions were decorated with more anti-virus globulin by placing grids (coated side down) onto a 30 µl drop of anti-virus IgG for 1-3 hrs at 37 C, then the excess was drained off. Prepared grids were negatively stained by adding 1 drop of a 2 % (w/v) ammonium molybdate solution pH 7.0, onto the coated side for 30 seconds. Excess stain was drained off and grids were examined in a Philips 201 transmission electron microscope (TEM).

2. Field Indexing to Determine the Extent of BBLMV Infection in Highbush Elueberry.

From a 10 hectare highbush blueberry field, a block of 450, 25-yr-old plants was indexed in May 1982 and early June 1983 for the presence of BBLMV. Breaks appeared in the field where diseased bushes had been cut to approximately 1-2 feet above ground level by the grower. Succulent leaf tissue or blueberry blossoms were sampled at several locations around the bush. Approximately 1 gm of tissue per plant was tested using the ELISA technique as previously described.

3. Pollen Transmission Studies

A. Detection of Virus in Pollen

1. Assay of Pollen Grains from Infected Blueberry Bushes

Pollen collected during anthesis was obtained from ELISA-indexed healthy and BBLMV-infected cv. Jersey highbush blueberry plants. Pollen was collected onto glass microscope slides by lightly twisting the blossom between the thumb and forefingers. Maternal tissue was separated from pollen using a stereoscopic dissecting microscope. Each sample was weighed (mg quantities) and ground in 100 µl virus extraction buffer, then assayed by ELISA. The dilutions used for the procedure were, 1 µg/ml per well anti-virus gamma globulin in coating buffer, a 1:400 enzyme-conjugate-buffer dilution and 1 mg/ml enzyme-substrate in substrate buffer. Plates were read 45 minutes after the addition of enzyme substrate. Other control samples included, known diseased and healthy pollen in a 1:10 (w/v) ratio of tissue to virus extraction buffer.

2. Serological Blocking to Determine the Location of Virus in Pollen

The purpose of these experiments was to determine if pollen collected from diseased bushes is infectious and whether pollen germination is necessary for the infection process. Determination of virus location on or within highbush blueberry pollen grains was obtained by a modified serological blocking technique used by Cole et al (35).

Highbush blueberry pollen was collected in 1983 from ELISA-indexed healthy and BBLMV-infected bushes as previously described. A 20 mg aliquot of each pollen sample collected was suspended in 0.4 ml of a 0.01

M phosphate buffered saline solution, pH 7.4 (PBS) (Figure 1). Pollen grains were examined using a stereoscopic dissecting microscope and appeared to be intact and not to contain maternal tissue. After a 30 minute incubation at room temperature (21 C), samples were centrifuged at low speed (6,000 rpm, 10 min.). Each pellet was given 3 washes in 0.04 ml PBS with gentle agitation followed by a low speed centrifugation after each wash. The supernatant from each wash was tested for BBLMV using the ELISA method. Pollen integrity was checked after each centrifugation. After washing, samples were resuspended in 0.4 ml PBS and mixed with equal volumes of either, 1) PBS containing polyvinylpyrolidone (PVP), 2) anti-BBLMV gamma globulin diluted to 0.5 µg/ml in PBS, or 3) anti-peach rosette mosaic virus (PRMV) gamma globulin diluted to 0.5 µg/ml in PBS. Samples were mixed and given a 6 hr incubation at 4 C. After a low speed centrifugation, samples were rinsed three times in PBS with gentle agitation to remove excess globulin. The supernatant was again ELISA-tested and the integrity of the pollen observed. Following a final rinse, samples were resuspended in 1 ml PBS and subdivided. One half of the sample, containing intact pollen grains, was ELISA-assayed, while the other half was given a high speed centrifugation (35,000 rpm, 10 min, Beckman No. 40 rotor), to release virus particles from within the pollen grains. Pollen was viewed microscopically and many grains remained intact after centrifugation. The samples were further divided and half were ground in porcelain spot plates using flat-ended glass rods for 30 seconds, while the other half were not ground. Both subsamples were assayed by RIA.

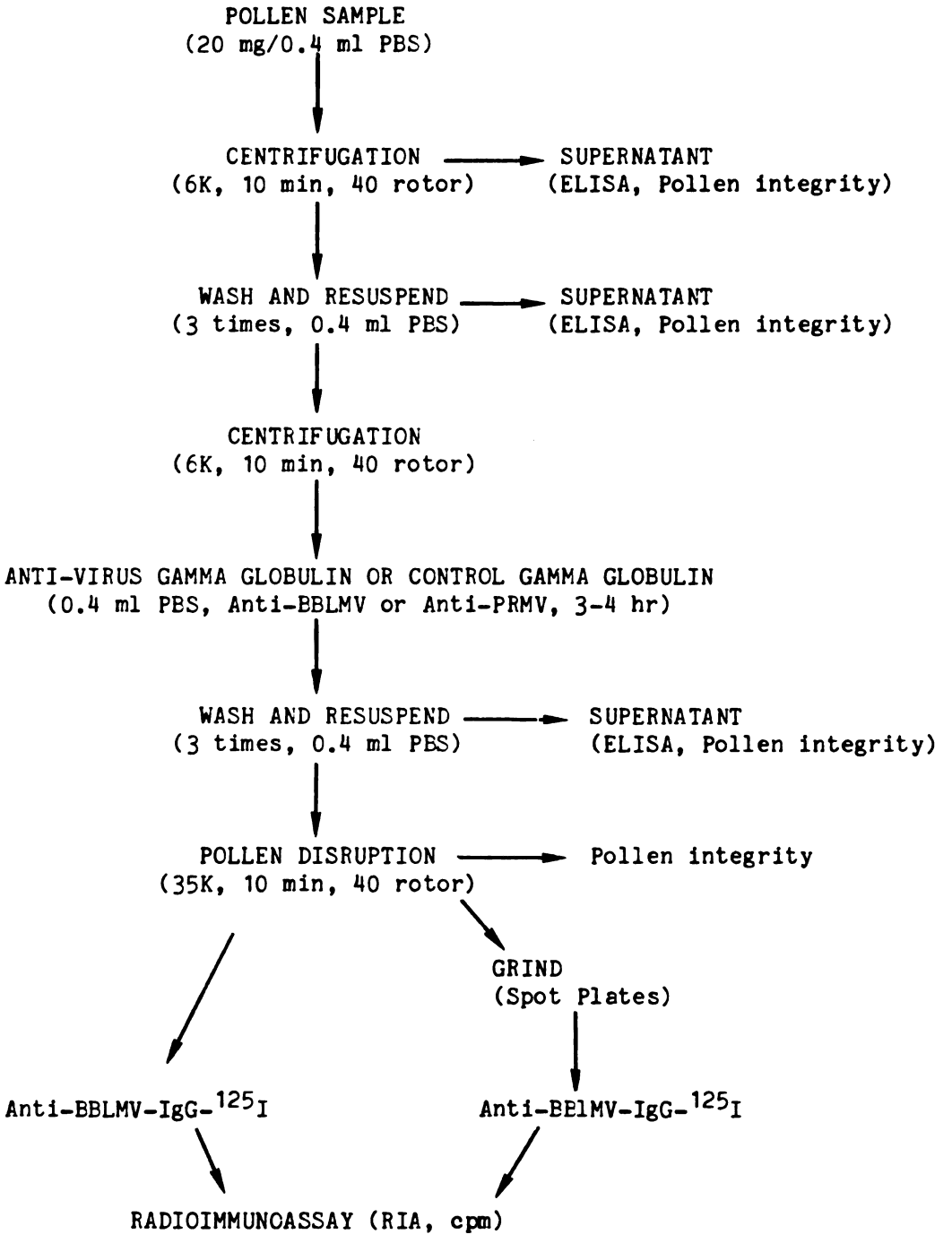

Figure 7. Procedure for the localization of blueberry leaf mottle virus in cv. Jersey highbush blueberry pollen using anti-virus globulin blocking of serologically reactive sites on the surface of intact pollen grains.

ELISA Procedure

Pollen and the washings were tested for BBLMV by methods described before. A 1 µg/ml (v/v) concentration of anti-BBLMV-IgG in coating buffer was incubated in polystyrene microtitre plates for 4 hrs at 37 C. Samples were suspended in either virus extraction buffer or PBS. A 200 µl amount of sample was added to individual wells and the plates were incubated for 8-16 hrs at 4 C. Enzyme-conjugated-anti-BBLMV-IgG at a 1:1000 dilution (v/v) in virus extraction buffer, was added to plates that were rinsed three times with PBS to remove the unbound sample. The plates were then incubated for 4 hrs at 37 C. Enzyme-substrate was added and plates were read after 30 min to 2 hr at A_{405nm}.

RIA Procedure

Radioimmunoassays of pollen resuspended in PBS were conducted with similar concentrations as previously described except for the addition of ^{125}I-IgG (55,000 cpm per well) diluted in a BSA-PBS buffer. Sample wells were rinsed with BSA-PBS-Tween-20 and counted in a gamma counter for 1 minute.

B. Pollen Infectivity

Blueberry pollen collected from cv. Jersey diseased bushes in 1983 was tested for the presence of BBLMV using herbaceous indicator hosts. The samples were obtained from the same infected bushes used for the pollen-virus localization experiment. Samples, diluted 1:5 (w/v) in buffer (0.1 M mono and dibasic phosphate buffer, pH 7.2) was ground with a

mortar and pestle and rub-inoculated onto carborundum (320 mesh)-dusted **Chenopodium quinoa** plants. Plants were kept 14 to 21 days under greenhouse conditions (22-30 C, cool-white fluorescent lights with a 15 hr day length). Leaf tissue, exhibiting symptoms, was ELISA tested for BBLMV.

C. Pollen Viability

If germination of the pollen grain is necessary for BBLMV infection, pollen viability will affect the spread of the disease. To determine the viability of BBLMV-infected pollen, 2.0-3.0 mg of healthy and diseased, air- dried pollen was diluted in 1 ml each of 12.5 % (w/v) sucrose in distilled water alone or with one of the following treatments: 1)100 parts per million (ppm) boric acid (H_3BO_3), 2) 100 ppm boric acid plus 300 ppm calcium nitrate ($Ca(NO_3)2.4H_2O$), 3) 100 ppm boric acid plus 200 ppm magnesium sulfate ($MgSO_4.7H_2O$) or 4) 100 ppm boric acid plus, 300 ppm calcium nitrate and 200 ppm magnesium sulfate. A portion of the pollen samples was pretested for virus content before germination using ELISA. Cells were counted after 3-4 hrs incubation at room temperature (22 C) in a 10X field of view, using a stereoscopic dissecting microscope. Each treatment was replicated three times.

D. Transmission of BBLMV in Infected Pollen to Healthy Blueberry Plants by Hand Pollination.

In 1981, pollen was obtained from healthy and BBLMV-infected cv. Jersey blueberry plants as previously described. Terminals on 15, 2-yr-old (pre-tested by ELISA) plants were tagged and newly opened blossoms were emasculated by cutting away the anthers. Pollen from infected bushes

was applied to the stigmas of marked flowers with a small, artist's brush. The plants were allowed to set fruit. Leaf tissue as well as the mature and immature berries were ELISA-tested for the presence of virus. Four healthy 2-yr-old bushes were pollinated following emasculation by the application of healthy pollen and were used as controls.

4. Vector Identification

The pattern of BBLMV spread in the field was more characteristic of a winged or active vector rather than by nematode transmission. Previous attempts to demonstrate nematode transmission of BBLMV were not conclusive. Several insects common to highbush blueberry fields were used for virus transmission studies.

A. Honeybee-Mediated Transmission

1. Detection of BBLMV in Honeybee Pollen Sacs

Pollination of blueberry fields by placement of honeybee (**Apis mellifera** fs. **ligustica**) hives in the field has been employed by Michigan growers since 1954. To determine whether honeybees carry BBLMV-containing pollen in their pollen baskets (sacs) foraging bees were trapped at bloom during the 1981 and 1982 seasons. The hind legs containing the pollen sacs, were tested using ELISA in 1981, while all three pairs of legs were removed and tested in 1982. Honeybee legs were placed in glass vials containing 400 µl (mean weight of legs = 31 mg) of virus extraction buffer and were ground with glass rods for 2 minutes. Healthy pollen samples were obtained from honeybees artificially reared at the Michigan State University apiary by Dr. R. Hoopingarner.

2. Transmission of BBLMV from Infected Pollen to Healthy Blueberry Trap-Plants via Honeybee Foraging.

It was postulated that BBLMV may be vectored by honeybees while foraging from plant to plant, and also by bringing infected pollen back to the hive. To test this hypothesis, containment cages were constructed and placed around known BBLMV-infected and healthy highbush blueberry bushes in the field before bloom in 1982 and 1983. Ten virus-free (ELISA tested), 2-yr-old cv. Jersey trap-plants (Tower View Nursery, So. Haven, MI 49090) were placed around each caged and selected non-caged bushes. All plants (source and trap-plants) were at the same stage of development. Terminals on the trap-plants, containing developing flower buds were tagged. Small hives or "nukes", containing 2,500-3,000 female workers and a queen (Buckfast queen honeybee, Weaver Apiary, Route 1, Box 256, Navasota, Texas 77868), were placed within some of the cages and near to other non-caged plants. A hive was also placed within the field so honeybees could forage wherever pollen was available. At three times during bloom, pollen was collected from pollen-traps attached to the hive entrance that scraped pollen from the hind legs as bees entered. Air dried pollen, stored at 4 C was tested by RIA as previously described. Any maternal tissue was separated by observation under a stereoscopic dissecting microscope.

Tissue samples, fruit and peduncles from tagged terminals on the trap-plants were ELISA tested for virus after fruit set and for one or two years after incubation in isolation.

B. Acquisition Access Study of BBLMV by the Elueberry Aphid **Illinoia pepperi** (MacGillivray)

The blueberry aphid, **Illinoia pepperi**, MacGil., is a common insect pest of commercial highbush blueberry fields. Control of this aphid is important due to its ability to transmit another virus, blueberry shoestring virus, causing disease in blueberries. Apterous, mature and immature aphids, cultured on healthy blueberry bushes in temperature-controlled incubators (18 hr. day photoperiod with day and night temperatures of 23 C and 18 C, respectively) were obtained for this experiment. Aphids given a starvation period of 1 hr were placed on the succulent terminals of BBLMV-infected and healthy 3-yr-old cv. Jersey blueberry bushes. Elueberry leaf mottle virus-infected source bushes had ELISA-tested positive for BBLMV and expressed symptoms typical of the virus. Two separate experiments were performed. During both experiments, aphids were allowed to feed for either a 72 hr or 120 hr acquisition access period (AAP). Following the feeding period, each individual aphid was placed in a glass vial, containing 200µl virus extraction buffer and ground with a glass rod for 2 min, similar to the technique described by Gillet et al (83). Samples were assayed by RIA using the same concentrations as described previously, (55,000 cpm per well of anti-BBLMV-^{125}I-IgG) and counted in a gamma counter for 1 minute.

C. Nematode Transmission Study to Determine if BBLMV is Vectored by Nematodes

1. Field Survey and Nematode Assay

Although success with nematode transmission of BBLMV has not been realized, the population dynamics of nematodes in BBLMV-infected highbush

blueberry fields were evaluated. A survey of plant parasitic nematodes was made by collecting soil samples 6 to 8 cm deep, at three locations below the drip-line, around each infected bush. Soil from underneath bushes in five commercial fields was sampled. Nematodes were extracted from 200 cm^3 of soil by the Jenkin's Sugar-Flotation technique (116). Extracted nematodes were identified and individually placed in 100 ul of extraction buffer. Each sample nematode was ground with a glass rod for 2 minutes than assayed for BBLMV by RIA. Healthy control nematodes were obtained from virus-free fields or from a culture provided by G. W. Bird.

2. Greenhouse Transmission Study

Xiphinema americanum and **Longidorus sp.** are vectors of several nepoviruses and are occasionally found in blueberry fields in Michigan. Two experiments were conducted where the nematodes, **X. americanum** and **Longidorus sp.** were extracted from soil beneath diseased bushes by methods previously described. At least 15 nematodes were transfered by a dental pick to sterilized, sandy soil in a 3 inch clay pot containing one **Nicotiana clevelandii** seedling. Leaves were removed after an incubation period of 12 days, ground in 0.01 M sodium-phosphate buffer, pH 7.3, and mechanically inoculated to carborundum-dusted **C. quinoa**. Inoculated plants were tested by RIA following a 14 day incubation period at 22 C and 15 hours of cool-white fluorescent light.

3. Nematode Survival in Different Soil Types

Xiphinema americanum is difficult to establish in culture due to its sensitivity to soil type and moisture. To determine the optimum soil type

for the survivability of **X. americanum** under greenhouse conditions, nematodes were placed in different combinations of soil types. Nematodes were extracted from sandy-loam soil, obtained from a fallow site located on the Michigan State University campus. They were extracted by the sugar-flotation (116) method. Soil treatments consisted of: 1) sterilized sand only, 2) 1:1 (v/v) sand and steam-sterilized sandy-loam (orchard) soil, 3) 1:1 (v/v) sand plus greenhouse mix (consisting of 1:1:1 (v/v/v) sand, peat and perlite), and 4) greenhouse mix only. Fifty nematodes (adults and late instar stages) were placed onto washed, healthy roots of **N. clevelandii** plants, previously transplanted to 10 cm clay pots, containing one of the soil types. Two pots per treatment were placed into plastic bags with small perforations to minimize moisture loss. These pots were incubated for 3 weeks at 22 to 26 C. Nematodes were extracted and the survival rate was determined at the end of this period.

4. Acquisition and Transmission Studies of BBLMV using **Xiphinema americanum** (Cobb)

Blueberry leaf mottle virus is mechanically transmitted to and induces systemic symptoms in **C. quinoa**. Blueberry leaf mottle virus or tomato ringspot virus (TmRSV) was rub-inoculated onto ten plants each of **C. quinoa**, in the fourth-leaf stage. Inoculated plants were incubated 8 to 14 days under greenhouse conditions. Excised roots from these plants were inoculated onto virus-free **C. quinoa** leaves to determine whether the virus was in the roots The leaves were ELISA-tested for virus after 14 days. Plastic beakers (100 ml) were filled one-third full with a 1:1 (v/v) greenhouse soil (peat:soil) and sand mixture. Virus-inoculated **C.**

quinoa plants were placed into these beakers, and the roots were spread upon the soil. One hundred **X. americanum** nematodes were applied to the roots with an eye-dropper. The remaining two-thirds of the soil was added over the roots. Non-inoculated and buffer-inoculated plants were used as controls. Nematodes were extracted after a 3-week AAP and tested for virus acquisition by ISEM. Individual nematodes were placed into glass vials containing 100 µl of virus extraction buffer and ground for 2 minutes. Antiserum coated prepared grids were placed onto 30 µl drops containing nematode extracts. The grids were then coated with either anti-BBLMV-IgG or anti-TmRSV-IgG, stained with ammonium molybdate and observed in the TEM.

5. Detection of BBLMV in cv. Jersey Highbush Blueberry Seed and Seedlings

A. Detection of BBLMV in Untreated and Surface Disinfected Blueberry Seed

Transmission of virus through seed to seedlings is an alternate mode of transmission for several virus groups. Experiments were conducted to determine percent BBLMV infection in highbush blueberry seed and transmission to the embryo and developing seedlings. Mature seeds were extracted from ripe cv. Jersey highbush blueberries harvested from diseased and healthy bushes in the field. To test whether BBLMV was a contaminant on the outside of the seed coat or contained within the seed, seeds were untreated or treated with a solution of 0.5 % NaOCl for 3 minutes. Each treatment was followed by three-3-minute washes in distilled water, and the seeds were air-dried. Individual seeds were ground in 125 µl of extraction buffer and tested by either ELISA or RIA.

B. Infectivity of Seeds and Seedlings

While seeds extracted from berries on infected bushes contained virus particles, the question arose as to whether the virus survives the germination process. Seeds extracted from mature blueberries from diseased and healthy bushes were placed 1/8 inch deep in flats of peat, watered and allowed to germinate. Approximately 0.5 gm of tissue (entire seedling plus roots), was ground in either extraction buffer and tested for virus with ELISA, or ground in 0.1 M phosphate buffer plus 2 % nicotine (v/v), then mechanically inoculated onto carborundum (320 mesh)- dusted **C. quinoa**. One week later, leaves from inoculated plants were ELISA tested for BBLMV infection.

To determine if BBLMV is in the seed of infected **C. quinoa** plants, the following inoculations were conducted. Seeds collected from infected **C. quinoa** plants were ground in sterilized distilled water containing 2 % nicotine (NAW) and inoculated onto healthy **C. quinoa** plants. Plants were observed 10-14 days later for virus symptoms.

Although virus particles were detected in seeds of highbush blueberry, infection by rub-inoculation of seedlings from these infected seed lots to **C. quinoa** was not observed. Therefore, to determine whether the seed contains infectious virions, seeds obtained from healthy and diseased seed lots were cut in half transversely using a sterile razor blade. One half of the seed was tested for virus by ELISA, while the other half was crushed in 200 µl 0.1 M phosphate buffer, pH 7.2 and inoculated onto **C. quinoa** plants. After 14 days, tissue samples from inoculated plants were ELISA tested for virus. Controls included healthy and BBLMV-infected **C. quinoa**.

6. Weed Survey for Alternate Hosts of the Blueberry Leaf Mottle Virus

Weeds commonly found in fields planted to highbush blueberry near Agnew, MI. were sampled and tested for the presence of blueberry leaf mottle virus during the 1982 and 1983 growing season. Leaf tissue, flower parts and seeds were tested separately by RIA. Weed samples were taken from underneath infected and healthy bushes as well as at random locations within and outside the field. However, these weed samples were taken near to and underneath infected bushes where potential vector nematodes were not present.

In addition, weeds from BBLMV-infected bushes were assayed, where potential vector nematodes were present. At Otter Lake, MI, a field containing two infected bushes, with one and three **Xiphinema americanum** per 100 cm^3 of soil respectively, were chosen for weed sampling to determine if they were infected, presumably by nematodes feeding on their roots. Weeds were also sampled beneath four BBLMV-infected bushes with soil containing one to four **Longidorus** spp. per 100cm^3 soil, in a highbush blueberry field located at Breedsville, MI.

C. RESULTS

1. Field Indexing For BBLMV In Commercial Blueberry Fields

The experimental block of cv. Jersey highbush blueberry bushes was located in the center of the northern half of the field and spanned 15 rows of plants (Figure 2A). Plants were ELISA-indexed for BBLMV in May 1982 and 1983. Of the 450 plants observed, 117 (26 %) were missing or cut back by the grower to 2 feet above the ground. Many suckers grew from these stumps and often blossoms were produced in the spring. Symptoms typical for the disease were obvious on these plants and many were ELISA-positive. Twenty-three plants (5.1 %) in this block were diseased and expressed symptoms. No symptomless, but infected bushes were detected. Although the rate of disease spread could not be determined due to such a large number of missing bushes, the pattern of spread appears to be random. This random pattern was also observed in parts of the field that were not indexed, but contained bushes expressing symptoms characteristic of the disease.

2. Pollen Transmission Studies

A. Detection of BBLMV in Highbush Blueberry Pollen

Fifteen pollen samples collected from BBLMV-infected bushes were tested for virus (Table 1). The average sample weight was 2.6 mg with a range between 0.17 mg and 7.2 mg. Healthy and diseased pollen control samples weighed 1.0 mg each. Enzyme-linked immunosorbent assay detected BBLMV particles associated with thirteen of the fifteen pollen samples

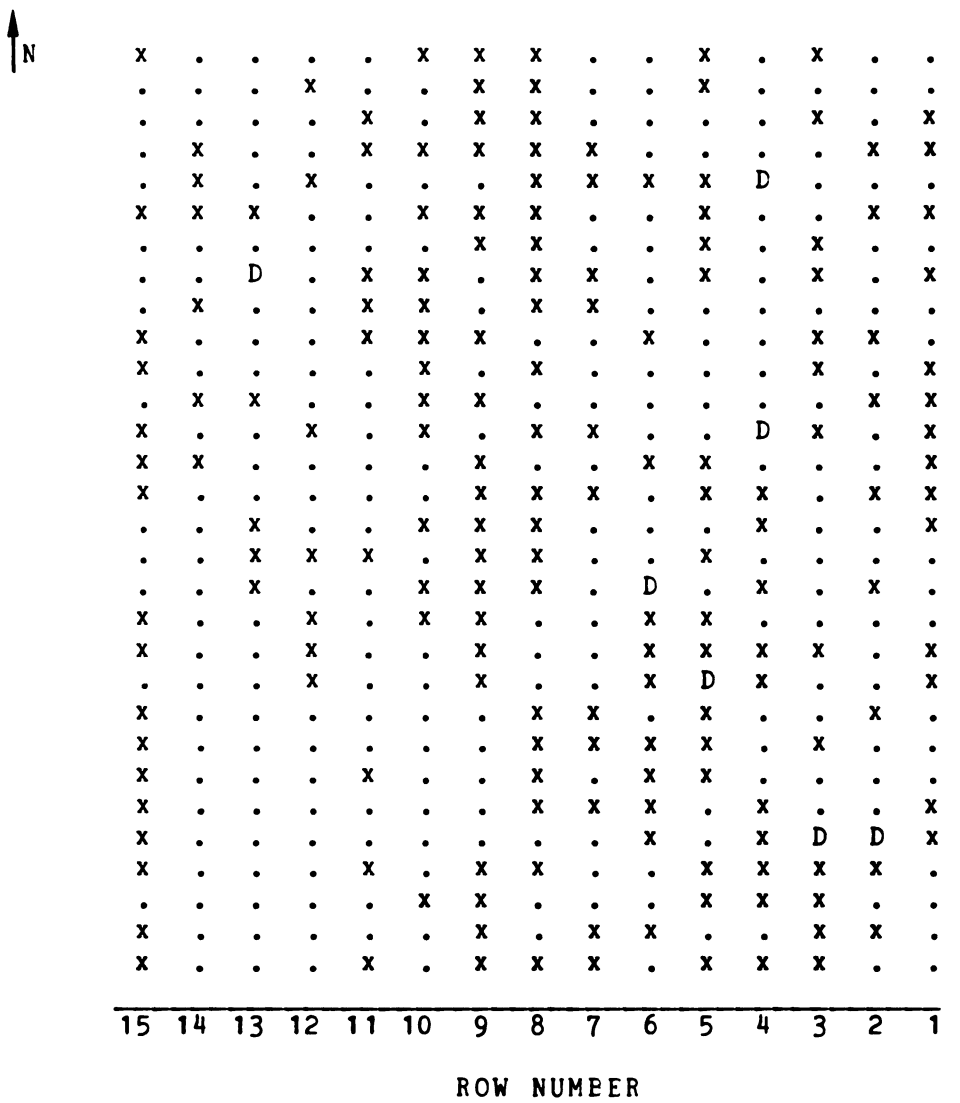

Figure 2A. ELISA-Indexed Highbush Blueberry Field for Analyzing the Spread of Blueberry Leaf Mottle Virus (BBLMV). Agnew, Michigan, 1983. D=diseased, x = missing, . = healthy.

TABLE 1. DETECTION OF BLUEBERRY LEAF MOTTLE VIRUS (BBLMV) IN CV. JERSEY HIGHBUSH BLUEBERRY POLLEN COLLECTED FROM ELISA INDEXED HEALTHY AND INFECTED BUSHES. Agnew, MI.[a].

POLLEN SAMPLE	WEIGHT (gm)[b]	ELISA VALUES[c]
1	.0040	0.07
2	.0046	1.60
3	.0060	1.94
4	.0020	1.89
5	.0020	1.94
6	.0002	0.26
7	.0011	1.87
8	.0018	0.14
9	.0018	1.85
10	.0072	1.75
11	.0025	1.91
12	.0011	0.12
13	.0010	0.07
14	.0010	1.86
15	.0020	0.58
HEALTHY CONTROLS		
1	.0010	0.06
2	.0010	0.05
3	.0010	0.07
4	.0010	0.07
DISEASED CONTROLS		
1	.0010	1.96
2	.0010	1.97
3	.0010	1.93

[a] Samples collected at full bloom, May 1981.

[b] Samples were diluted in 100 µl of a 0.01 M sodium-potassium phosphate buffer pH 7.4.

[c] Absorbance readings at A_{405nm}, represent a mean of two subsamples. The values of the diseased samples are greater than the mean plus standard deviations of healthy controls ($X + 3sx = 0.09$).

(86.7 %) tested. ELISA readings, positive for BBLMV were greater than 0.09 (A_{405nm}), the mean plus three standard deviations of healthy control values. The mean reading at A_{405nm} for the pollen samples was 1.36 with a range between 0.12 and 1.94. The mean A_{405nm} value of the diseased control samples was 1.95.

E. Serological Blocking to Determine the Location of BBLMV in Blueberry Pollen

Highbush blueberry pollen was assayed for BBLMV inside the pollen grain, following serological blocking of the outer surface of the cells with either anti-BBLMV-IgG, or "sham blocking" with anti-PRMV-IgG (a serologically unrelated nepovirus) or PBS, as controls (Table 2). The phosphate buffered saline solution in which pollen from BBLMV-infected bushes was stored initially, contained a high virus content with an average A_{405nm} reading of 1.34. At least two separate samples of pollen per treatment were sham-blocked with either anti-PRMV-IgG or PBS. Eight samples of pollen from diseased bushes, were blocked with anti-BBLMV globulin. Many virus antigens were removed from the surface of the pollen tetrads following the three preblocking washes with PBS (Table 3). Blueberry leaf mottle virus was present on intact cells sham-blocked with either PBS or the non-specific anti-PRMV globulins. Virus was not detected on the surface of intact pollen grains blocked with anti-BBLMV gamma globulins. Blueberry leaf mottle virus particles were not detected in post-treatment washings, for all three treatments. Most of the virions were removed from the pollen surface during the previous three washings, but could be detected in the residual pollen collected from the washing

TABLE 2. ASSAY OF HEALTHY AND BLUEBERRY LEAF MOTTLE VIRUS-INFECTED BLUEBERRY CV. JERSEY POLLEN FOR VIRUS LOCALIZATION USING RADIOIMMUNOASSAY, FOLLOWING PRE-TREATMENT WITH ANTI-VIRUS-SPECIFIC AND NON-SPECIFIC GAMMA GLOBULIN.

POLLEN SOURCE	PRE-TEST[a] ELISA VALUE	INFECTIVITY[b]	VIRUS-BLOCKING[c] GAMMA-GLOBULIN and CONTROL GLOBULIN	POST-BLOCKING TREATMENT	RIA[f] VALUES
Healthy	0.07	-	PBS	washed, not ground[d]	0.00
	0.07	-	Anti-BBLMV	washed, not ground	0.00
	0.07	-	PBS	washed, ground[e]	0.00
	0.09	-	Anti-PRMV	washed, ground	0.00
	0.07	-	Anti-BBLMV	washed, ground	0.00
BBLMV-infected	1.60	+	PBS	washed, not ground[d]	0.00
	1.82	+	Anti-PRMV	washed, not ground	108.00
	1.07	+	Anti-BBLMV	washed, not ground	332.20
	1.26	+	PBS	washed, ground[e]	5135.55
	1.01	+	Anti-PRMV	washed, ground	3897.65
	1.25	+	Anti-BBLMV	washed, ground	3350.93

[a] Diseased sample values are greater than the mean plus three standard deviations of healthy samples (A_{405}nm =0.09).

[b] Chenopodium quinoa mechanically inoculated, two replicates per sample.

[c] Equal volumes (0.02 gm/0.4 ml) of either anti-BBLMV, incubated with pollen to block surface antigens, or anti-PRMV gamma globulin or phosphate buffered saline (PBS) added as a sham-block control.

[d] Pollen samples washed in 0.4 ml PBS three times, but not ground with a mortar and pestle.

[e] Pollen samples washed three times in 0.4 ml PBS and ground with a mortar and pestle to disrupt cells.

[f] Samples tested by radioimmunoassay (RIA), counts per minute (cpm) after background counts were subtracted. Diseased samples are greater than the $\bar{X} + 3sx = 2649.8$ cpm.

TABLE 3. ENZYME-LINKED IMMUNOSORBENT ASSAYS OF PHOSPHATE BUFFERED SALINE WASHINGS OF BLUEBERRY POLLEN, BEFORE AND AFTER BLOCKING OF POLLEN SURFACE WITH ANTI-BBLMV GAMMA GLOBULIN.

		ELISA VALUES (A_{405}nm)						
		PRE-BLOCKING[c] WASHINGS				POST-BLOCKING[e] WASHINGS		
POLLEN[a] SOURCE	BLOCKING[b] TREATMENT	1	2	3	POLLEN[d] RESIDUE	1	2	3
Healthy	PBS	0.06	0.05	0.06	0.04	0.04	0.06	0.04
	Anti-PRMV-IgG	0.05	0.05	0.05	0.04	0.04	0.04	0.04
	Anti-BBLMV-IgG	0.07	0.06	0.05	0.04	0.05	0.04	0.05
BBLMV	PBS	0.54	0.22	0.09	0.13	0.05	0.05	0.05
	Anti-PRMV-IgG	0.54	0.10	0.06	0.12	0.05	0.05	0.04
	Anti-BBLMV-IgG	0.46	0.19	0.10	0.04	0.04	0.04	0.04

[a] Pollen collected from ELISA tested BBLMV-infected and healthy cv. Jersey blueberry bushes, May 1983.

[b] Antigenic sites were blocked on the surface of BBLMV-infected pollen with: anti-BBLMV-IgG or sham-blocked with phosphate buffered saline (PBS) or anti-PRMV-IgG as controls.

[c] Pollen grains washed three times (0.4 ml PBS) and centrifuged (6,000 rpm, 10 min, 40 rotor), to remove BBLMV from the pollen surface prior to anti-virus blocking.

[d] Residual, intact pollen collected from supernatant following centrifugation and after blocking with anti-virus globulin.

[e] Pollen grains washed (0.4 ml PBs) and centrifuged (6K, 10 min, 40 rotor) three times, to remove excess anti-virus globulin.The ELISA values of diseased samples are greater than mean plus three standard deviations of the healthy control values ($X + 3\ sx. = 0.10$).

solutions. Blueberry leaf mottle virus was not detected in washings or residual pollen from healthy bushes treated with PBS alone, anti-PRMV-IgG or anti-BBLMV-IgG.

Pollen samples from infected bushes that were not ground following incubation with one of the treatments, were negative for BBLMV when tested by RIA (Table 2). Blueberry leaf mottle virus was detected in 100 % (16/16) of the pollen samples ground and treated with phosphate buffered saline (PBS) and in 100 % (10/10) of the samples treated with anti-PRMV globulins. The values 108.0 cpm for anti-PRMV sham-blocked pollen and 332.2 cpm for anti-BBLMV-blocked pollen are not significantly different from healthy controls, where the mean plus three standard deviations was equal to 2,649.8 cpm. After grinding pollen samples with a mortar and pestle, 8 of the 16 samples (50 %) blocked with anti-BBLMV-IgG were RIA-positive for BBLMV. Blueberry leaf mottle virus was not detected in pollen from virus-free fields, whether the cells were intact or disrupted by grinding. Results demonstrated that while BBLMV is found on the inside of blueberry pollen not all pollen grains contained virus within the cells. Virus particles were easily removed from the pollen's surface during washing with a buffer solution.

C. Infectivity of BBLMV-Contaminated Blueberry Pollen

Two subsamples of each pollen sample were collected from diseased blueberry bushes. Samples previously collected from these same bushes were used in the serological blocking experiment. Samples were rub-inoculated onto **C. quinoa** plants in the fourth leaf stage. Fourteen days after inoculation, 43 % (6/14) (Table 2) of the inoculated plants

exhibited leaf mottling and local lesions typical of BBLMV infection. All six samples were ELISA-positive for the virus. Virus was not detected in plants inoculated with buffer or healthy pollen.

D. Pollen Viability

To determine pollen viability, healthy and BBLMV-containing pollen samples were observed for germ tube formation in various nutrient solutions. Percent germination was determined by the number of cells forming germ-tubes divided by the number of cells counted in each field. Three subsamples of pollen per each treatment were observed and recorded. Fewer pollen grains from diseased plants germinated in all treatments than pollen from healthy bushes. Only 6.2 % (39/629) of the pollen from diseased bushes germinated, while 44 % (266/604) of the healthy pollen germinated in sucrose alone (Table 4). Germination increased with the addition of boric acid plus other nutrient salts for both healthy and diseased pollen samples, but decreased with the addition of boric acid alone. The highest germination was obtained in sucrose, boric acid and calcium nitrate and in sucrose, boric acid, calcium nitrate and magnesium sulfate. More than one germ tube per pollen tetrad was formed in these treatments, while only one germ tube per tetrad was produced in other treatments. Pollen collected from diseased bushes tested positive for BBLMV antigens by ELISA, with an average A_{405nm} reading of 1.64.

E. Hand Pollination Study

Fruit set on each of the four healthy control bushes pollinated with virus-free pollen. Fourteen of the 15 test plants pollinated with pollen

TABLE 4. COMPARISONS OF POLLEN VIABILITY BETWEEN HEALTHY AND BLUEBERRY LEAF MOTTLE VIRUS (BBLMV)-INFECTED POLLEN, IN VARIOUS NUTRIENT SOLUTIONS.

TREATMENT[a]	GERMINATION[b] (%) HEALTHY SOURCE 1	2	3	MEAN[c]	DISEASED SOURCE 1	2	3	MEAN[c]
Sucrose	39	35	58	44	5	10	4	6
Sucrose, boric acid	20	16	21	19	13	10	18	14
Sucrose, boric acid, calcium nitrate	90	78	81	83	50	66	52	56
Sucrose, boric acid, magnesium sulfate	42	58	72	57	26	31	30	29
Sucrose, boric acid, calcium nitrate, magnesium sulfate	80	77	94	77	60	57	58	58

[a] A 12% sucrose solution diluted in sterile distilled water alone or containing one or a combination of the following nutrients; 0.01% H_3BC_3, 0.03% $Ca(NO_3)_2$ $4H_2O$, 0.02% $MgSO_4$ $7H_2O$.

[b] Pollen samples (2mg) were incubated in 1.0 ml of the treatment solution for 3-4 hrs at 21 C. Samples were pretested by enzyme-linked immunosorbent assay (ELISA).

[c] Mean of three subsamples; percentage of pollen grains forming germ-tubes per number of cells counted in one field of view with a light microscope (X250).

obtained from BBLMV-infected bushes also set fruit (Table 5). The mean number of blossoms pollinated for all 15 plants tested was 22.5, with an average of 5.1 corymbs tagged per plant. This resulted in a mean of 9.9 berries set per virus-infected pollen-treated plant. A mean of 35.5 blossoms with 9.8 tagged clusters was obtained for healthy control plants. An average of 33.8 berries were set per healthy control plant. The mean number of blossoms per group was similar for both healthy and diseased-pollen treated plants. Twice as many fruits were set per tagged terminal on healthy plants than those treated with pollen from diseased plants. Berries and leaves on tagged terminals were ELISA tested August 1981, while only leaves were tested June 1982, August 1982 and April 1983. Each year, plants were given a six-month cold dormancy (4 C) treatment. Blueberry leaf mottle virus was detected by ELISA, June 1982, in leaf tissue sampled from two of the tagged blossom clusters on plant 13, pollinated with BBLMV-containing pollen (Table 5). Those two terminals were dead in 1983.

3. Vector Identification

A. Transmission and Virus Acquisition Studies

1. Detection of BBLMV in Honeybee Pollen Sacs

Honeybees were collected in May 1981 and 1982 during bloom in highbush blueberry fields containing bushes infected with BBLMV (Table 6). Only the hind legs, containing the pollen sacs, were tested in 1981. Blueberry leaf mottle virus was detected in 15.5 % (9/58) of these samples. All three pairs of honeybee legs were tested for BBLMV-containing pollen in May 1982. Enzyme-linked immunosorbent assays

Table 5. Hand-Pollination of Healthy Two-Year-Old cv. Jersey Blueberry Bushes with Blueberry Leaf Mottle Virus (BBLMV)-Contaminated Pollen[a].

Plant Number	Number of Blossoms/ Number of Tagged Corymbs	Number of Berries Set	BBLMV[b] Infected
1	18/5	8	-
2	17/5	3	-
3A	42/7	9	-
3B	33/7	17	-
4	8/2	0	-
5	13/1	6	-
6	15/5	3	-
7	38/10	3	-
8	18/4	3	-
9	33/9	26	-
10	13/5	2	-
11	14/3	12	-
13A	17/3	10	+[c]
13B	26/4	13	-
14	33/6	34	-
Controls			
1	24/5	3	-
2	58/19	46	-
3	45/12	68	-
4	15/13	18	-

[a] Pollen transfered with an artist's brush to emasculated blossoms. Leaves and berries tested by enzyme-linked immunosorbent assay (ELISA) in 1981, 1982. Leaves tested by radioimmunoassay (RIA) 1983.

[b] Mean A_{405nm} value of healthy =0.05; diseased **C. quinoa** (Positive control) = 1.88. Diseased sample values are greater than the mean plus three std. deviations of healthy controls, A_{405nm} = 0.07.

[c] Leaves from 2 tagged shoots ELISA positive for BBLMV in 1982. A_{405nm} = 0.17, 0.18.

TABLE 6. DETECTION OF BBLMV-INFECTED POLLEN ON FORAGING HONEYBEES (**APIS MELLIFERA**), TRAPPED IN A BBLMV-INFECTED CV. JERSEY HIGHBUSH BLUEBERRY FIELD, AGNEW, MI.

SAMPLING DATES	NUMBER TESTED	NUMBER WITH VIRUS-INFECTED POLLEN	PERCENT WITH VIRUS -INFECTED POLLEN
May 1981[a]	58	9	15.5
May 1982[b]	74	38	51.4
Control[c]	10	0	0.0

[a] The hind legs (3rd pair) of honeybees, containing the pollen baskets were ground in 200 µl of extraction buffer for 2 minutes. Samples were tested by enzyme-linked immunosorbent assay (ELISA). Mean A_{405nm} value plus three std. deviations of the healthy control = 0.1, diseased = 0.1-0.4

[b] all three pairs of honeybee legs were assayed by ELISA.

[c] Honeybees obtained from blueberry leaf mottle virus (BBLMV)-free cv. Jersey blueberry fields.

detected BBLMV in 51.4 % (38/74) of the samples of pollen from bees legs in 1982. Samples of honeybee legs collected from virus-free fields did not contain infected pollen.

2. Transmission of BBLMV in the Field Using Trap-Plants, BBLMV-Infected Source Plants and Beehives

Trap plants were removed from around caged and noncaged BBLMV-infected source plants at the end of the bloom period in both 1982 and 1983. Shoot terminals were tested by RIA, while mature and immature berries were tested by ELISA. Twelve of the 138 (8.7 %) tagged terminals on the 1982 (Table 7) trap-plants placed around uncaged, diseased bushes with a beehive near, were positive for BBLMV in July 1982. From these twenty bushes, only one berry of the 537 tested (0.19 %) was ELISA-positive. A slightly higher percentage of the terminals (13.4 %) and berries (0.45 %) on plants placed in cages with a diseased bush plus hive assayed BBLMV-positive. Almost twice as many of the berries set on plants in cages with hives, compared to noncaged plants with bees nearby. Only two of the 93 terminals were BBLMV-positive (2.2 %) on trap-plants placed around caged, diseased source plants without a beehive inside. It is probable that some pollination and subsequently infection occurred without the aid of bees. None of the mature or immature fruit that developed (0/95) contained BBLMV. A blueberry plant in another field, 9 miles away, without the disease present and previously assayed negative for BBLMV was used as the control. Leaf tissue from the 55 tagged terminals on trap-plants surrounding this plant assayed virus-free. Virus was not detected in any of the 238 berries that set on these terminals.

TABLE 7. INFECTION OF HEALTHY BLUEBERRY cv. JERSEY TRAP-PLANTS SURROUNDING BBLMV-INFECTED SOURCE PLANTS VIA BBLMV-CONTAINING POLLEN DURING HONEYBEE FORAGING, AGNEW, MI 1982.

TREATMENT[a]	NUMBER OF TRAP PLANTS	INFECTED[b] TERMINALS	%	INFECTED[c] BERRIES	%	INFECTED[d] POLLEN	%
Diseased-no cage, hive near	20	12/138	8.7	1/537	0.19	1/17	5.9
Diseased-cage, hive inside	20	23/172	13.4	6/1338	0.45	0/6	0.0
Diseased-cage, no hive	19	2/93	2.2	0/95	0.0	0/0	0.0
Healthy-cage, hive inside	10	0/55	0.0	0/238	0.0	0/13	0.0

[a] Two-year-old trap plants placed around source plants 19 May 1982, at 75% bloom.

[b] Number of blueberry leaf mottle virus (BBLMV)-infected terminals per number tagged on trap plants, tested by radioimmunoassay, 1983. Mean cpm plus three standard standard deviations of healthy controls = 329; diseased = 368-685cpm.

[c] Number of berries assayed positive for BBLMV, per number of berries tested by ELISA, 1982. Mean A_{405nm} plus 3 standard deviations = 0.07, diseased = 0.09-0.14.

[d] No pollen assayed positive by ELISA, 9 March 1983. Mean A_{405nm} plus 3 standard deviations of healthy controls = 0.04.

Trap-plants from the 1982 experiment were given a cold dormancy (4 C) for 5 months in 1982 and 1983. Plants were forced in the greenhouse (22-26 C, and a daylength of 15 hrs under cool-white fluorescent light). After each cold period, succulent tissue was tested for virus. Blueberry leaf mottle virus was not detected in any of the leaf tissue sampled from terminals of trap-plants in 1983 or 1984. Pollen collected from pollen-traps placed in front of hives was ELISA assayed in March 1983. One of the 17 (5.9 %) pollen samples collected from hives placed near uncaged, diseased plants, contained BBLMV-infected pollen. Bees were allowed to forage freely within the field which allowed them to collect pollen from other sources. Blueberry leaf mottle virus was not detected in any of the six pollen samples collected from hives placed in cages with a diseased source plant, surrounded by trap-plants. Although blueberry pollen may be stored for a year, the pollen samples were stored for 5 mo at 4 C and the virus may have degraded after storage for this period of time. Thirteen pollen samples were collected from the beehive placed within the cage with the healthy source plant. Virus was not detected in any of the samples tested.

Fewer leaf terminals were tagged on all trap-plants used for the transmission study conducted in 1983 (Table 8), than in 1982. Two of the 47 terminals (4.3 %) on noncaged trap-plants surrounding a diseased source plant and a hive nearby, were ELISA-positive for BBLMV. These values are greater than the mean value plus 3 standard deviations of the mean values of healthy controls. Terminals on trap-plants placed within cages with a diseased source plant and beehive were assayed by ELISA in March 1984, after a 4-5 month dormancy period. Two plants from which leaf tissue from the entire plant was sampled tested positive for BBLMV infection. These

TABLE 8. TRANSMISSION OF BBLMV-CONTAINING POLLEN FROM INFECTED cv. JERSEY BLUEBERRY SOURCE PLANTS TO HEALTHY TRAP PLANTS VIA FORAGING HONEYBEES (Apis mellifera), AGNEW, MI 1983.

SOURCE PLANTS[a]	NUMBER OF[b] TRAP PLANTS	INFECTED[c] TERMINALS	%	INFECTED[d] BERRIES	INFECTED[d] PEDUNCLES	INFECTED[e] POLLEN	%
Diseased-no cage, hive near	20	2/47	4.3	0/13	0/10	13/56	23.2
Diseased-cage, hive inside	20	2/71	2.8	0/350	0/290	6/20	30.0
Diseased-cage, no hive	20	0/65	0.0	0/3	0/3	0/0	0.0
Healthy-cage, hive inside	10	0/24	0.0	0/125	0/93	0/8	0.0

[a] Blueberry leaf mottle virus (BBLMV)-infected or healthy source plants in the field, caged or non-caged with beehives placed in some of the cages.

[b] Healthy two-year-old potted trap plants surrounding source plants, placed in the field 24 May 1983 at 75 % bloom.

[c] Ratio of terminals ELISA assayed positive for BBLMV/number of terminals assayed in March 1984. Mean A_{405nm} value of healthy control plus three std. deviations = 0.05, diseased =0.05-0.46.

[d] Number of berries and peduncles radioimmunoassayed (RIA) positive for virus/number individually tested June 1983. Mean A_{405nm} value of healthy control plus three std. deviations = 133 cpm. No samples were diseased.

[e] Number of pollen samples infected with BBLMV/number of samples collected from beehives, tested by RIA June 1983. Mean A_{405nm} value of healthy control plus three std. deviations = 320 cpm., diseased = 322-440 cpm.

plants were not positive, when tested in June of the same year. Blueberry leaf mottle virus was not detected in leaf terminals on trap-plants surrounding caged, diseased source plants with no hive, nor surrounding caged, healthy source plants with a beehive inside.

A greater number (350) of berries was set on trap-plants placed within cages with a diseased source plant and beehive, compared to plants surrounding noncaged source plants. However, none of these berries were ELISA-positive for BBLMV. Peduncles attached to these berries also assayed negative. Blueberry leaf mottle virus was not detected in the berries or peduncles collected from trap-plants surrounding noncaged diseased source plants or caged diseased source plants with no honeybee hive inside. However, 23 % (13/56) of the pollen samples collected from pollen-traps placed in front of hives near noncaged diseased source plants were positive for BBLMV when assayed by RIA. Thirty percent (6/20) of the samples obtained from pollen collected from hives caged with BBLMV-infected bushes, contained virus. Pollen was not collected from within cages that did not contain a honeybee hive. Eight pollen samples were collected and assayed from the healthy control treatment. Virus was not detected in any of these samples. Honeybees did not survive well in the screen cages and the population of 2500 bees per hive had decreased considerably after two weeks in the field.

B.Results of BBLMV-Acquisition Study by the Blueberry Aphid

Adult and late instar stages of the blueberry aphid, (**I. pepperi.** MacGil.), were fed on succulent leaf-terminals of symptomatic, BBLMV-infected caged blueberry bushes for an acquisition access period (AAP) of 72 or 120 hrs. Individual aphids were removed and tested by ELISA for

BBLMV uptake. Blueberry leaf mottle virus was not detected in any of the 63 aphids assayed July 1983 nor in the 80 assayed September 1983, after an AAP of 72 hr (Table 9). Blueberry aphids did not appear to acquire the virus when given a longer acquisition access period than 72 hours. Of the 68 and 72 aphids given an AAP of 120 hrs and tested on 22 September and 29 September, respectively, none were ELISA positive.

C. Results of Nematode Transmission Studies

1. Field survey and nematode assay

Six commercial highbush blueberry fields in southwestern Michigan containing BBLMV-infected cv. Jersey bushes, were surveyed for plant parasitic nematodes as possible virus vectors in 1983 (Table 10). One field, located at Agnew, Mi. with a high incidence of virus infection was surveyed three times: September 1982, July 1983 and September 1983. Three different genera of plant parasitic nematodes were observed; **Xiphinema** spp., **Longidorus** spp. and **Trichodorus** spp. The percent occurrance represents the number of soil samples yielding a particular nematode divided by the number of samples tested. Nematode density was the mean number of nematodes per 100 cm^3 of field soil. **Xiphinema americanum** was only found in 33.3 % of the soil samples (2 of 6 bushes) taken from beneath infected bushes from only one field (Otter Lake, MI.) and the nematode density of these nematodes was low (1 and 3/100 cm^3 soil, respectively). Soil samples from a field near Breedsville, MI. had a high incidence (66.7 %) of **Longidorus** spp., but again the number of nematodes per sample was low (0-4/100 cm^3 soil). A high percent of **Trichodorus** spp., the stubby root nematode, was found in all fields

Table 9. Radioimmunoassay (RIA) of Blueberry Aphids (**Illinoia pepperi** MacGil.), For Uptake of BBLMV After Feeding on Two-Year-Old BBLMV-Infected Highbush Blueberry Plants.

Acquisition Access[a] Time (hrs.)	Date (1983)	Number of Aphids BBLMV-Positive[b]/ Number of Aphids Assayed
72	29 July	0/63
72	16 Sept.	0/80
120	22 Sept.	0/68
120	29 Sept.	0/72

[a] Aphids fed for 72 or 120 hours on blueberry leaf mottle virus (BBLMV)-infected cv. Jersey bushes in the greenhouse (22 to 26 C), following a 24 hour pre-acquisition fast.

[b] Sixteen aphids fed for 72 hours on virus-free blueberry plants and were used as healthy controls. Individual aphids tested by RIA. Mean of healthy control = 0.0 cpm.,diseased **C.quinoa** = 2643.0 cpm., after the subtraction of background.

TABLE 10. SURVEY OF NEMATODE POPULATIONS ASSOCIATED WITH BLUEBERRY LEAF MOTTLE VIRUS (BBLMV)-INFECTED cv. JERSEY HIGHBUSH BLUEBERRY BUSHES IN MICHIGAN PLANTINGS.

DATE SAMPLED	LOCATION	NO. BUSHES SAMPLED	NEMATODE GENERA	OCCURRENCE[a] (%)	DENSITY[b]
3 Sept 82	Agnew-1	7	Xiphinema	0	0
		7	Longidorus	0	0
		7	Trichodorus	42.9	0.4
5 July 83	Agnew-1	3	Xiphinema	0	0
		3	Longidorus	0	0
		3	Trichodorus	100.0	6.3
	Agnew-2	10	Xiphinema	0	0
		10	Longidorus	0	0
		10	Trichodorus	70.0	4.0
26 July 83	Breedsville	9	Xiphinema	0	0
		9	Longidorus	66.7	1
		9	Trichodorus	100.0	13.3
	Hartford	11	Xiphinema	0	0
		11	Longidorus	0	0
		11	Trichodorus	100.0	4.0
17 Aug 83	Otter Lake	6	Xiphinema	33.3	2.0
		6	Longidorus	0	0
		6	Trichodorus	100.0	13.5
26 Sept 83	Agnew-1	9	Xiphinema	0	0
		9	Longidorus	0	0
		9	Trichodorus	100.0	56.2
20 Oct 83	Agnew-2	7	Xiphinema	0	0
		7	Longidorus	0	0
		7	Trichodorus	100.0	69.0

[a] Number of samples with nematode genera indicated/number of soil samples collected.

[b] Mean number of nematodes/100 cm^3 of field soil. Three samples taken per bush at 20.3 cm (8 in) depth.

tested. In two of these fields, where the disease was particularly severe, a high density of **Trichodorus** spp. nematodes (56.2 and 69.0 per 100 cm^3) was present in soil samples. While **X. americanum** and **Longidorus** spp. may be potential vectors of BBLMV, the populations are considered very low and are not always found with the disease in the field. Although **Trichodorus** spp. was found frequently in BBLMV-infected blueberry fields, there has been no report of transmission of a nepovirus by this nematode.

Nematodes extracted from field soil beneath BBLMV-infected bushes were tested individually for virus by RIA in 1983 (Table 11). None of the 60 individual **X. americanum** nor the 133 **Trichodorus** spp. tested, contained BBLMV. These preliminary results suggest that if nematodes vector BBLMV, it is only after populations have reached a higher level than that found in infected fields.

2. Greenhouse Transmission Study

Ten individual **N. clevelandii** plants were assayed for BBLMV after a 14 day inoculation access period with approximatedly 15 **X. americanum** per pot. None of these seedlings assayed positive for BBLMV (Table 12). A total of 17 **X. americanum** were recovered from these pots and did not contain BBLMV when tested individually by RIA. None of the **N. clevelandii** plants tested positive for BBLMV after incubation with **Longidorus** spp. extracted from soil beneath BBLMV-infected highbush blueberry bushes. No **Longidorus** spp. nematodes were recovered.

Table 11. Nematode Genera Extracted From Soil Beneath Blueberry Leaf Mottle Virus (BBLMV)-Infected cv. Jersey Blueberry Bushes and Tested by Radioimmunoassay (RIA). 1983.

Date Extracted	Nematode Genus[a]	Number Assayed	Number BBLMV[b]-Positive
14 Oct.	Xiphinema americanum	60	0.0
8 Nov.	Trichodorus spp.	48	0.0
11 Nov.	Trichodorus spp.	85	0.0

[a] Thirty-two **Xiphinema americanum** obtained from a virus-free field culture, were used as controls. Mean counts per minute (cpm) plus three standard deviations = 223.9 cpm.

[b] Mean value for individual nematodes assayed = 0.0 cpm, after the background was subtracted. BBLMV-infected **Chenopodium quinoa** = 2106.2 cpm.

Table 12. Radioimmunoassay of **Nicotiana clevelandii** Plants following a 14 Day Inoculation Access Period with **Xiphinema americanum** or **Longidorus** spp. Obtained From Beneath BBLMV-Infected Blueberry Bushes.

Sample Number	Nematode Genus	Number of Nematodes/[a,b] Added Per Plant	RIA[c] (cpm)
1	Longidorus spp.	10	433.5
2	Longidorus spp.	10	448.0
3	Longidorus spp.	8	414.5
4	Longidorus spp.	10	409.5
5	Longidorus spp.	10	360.5
6	Longidorus spp.	8	389.0
7	Longidorus spp.	8	398.0
8	Longidorus spp.	7	427.0
9	Longidorus spp.	5	---- [d]
10	Xiphinema americanum	9	---- [d]
11	Xiphinema americanum	15	498.5
12	Xiphinema americanum	15	399.5
13	Xiphinema americanum	15	394.5
14	Xiphinema americanum	15	419.0
15	Xiphinema americanum	15	446.5
16	Xiphinema americanum	15	---- [d]
17	Xiphinema americanum	15	390.5
18	Xiphinema americanum	15	421.5
19	Xiphinema americanum	15	402.0

[a] Nematodes applied to **Nicotiana clevelandii** roots. After a fourteen day inoculation access period, leaf tissue was tested for BBLMV by radioimmunoassay (RIA).

[b] No **Longidorus** spp. nematodes were recovered after 14 days, while between 0 to 6 **Xiphinema americanum** were recovered from inoculated pots.

[c] Diseased samples are greater than twice the mean, 2X = 1056.7 counts per minute (cpm) of the healthy control plants.

[d] Plant dead; not tested.

3. Nematode Survival in Different Soil Types

Previous greenhouse studies indicated a sensitivity of **X. americanum** to different soil regimes. To optimize populations of **X. americanum** for BBLMV transmission studies, fifty nematodes (adults and late instar larvae) were placed on the roots of **N. clevelandii** plants potted in four different soil types. The plants containing nematodes were allowed to incubate for 3 weeks at 22 to 26 C. The results showed that a 1:1 (v/v) mixture of sand and greenhouse mix, supported the nematode populations the best, albeit at a low level. It also provided a good medium for plant growth, in comparison to other soil types. Five percent of the 100 nematodes for each replicate were recovered from this soil type 1 (1:1 sand and greenhouse mix). **Xiphinema americanum** was not recovered from other soil types, and generally plant growth was poor. No evidence of nematode feeding on root tissue was observed during this experiment.

4. Detection of BBLMV in Seed and Seedlings

Individual blueberry seeds collected from berries on healthy and BBLMV-infected bushes were assayed directly by ELISA or sterilized with a 0.5 % NaOCl solultion to remove virus particles on the seed coat (Table 13). Blueberry leaf mottle virus was detected in 28.7 % (31/108) of the untreated seed and 25.0 % (25/100) of the NaOCl-treated seeds. These results indicate that virus particles are present not only as a contaminant on the seed coat, but possibly within the seedcoat or embryo as well. Neither healthy untreated nor treated seeds contained EBLMV.

TABLE 13. DETERMINATION OF THE PRESENCE OF BLUEBERRY LEAF MOTTLE VIRUS (BBLMV) IN INDIVIDUAL HIGHBUSH BLUEBERRY SEED AND GERMINATED SEEDLINGS.

SAMPLE	NUMBER OF[b] SAMPLES ASSAYED	NUMBER BBLMV-INFECTED	% INFECTED
Healthy seed	50	0	0
Diseased seed	108	31	28.7
Healthy seed[a]	50	0	0
Diseased seed[a]	100	25	25.0
Healthy seedlings	158	0	0
Diseased seedlings	90	0	0
Diseased seedlings[c]	341	5	1.5
CONTROLS			
Healthy *Chenopodium quinoa*	3	0	0
BLMV-infected *Chenopodium quinoa*	2	2	100.0
Buffer	3	0	0

[a] Seeds treated for three minutes in a 0.5 % NaOCl solution followed by three washes in distilled water to remove surface antigens.

[b] Samples tested by enzyme-linked immunosorbent assay (ELISA). Positive samples are greater than the mean plus three standard deviations of healthy controls = 0.05; diseased = 0.08-0.74.

[c] Samples tested by radioimmunoassay (RIA) 1983. Diseased samples are greater than the mean plus three standard deviations of healthy controls =442 cpm.; diseased = 444-515 cpm.

a. Infectivity of seeds from EBLMV-Infected Bushes

All **C. quinoa** plants inoculated with non-surface sterilized blueberry seeds obtained from diseased bushes assayed negative for EBLMV after 14 days. Individual seeds from diseased bushes were cut transversly; one half was assayed by ELISA for BBLMV, while the other half was rub-inoculated to **C. quinoa** plants. Of the 40 seeds tested, 35 % (14/40) assayed positive for BBLMV, but none of the **C. quinoa** plants inoculated with the other half of the seed became infected. All controls were negative for BBLMV when assayed.

b. Results of Germinated Seedlings Assayed for the Presence of BBLMV

Seedlings from seeds produced on healthy and BBLMV-infected blueberry bushes germinated slowly (3-4 months) in peat under greenhouse conditions. Ninety of the seedlings from diseased bushes were negative for BBLMV when assayed by the ELISA technique (Table 13). However, 1.5 % (5/341) of the seedlings tested by RIA were positive for BBLMV. **Chenopodium quinoa** inoculated with seedlings from infected seed lots did not become infected after 14 days and no symptoms were observed on these plants.

5. Weed Survey for Alternate Hosts of BBLMV

The flowers, leaves and/or seeds of 18 weed genera and species from a field near Agnew, MI. were ELISA assayed in 1982 and 1983 for BBLMV (Table 14). Samples representing 11 families were obtained from within and surrounding a field with several BBLMV-infected highbush blueberry bushes. None of the infected bushes from under which weeds were sampled harbored vector-type nematodes e.g. **Xiphinema** spp. or **Longidorus** spp. Carolina horsenettle leaves and flowers were ELISA-positive for BBLMV in 1982, but

TABLE 14. SURVEY OF WEEDS COMMONLY FOUND UNDER BBLMV-INFECTED cv. JERSEY HIGHBUSH BLUEBERRY BUSHES WITHOUT POTENTIAL VECTOR NEMATODES PRESENT. AGNEW, MI 1982, 1983[a].

Latin Name	Common Name	Plant Part[b] Tested	Radioimmunoassay[c] Results (cpm)
Acnida altissima	water hemp	L,F,S	164.75
Amaranthus retroflexus	rough pigweed	L,F	174.50
Cyperus esculentus	yellow nutgrass	L,F	201.00
Digitaria sanguinalis	hairy crabgrass	L,F	167.50
Echinochloa crusgalli	barnyard grass	L,F	181.00
Fragraria virginiana	strawberry (wild)	L	213.00
Galium aparine	bedstraw	L	195.00
Gnaphalium obtusifolium	cud weed	L,F	166.50
Juncus tenuis	slender rush	L	190.00
Phytolacca americana	pokeweed	L,F	163.00
Polygonum pennsylvanicum	Penn. smartweed	L,F	168.50
Portulaca oleracea	purslane	L,R,F	149.00
Rumex acetosella	red sheep sorrel	L	190.00
Solanum carolinense	horsenettle	L,F	213.30
Solidago nemoralis	gray goldenrod	L,F	179.50
Solidago tenuifolia	narrow leaved goldenrod	L,F	181.00
Taraxacum officinale	dandelion	L,R,F	190.50
Vitis riparia	riverbank grape	L	191.00
CONTROLS			
Healthy blueberry		L	171.50
BBLMVinfected blueberry		L	3297.50
BBLMVinfected Chenopodium quinoa		L	2927.00

[a] Potential vector nematode genera (**Xiphinema** spp. and **Longidorus** spp.) were not present in the soil from which weeds were tested.

[b] Plant parts tested for virus using radioimmunoassay(RIA). F=flowers; L=leaves; S=seeds; R=roots.

[c] Diseased samples are greater than 243.5 cpm, the mean plus three standard deviations of the healthy controls.

when assayed in 1983 by RIA they were negative. Carolina horsenettle may contain natural interfering compounds and may give "false positives" when assayed by ELISA. None of the weed samples tested were positive for BBLMV infection. Weeds commonly found within a blueberry field without potential vector nematodes present did not appear to be reservoirs for BBLMV. The field near Otter Lake, MI., which contained two infected bushes supporting a low population of **Xiphinema americanum**, did not yield BBLMV-infected weeds, when assayed by sap-inoculating their leaves to **C. quinoa** (Table 15). Weed species in the field near Breedsville, MI., where four infected bushes supporting a low population of **Longidorus** spp. were also tested by sap-inoculation to **C. quinoa**. None of these weed samples were positive for BBLMV (Table 16). However, some of these weeds became infected with a sap-transmissible virus, which did not react in gel double diffusion tests with antisera to BBLMV, Tomato ringspot virus, tobacco ringspot virus or peach rosette mosaic virus. In these tests, a positive BBLMV control antigen source did react with BBLMV antiserum.

DISCUSSION

While BBLMV appears to be spread extensively within the indexed field (Figure 2A), only two newly infected bushes were detected by ELISA between 1982 and 1983. Cameron (24) reported a geometric increase in the number of cv. Montmorency cherry trees infected with prunus necrotic ringspot virus (PNRSV) over an initial 4 year period, when the trees were allowed to flower. The number of infected trees declined after this period and was attributed to the fact that over 50 % of the trees in the experimental block were already infected, reducing available sites for infection. A

Table 15. Assay of Weeds Associated with Two Blueberry Leaf Mottle Virus (BBLMV)-Infected cv. Jersey Blueberry Bushes Supporting a Low Population of **Xiphinema** spp. Otter Lake, MI. 1984.

Bush Number	Latin Name	Common Name	Number of Infected Indicator Hosts[a]/ Number of Weeds Tested
1[b]	Plantago major	broadleaf plantain	0/8
	Rumex acetosella	red sheep sorrel	0/10
	Taraxacum officinale	dandelion	0/10
2[b]	Fragraria virginiana	wild strawberry	0/2
	Potentilla sp.	potentilla	0/1
	Rumex acetosella	red sheep sorrel	0/10
	Taraxacum officinale	dandelion	0/7

[a] Leaf tissue from each weed plant was ground in 0.1 M sodium-phosphate buffer containing 2 % nicotine (NAB), pH 7.0 and mechanically inoculated onto **Chenopodium quinoa**.

[b] Weeds collected from beneath a BBLMV-infected blueberry bush, September 1984. Soil samples collected beneath these infected bushes contained one to three **Xiphinema** spp. per 100 cm^3 of soil.

Table 16. Assay of Weeds Associated With Four Blueberry Leaf Mottle Virus (BBLMV)-Infected cv. Jersey Blueberry Bushes Supporting a Low Population of **Longidorus** spp. Breedsville, MI. 1984.

Bush Number	Latin Name	Common Name	Number of Infected Indicator Hosts[a]/ Number of Weeds Tested	Ouchterlony[b] Reaction
1[c]	Plantago major	broadleaf plantain	0/5	-
	Polygonum sp.	Penn. smartweed	0/2	-
	Solanum carolinense	Carolina horsenettle	0/2	-
	Taraxacum officinale	dandelion	0/4	-
2[c]	Plantago major	broadleaf plantain	2/6	-
	Solanum carolinense	Carolina horsenettle	0/6	-
	Taraxacum officinale	dandelion	0/6	-
3[c]	Plantago lanceolata	narrowleaf plantain	1/4	-
	Plantago major	broadleaf plantain	3/6	-
	Taraxacum officinale	dandelion	4/6	-
4[c]	Glechoma hederacea	ground ivy	0/8	-
	Plantago major	broadleaf plantain	0/4	-
	Stellaria media	chickweed	1/3	-
	Taraxacum officinale	dandelion	0/3	-

[a] Leaf tissue from each weed plant was ground in 0.1 M sodium-potassium buffer containing 2 % nicotine (NAB), pH 7.0 and mechanically inoculated onto **Chenopodium quinoa**.

[b] Plant sap from infected **C. quinoa** plants were tested against antisera to BBLMV, TmRSV, TRSV and PRMV by the Ouchterlony gel double diffusion test. No reaction with any of these viruses occured at antiserum dilutions of 1:16, 1:32 or 1:64.

[c] Weeds collected from beneath a BBLMV-infected blueberry bush September 1984. Soil samples collected from beneath these infected bushes contained one to four **Longidorus** spp. per 100 cm^3 of soil.

decrease in the number of new infection sites as the number of available host sites decreases has been observed for several diseases (87,219). It would appear that the portion of the field indexed was in the stationary phase of infection with few healthy bushes remaining to become infected.

An estimate of the rate of spread of BBLMV could not be made due to the number of missing bushes, as a result of removal by the grower. It appears to be spread by an active, randomly moving vector either initiating new infection foci or increasing areas of established infection. Mink (151) found patches of cherry trees infected with the cherry rugose mosaic strain of PNRSV. Virus diseased cherry trees were located around sites of initially infected trees. A similar pattern was observed in highbush blueberry fields not as extensively infected with BBLMV as the field indexed. This pattern of disease spread might indicate mediation of BBLMV infection by the foraging activities of honeybees during bloom. It has been documented (105) that honeybees maintain a particular foraging area, i.e. a certain part of the field, group of bushes or even a single bush where they visit. This behavior may be influenced by the size of the area, number of flowers or other foragers, but bees usually return to this same area following each visit to the hive. Honeybees have been observed to maintain a 4 to 5 square yard area (105). Therefore, a single honeybee foraging on an infected bush would have the potential to spread virus-contaminated pollen to several bushes in a single season. This may involve several thousands of visits per bee each season. In addition, the random spread of BBLMV does not resemble that characteristic of virus spread by a nematode vector, but may be an alternate mode of spread in the absence of a vector nematode. Cherry leaf roll virus (CLRV) has also been placed as a putative member in the

nepovirus group, although transmission by a nematode vector has not been demonstrated conclusively (122). Evidence suggests that CLRV has evolved to a more efficient mode of spread, presumably by pollen. The virus has been associated with pollen. Although transmission to healthy **Prunus** species via CLRV-contaminated pollen has not been successful in the United States, Mircetich and coworkers (148) have demonstrated seed and pollen transmission of the CLRV-W strain causing black-line disease in English walnut (**Juglans regia**) in California orchards. Several different strains of CLRV have been found naturally infecting many woody plant species in Europe, North America and New Zealand (41). Experimental evidence suggests that BBLMV and CLRV are examples of viruses that may have evolved to survive in the absence of the nematode vector by a more efficient mode of transmission, e.g. pollen.

Enzyme-linked immunosorbent assay of blueberry pollen demonstrated that a high percentage of the samples collected from BBLMV-infected bushes within the field contained virus particles. Blueberry leaf mottle virus was easily removed from the pollen surface when given a series of saline washes with PBS. Cole et al (35) detected prune dwarf virus (PDV) as a contaminant on the surface of cherry pollen and it was also easily removed by serial washing in saline. Serological blocking of the virions on the surface of pollen with anti-PDV gamma globulin, followed by homogenation resulted in no PDV being detected in disrupted cells. They concluded that germination of the pollen, resulting in fertilization of the ovule was not necessary for PDV infection. The antigenic sites of EBLMV particles located on the surface of blueberry pollen grains were blocked with anti-BBLMV gamma globulin, but not by anti-PRMV-IgG or PBS. Radioimmunoassay of disrupted cells detected significant numbers of BBLMV particles either

within the pollen grains or possibly released from the lipid layer of the pollen exine.

The pollen grains of many entomophilous flowers have a sticky coating, enabling the pollen to adhere to the insect during dissemination, to the style during pollination, as a protective layer against desiccation, or it may be involved in pollen style recognition. Dickinson (55) has discussed the role that tapetal plastids play in the composition of the exine layer of the pollen grain. In the tapetum, specialized cells of the anther, plastids disintegrate and the microspore may be covered with a mixture of lipid, proteins, and tapetal fragments, depending on the type of pollen development. In addition, the surface of the pollen exine contains a fibrous layer of protein that facilitates the compaction of this sticky coating. Interestingly, Dickinson observed that the coating does not usually cover the orbicules or bacula portions of the pollen grain (55). Hamilton et al (91) used the electron microscope to show the association of southern bean mosaic virus (SBMV) with the bacula of the exine and coating the surface of the exine in mature bean (**Phaseolus vulgaris** cv. Bountiful) pollen. Virus was not located within all of the anthers observed and 100 % infection was not obtained when pollen from SBMV-infected bean was inoculated to healthy Pinto bean.

Inoculation of pollen samples from BBLMV-infected bushes to **C. quinoa** indicator plants demonstrated the infectivity and ease with which virus particles are removed from the pollen surface. However, only half of the plants became infected. The results of the serological-blocking experiment indicates that BBLMV may be located within the bacula or other parts of the pollen grain, not covered by the exine, and easily removed during gentle agitation in PBS. Disruption of the exine, either

mechanically or during the germination process, may be required to release unblocked virus particles from within the exine. Observations of pollen from BBLMV-infected bushes with the electron microscope (EM) would be necessary to determine whether virions are located within the cells of the tetrad. Yang and co-workers (231) used the EM to locate TRSV in the intine of the pollen wall, as well as being associated with the parts of the generative and vegetative cells of soybean pollen. They also observed that infected soybeans produced less pollen and that fewer pollen cells germinated or the germ-tube did not elongate as rapidly as pollen from healthy plants.

The microspore of the Vaccinium species is composed of four cells, usually a tetrahedron. Each cell is capable of germination, but in highbush blueberry, usually only one germ-tube is produced. Pollen collected from BBLMV-infected bushes and germinated in a 10 % sucrose solution, produced considerably fewer germ-tubes than pollen collected from healthy plants. Although no morphological alterations of the pollen cell or germ-tube were observed, BBLMV-infection may alter the physiological processes of the cell, and its effect may be overcome by the addition of certain amino acids or enzymes. These results suggest a decreased viability of virus-contaminated pollen due to the presence of the virus directly, or as a result of the infected condition of the parent plant. Reduced pollen viability, abnormalities or pollen abortion has been described for several virus-host interactions (67,163,231). Cole et al (35) postulated that morphological and physiological alterations of cherry and almond pollen by PNRSV may depend on the cultivar of the host and the virus isolate. They also concluded, however, that viable pollen may not be necessary for PNRSV-infection and that mechanical transmission could be possible by wounds created during honeybee foraging.

The delay in germ-tube formation of highbush blueberry pollen from BBLMV-infected bushes would indicate that contaminated pollen could not compete as well as pollen from healthy plants. This would be a disadvantage for transmission of virus through infection of the ovule during the fertilization process. Transmission through wounding of the undeveloped ovary or other flower parts directly during honeybee foraging, would be a more efficient mode of transmission. However, hand pollination of healthy blueberry bushes with BBLMV-contaminated pollen raised the possibility of transmission through fertilization of the ovule. Virus was detected in the leaves of the parent plant one year after hand-pollination, but was not detected in the fruit. Wounds might have been created during pollen application. Entry of virus through wounds cannot be ruled out. However, inconclusive results indicate that virus may have been passed through the ovule into the mother plant and was excluded from the developing seeds or berry.

Several avenues of virus entry into blueberry via pollen may be proposed: 1) by mechanical inoculation by the germ-tube during disruption of the exine as the tube elongates; 2) infection of the stigma by contact with virus-contaminated pollen; 3) infection of the integuments of the ovule, carrying virus internally from the sperm cell to the egg cell, resulting in transmission to the developing zygote, and 4) finally by transmission through the cytoplasm of the vegetative cell, then to the embryo. Carroll et al (26) found that barley stripe mosaic virus is more often in the cytoplasm than in the nucleus of the vegetative cell. It was also found in more than 41 % of the sperm nuclei of barley pollen. Indicating virus infection may occur in the seed by either maternal

transmission through the vegetative cells or during the pollination process.

Honeybees foraging in BBLMV-infected fields were shown to contain virus-contaminated pollen, not only within the corbicula (pollen baskets), but associated with all three pairs of legs. The anthers in highbush blueberry flowers are located in front of the nectaries and nectar cannot be gathered without pollen becoming attached to the honeybee's body. Mink (151) observed that honeybees leaving commercial hives in cherry orchards had pollen attached to their bodies. He postulated that PNRSV-containing pollen may be spread for several days in this manner without pollen viability being important. They found that while the infectivity of PNRSV decreased with time on pollen stored in the hive, it remained infectious on pollen collected within the field for 14 days.

Pollen collected from pollen-traps placed in front of hives located within cages containing BBLMV-infected source plants or near to uncaged source plants, produced symptoms on **C. quinoa**. The length of time BBLMV remains infectious in the field was not determined. Mink (151) concluded that pollen stored in the hive did not germinate in a dilute sucrose solution and may not be important in the spread of cherry rugose mosaic disease. It cannot be ruled out that virus-contaminated pollen is spread throughout the bee colony through physical contact alone. However, Mink (151) found that while over 50,000 hives were transported from PNRSV-infected cherry orchards in California to orchards in Washington state, only a low number of new infections were observed. When one of these hives was placed within a cage with a healthy cherry tree in full bloom, only 5 seeds from the 120 fruits that set contained prune dwarf virus (PDV) and none contained PNRSV. A similar low occurrance of virus

transmission was observed on healthy trap-plants surrounding caged or noncaged BBLMV-infected blueberry source plants with honeybee hives nearby. The highest percentage of infected leaf shoots (13.4 %) and berries (0.45 %) was on trap-plants in cages with a hive of foraging bees in 1982. Only a low amount (2.5 %) of the leaf shoots on trap-plants associated with honeybee activity was infected with BBLMV the following year in 1984. None of the berries or peduncles tested in 1983 were infected with BBLMV. Symptoms were not observed on these infected leaf shoots and several years may be necessary for symptom expression to occur. George and Davidson (78) detected PNRSV in the leaf tissue of female cherry plants 2 weeks after pollination with NRSV-contaminated pollen. They concluded that infection occurs from the pollen to blossoms and then to the fruit and twigs shorly after full bloom. They also found symptoms were not always produced on the same branches the following year. This might be expected since virus has been shown to move to the roots during dormancy and then to follow the flow of nutrients upward as the plant breaks dormancy in the spring (188).

A survey for nematodes known to vector nepoviruses in five highbush blueberry fields containing BBLMV-infected bushes, revealed that either vector nematodes were not present or that the population was so low as to not be considered the primary vehicle of virus transmission at this time.

Radioimmunoassay did not detect BBLMV in the individual samples of **X. americanum** spp. or **Trichodorus** spp. obtained from soil in the root zone of BBLMV-infected bushes (Table 11). Blueberry leaf mottle virus was not observed by ISEM in preparations of **X. americanum** and **Longidorus** spp. allowed a 3 week feed on BBLMV-infected roots of **C. quinoa**. However, these results are not conclusive evidence that nematodes do not vector

BBLMV, since both of these genera of nematodes did not survive well under experimental conditions. In addition BBLMV particles were not observed on EM grids prepared with purified virus. Less than 1.0 % (10/1000) of the nematodes were recovered in these experiments. Furthermore, it is common for nematode-transmitted virus diseases to be associated with weed species near the sites of infection, which may act as "hold-over" sources of the virus. A two year survey of weeds commonly found in blueberry fields did not reveal BBLMV infection.

Blueberry leaf mottle virus was not detected by RIA in individual aphids allowed an acquisition access period of 72 or 120 hours. This does not rule out the possibility of virus uptake at shorter feeding periods, although aphids are not usually associated with transmission of nepovirus diseases. In addition, aphids did not appear in abundance in heavily infected fields.

Blueberry leaf mottle virus was detected in 26.9 % of the seeds from fruit on infected bushes. Location of BBLMV within the seed was not determined conclusively since homogenization of surface disinfected seeds may release virions from the integument of the seed coat and not the endosperm, embryo or cotyledons. Only 1.5 percent (5/341) of the seedlings assayed were positive for BBLMV. Further experiments are warranted to determine whether this low number of infected seedlings was due to reduced viability of BBLMV-contaminated pollen or direct inhibition of the virus during the seed germination process. Several physiological and morphological theories have been proposed to explain why some viruses are excluded from the developing seedling (39,45,222). Crowley (44) showed that bean mosaic virus (BCMV) transmission to seed varied between 0 and 25 % depending on the temperature. Bennet (11) found that highly infectious viruses may be excluded from the seed due to inability to

infect the developing gametophytic and meristematic tissue of the host. Further studies to investigate the effect of seed germination on the survival of BBLMV in highbush blueberry seeds would be interesting.

Pollen may represent an alternate mode of BBLMV transmission to highbush blueberry in the absence of the nematode vector, or may represent an ecological adaptation for a more rapid mode of spread. It does not appear that virus needs to be located within the pollen grain or that fertilization of the ovule is required, for virus transmission to occur. Honeybees foraging for nectar and pollen do wound the floral tissue, and BBLMV may be directly introduced to the parent plants through these wounds. Virus spread, through the fertilization process and developing seed and seedling seems to be a less efficient mechanism. Hamilton et al (91) described several criteria or consequences affecting the efficiency of virus spread via pollen: 1) transmission would be higher in open-pollinated than self-pollinated plants; 2) higher virus titer and method of attachment to the pollen grain would be more efficient; 3) wind blown pollen is a less efficient vehicle or mode of transmission than that mediated by a pollinator; and 4) in most cases, transmission of virus to seed is more efficient when virus is introduced to the egg during the fertilization process. The blueberry leaf mottle virus-incited disease of highbush blueberry appears to be a prime example of a virus being efficiently spread via pollen. Blueberry plants may be either self or open-pollinated. Blueberry leaf mottle virus maintains a high titer in the infected leaf tissue and pollen grains. Virus-contaminated pollen may be transmitted to susceptible hosts via honeybees. Finally, BBLMV is readily transmitted to the seed but the mode of virus entry may be via wounds induced during honeybee foraging rather than by fertilization of the

ovule. Further examination of this last point would be interesting in terms of seed transmission and the potential role of birds as mediators of long distance spread of BBLMV.

Chapter III. Extraneous Sources of Spread Influencing the Epidemiology of Peach Rosette Mosaic Virus (PRMV) in **Vitis labrusca** L. cv. Concord Vineyards.

A. INTRODUCTION

Peach rosette mosaic virus (PRMV) was reported in 1974 in Michigan as being associated with the disorder of Concord grapes,(**Vitis labrusca** L.), refered to as "grapevine degeneration" (54). The Concord cultivar is very susceptible to PRMV and comprises most of the 18,000 acres of grapes grown in Michigan. Peach rosette mosaic virus has been detected serologically in over 27 vineyards and economic loss is due to reduced yield as well as replacing unproductive vines.

Symptoms consist of delayed bud break, shortened internodes, crooked canes and a condition called "berry cluster shelling". Abnormal development of the main sinus of affected leaves results in a downward cupped-like appearance. Symptoms are usually expressed in the field after a four year latent period.

Peach rosette mosaic virus is a multicomponent virus, with isometric particles that are approximately 28 nm in diameter. Characteristic of members in the nepovirus group, PRMV possesses two infectious single-stranded RNA species. The virus is vectored by the nematode, **Xiphinema americanum** (Cobb) (53) and **Longidorus diadecturus** (4). Spread of the disease is in a slow circular pattern, typical of this group. Transmission to healthy vines may occur from nematode vectors feeding on the roots of infected vines and/or the roots of several weed species indigenous to vineyards in Michigan (175).

Most of the Concord grapes grown in Michigan and neighboring states are processed to make juice and jelly products. The price of each crop depends on the percent sugar content of the berries. Pulp and seed, residue after processing, have been used as mulch in productive vineyards. The seeds may germinate in the field. An important concern is the spread of PRMV-infected seed by the application of this residue to healthy vineyards. In vineyards where vector nematode populations are established, the spread of this disease could result in serious economic loss.

Experiments were conducted to determine whether PRMV infects the seeds produced in the fruit on infected vines, and if after processing, the virus remains infectious in germinated seedlings. Pre-selected plots were also indexed to determine the rate and spread of PRMV in infected vineyards. Crop loss assessments were performed by measuring yield and sugar content differences between ELISA-indexed healthy and PRMV-infected vines during the 1981 and 1982 growing season.

B. MATERIALS AND METHODS

1. Virus Purification and Assay Techniques

Peach rosette mosaic virus was isolated and purified from infected **C. quinoa** leaves according to the methods described by Dias and Cation (54). Ten days after inoculation with a Michigan isolate of PRMV, leaf tissue exhibiting symptoms, was harvested and 1 gm was homogenized in 2 ml 0.02 M monobasic and dibasic sodium phosphate buffer, pH 7.0 The extract was filtered through two layers of cheese cloth and frozen overnight to coagulate plant debris. Following a 24 hr slow thaw at 4 C, the extract

was centrifuged at low speed (10,000 rpm, 15 min.). The supernatant was dialysed in 5 volumes of 0.02 M sodium phosphate buffer containing 20 % (w/v) ammonium sulfate, pH 7.0 for 24 hr. After a low speed centrifugation (10,000 rpm., 15 min) of the dialysate, the supernatant was given a high speed centrifugation at 28,000 rpm for 2.5 hr (Beckman 30 rotor). The pellet was resuspended in 0.02 M sodium potassium buffer, pH 7.0 overnight, and was centrifuged at low speed (8,000 rpm., 30 min.), followed by a high speed centrifugation (38,000 rpm., Beckman no. 40 rotor, for 90 min) of the resultant supernatant. The pellet from the high speed centrifugation was resuspended overnight in 2 ml of 0.02 M sodium phosphate buffer. Following a final low speed centrifugation (8,000 rpm., 10 min.), the virus-supernatant was passed through 0-30 % linear-log sucrose gradients by ultracentrifugation (38,000 rpm., Beckman SW-41 rotor, 90 min.). Virus fractions were diluted 3:1 (v/v) with 0.02 M sodium phosphate buffer and centrifuged at 38,000 rpm for 4-5 hr in a Beckman no. 30 rotor to pellet the virus out of the sucrose. The final virus pellet was resuspended in 1.0 ml total volume of buffer. Virus concentration was determined spectrophotometrically and adjusted to 1mg/ml using a molar extinction coefficient of $E^{0.1\%}_{260nm} = 10$.

2. Preparation of Anti-PRMV Gamma Globulin and Conjugate

Pre-immune blood serum was obtained from a female, New Zealand white rabbit. An initial intramuscular injection was made with 1 ml of a 0.5 mg/ml concentration of purified PRMV emulsified in an equal volume of Freund's complete adjuvant. Three additional intramuscular injections were made 7-10 days apart with the same amount of PRMV emulsified 1:1 (v/v) in Freund's incomplete adjuvant. Blood serum was collected four

times at seven day intervals, beginning seven days following the last injection. Serum was separated from coagulated blood cells and titered by immunodiffusion tests in Ouchterlony plates as previously described in chapter II.

Anti-PRMV-gamma globulin was obtained and conjugated to the enzyme alkaline phosphatase or radioactive (^{125}I) as previously described in Chapter II.

3. Field Indexing of Grapevines

Sections of two vineyards containing PRMV-infected cv. Concord vines were ELISA-indexed for virus each year between 1981 and 1983. One site was located at Lawton, MI. and the other near Mattawan, MI. Vines were sampled in a block containing 165 plants at the green-tip stage, located within a 20 acre vineyard at the Lawton site. The vineyard had a previous history of PRMV infection and a history of **Xiphinema americanum**. A portion of the vineyard was indexed for PRMV between 1974 and 1977 (175). In 1981, six cuttings from each dormant vine were removed in early January, and forced under greenhouse conditions prior to assaying for virus. Cuttings were placed in moist sand at 27 C for 2 wk until they were at the green-tip stage of growth. A 0.5 gm sample of green, immature leaf tissue was homogenized in 5 ml [1:10 dilution (w/v)] virus extraction buffer containing 2 % (v/v) nicotine alkaloid. Samples were tested by ELISA as previously described. Young leaf tissue was assayed from six sites on each vine in 1982 and 1983. The Mattawan vineyard, containing 713 vines was also indexed only in 1982. This vineyard was known to have a history of PRMV-infection and the presence of the vector **Xiphinema americanum**. Maps of these plots were also used for ground truth for

aerial surveys of PRMV-infected vineyards.

4. Detection of PRMV in cv. Concord Grape Seeds Prior to and Following Heat Processing for Juice Products.

Ramsdell and Myers (175) reported 10.5 % (4/38) transmission of PRMV from grape seedlings which were collected as grape seeds from fruit set on diseased vines, using mechanical inoculation to C. **quinoa.**

Following heat processing of grape berries for juice products at the Welch grape cooperative plant at Lawton, MI., residual pulp and seed is returned to the vineyards as mulch. An important concern is the dissemination of infected seed as a result of this practice. Several questions concerning the role of residual seeds in the further spread of PRMV were addressed: 1) Does a significant percentage of seeds contain PRMV and is the virus located as a contaminant on the seed coat or located within the seed? 2) Is the virus inactivated during processing at 60-65 C for 2 hr, the time and temperature used to extract the juice? and 3) What percentage of the seeds are viable and infective after this process? To address these questions, the following experiments were conducted.

A. Detection of PRMV in Concord Grape Seeds

Seed samples collected from fruit on diseased and healthy vines in the field, were assayed to assess the occurance of PRMV in cv. Concord grape seeds and to determine whether the virus is located as a contaminant on the seed coat. Following extraction from the fruit, seeds were either assayed directly or treated for 3 min. in a 0.5 % (v/v) solution of NaOCI, followed by three-3-min. washes in sterilized distilled water.

Grape seeds were assayed by placing one seed into individual 0.6 cm diameter, 1.0 cm deep wells of an aluminum block. A matching aluminum block, with 0.6 cm diameter rods, 3.75 cm long was seated into each well to form a press. The dry seeds were crushed at 210 kg/cm^2 of pressure. A 300 µl aliquot of virus extraction buffer was added to each well and the samples were agitated. Samples were tested for virus by ELISA.

B. Survival of PRMV in Seed and Seedlings, Following Heat Processing.

Mature grapes are processed for juice and jam products by the Welch grape cooperative. Juice is extracted from the berries by treatment with a pectinase (Klearzyme^R, personal communication, W. Grevelding) which breaks down the pulp during heat treatment for 2 hr at 60-65 C. Remaining pulp and seed is pressed and separated from the liquid. The liquid is concentrated and stored in sterilized containers. Sixteen tons of grapes are processed at one time. During this process, residual pulp and seed may be collected by growers at the cooperative and spread as mulch over the soil.

Pomace samples were collected at timed intervals during processing in 1981 and 1982, to determine the percentage of PRMV-containing seeds present in each sample lot. Six tons of fruit from pre-tested PRMV-infected vines were dumped into the hopper, followed by 10 tons of fruit from healthy vines. In 1981, pomace samples containing seed were obtained at 3 min. intervals for up to 35 min. after heat processing was completed. Samples were collected at 0, 10, 16 and 22 min. intervals after the grapes were heat processed in 1982. Seed samples were collected from two different conveyer belts at each time interval sampled.

To simulate heat processing as done at the Welch plant, grapes were collected from virus-infected and healthy vines in the vineyard during the 1981 and 1982 seasons. The fruit was crushed at the laboratory at Michigan State University and 2-liter aliquots of sample, containing seed and pulp were heated at a constant 60 C temperature for 2 hrs without Klearzyme^{R} added. Seeds were removed, dried and assayed for PRMV.

Seed lots from both the Welch plant and laboratory heat treatment were divided. Half of the seed samples from each lot were ELISA tested directly for PRMV, while the remaining seeds were stratified at 4 C in moist sand for 3 mo and allowed to germinate. Emerging seedlings were then assayed for virus.

1. Seed stratification

Approximately 200 seeds from each time interval sampled after processing at the Welch plant and also from the laboratory treatment were stratified in 1981. Twelve hundred (1200) seeds from each treatment were stratified in 1982. In 1981, seeds were wrapped in 2 layers of cheesecloth and placed 5 cm deep in moist, sterilized sand in wooden flats. These flats were refrigerated at 4 C for 3 mo. to fulfill the cold dormancy required for grape seeds to germinate. Seeds were stratified in 1982 by placing seeds into wire-mesh screen envelopes, and placing them 5 cm deep in sterilized, moist sand. The flats were covered with plastic bags and placed outside for 3 mo to mimic natural field temperatures.

A. Seed Germination

Stratified seeds from both the 1981 and 1982 experiments were germinated in flats containing a sterilized soil mixture of 1 part sand to 1 part greenhouse soil. In addition, 1200 non-stratified seeds from each

time treatment were germinated under the same conditions. The soil pH was between 6.7 and 6.8. Twelve rows of seeds were planted in each flat. Fifteen seeds were placed 1.3 cm deep in each row. Each treatment was replicated three times in different flats. Flats from the 1981 experiment were placed into a growth chamber with 9 hr illumination and a 26 C day temperature followed by a 17 C night temperature. In 1982, flats containing seeds were placed in plastic bags to conserve moisture and placed in the greenhouse at approximately 22 C with 15 hr illumination. Germinated seedlings were tested by ELISA for PRMV.

2. Detection of PRMV in Seeds and Germinated Seedlings

Laboratory heat treated seeds were cut in half, longitudinally through the raphé, dividing the seed into symmetrical halves. One half of the seed was ground with a mortar and pestle with 0.5 ml virus extraction buffer and ELISA tested. The other half was ground in 0.5 ml of a 0.1 M potassium phosphate buffer (pH 7.0) and rub-inoculated onto carborundum dusted **C. quinoa** indicator plants. Inoculated plants were incubated at 22 C under greenhouse conditions for 14 days then virus assayed by ELISA.

Grape seeds were allowed to germinate for 2.5 mo under the conditions previously described. Leaf tissue was homogenized in a 2 % NPB solution and rub-inoculated onto carborundum-treated **C. quinoa** at the fourth true leaf stage. Two weeks later, inoculated plants were assayed by ELISA for virus infection.

5. Yield and Sugar Content of Fruit from Healthy and PRMV-Infected Grapevines

Monetary return to grape growers is determined by the current price of a given seasons crop. This price depends not only on that seasons

yield but also on the sugar content, resulting in a higher price for sweeter grapes. The effect of PRMV-infection on yield per vine and sugar content was determined in 1981 and 1982. Ten healthy and ten virus-infected vines were selected within an area of heavy infection. All grape clusters, including ripe and immature fruit were harvested and weighed in plastic lugs.

To measure sugar content, refractive measurements were made of samples collected from diseased and healthy vines. The juice from a 454 gm sample of mature grapes from each vine was placed into a refractometer and percent sugar content was read in brix units.

C. RESULTS

1. Virus Purification and Antiserum Production

Following centrifugation through a second sucrose density gradient to remove impurities, the final concentration was 2.0 mg per 400 gm of starting tissue. Antiserum, prepared against purified PRMV in the rabbit, reacted at a 1:1024 dilution end point to expressed sap from infected C. **quinoa** in agar gel double diffusion tests. A reaction with antiserum to sap from healthy C. **quinoa** at a dilution end point of 1:2 (v/v) was observed, but not at higher dilutions.

2. Field Indexing Grapevines to Determine the Spread of PRMV

A total of 165 vines were indexed by ELISA for PRMV infection in the vineyard located near Lawton, Michigan between 1981 to 1983 (Figure 3A). Initially, 14 of the 165 vines were missing (probably due to PRMV infection) at the 1981 sampling. Bordering rows were incorporated into

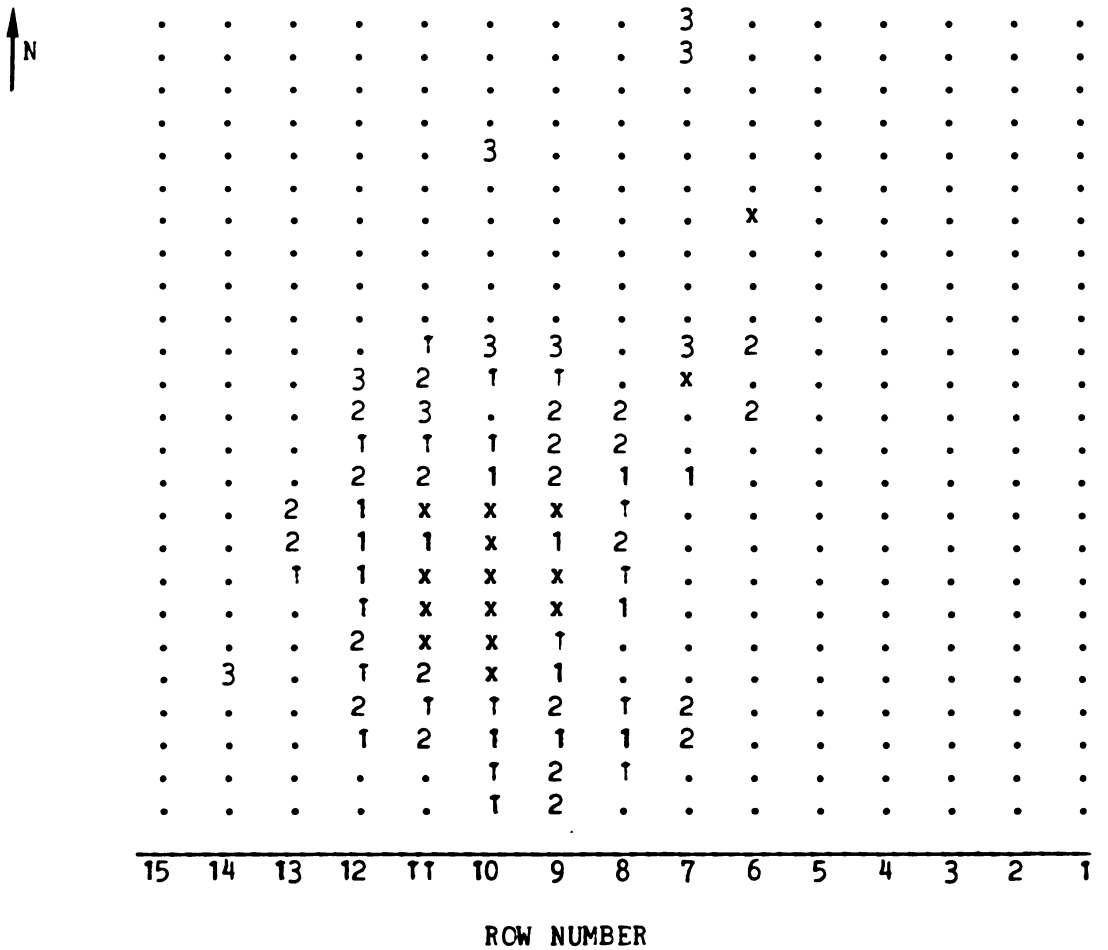

Figure 3A. Pattern of spread of peach rosette mosaic virus via **Xiphinema americanum** in a cv. Concord vineyard . = healthy, x = missing and 1,2,3 = date vine was ELISA positive for PRMV in 1981, 1982 and 1983, respectively. Lawton, MI

the survey area that did not contain infected vines in order to clearly define the area of infection.

ELISA indexing in 1981 revealed 32 PRMV-infected vines exhibiting symptoms. Many of these vines were located next to or in proximity to the area where vines were missing. In 1982, virus was detected in 22 additional vines which previously assayed virus-free. Again, these vines were clustered around previously infected vines, but the area of infection had expanded in all directions. Virus was detected in only nine previously virus-free vines the following year in 1983. Interestingly, most of these newly infected vines in 1983, were located down the rows containing several infected vines. The area of infection did not expand in all directions as previously described. In addition, isolated and infected vines were detected in 1983, well away from the initial sites of infection and creating new infection foci. Results of tests for randomness of infected plants, based on run analysis (140), indicated that the increase of PRMV spread in the field is nonrandom. For example, as would be the movement of the nematode vector from plant to plant. In all cases tested, the observed number of runs (U, a succession of diseased vines) was considerably less at the 5% level of probability, than the expected number [E(U)], indicating a nonrandom condition.

The second vineyard indexed for PRMV infection in 1982, and located near Mattawan, demonstrated a pattern of infected vines typical for the disease (Figure 4A). Of the 312 vines assayed in 1982, 185 vines were infected (59.3 %) with PRMV and 24 were either dead or missing. In most instances, diseased vines followed in a succession. The heaviest area of infection was located in a corner of the vineyard, dissipating further within the field. Only a few infected vines were located outside this area of infection and were surrounded by healthy vines.

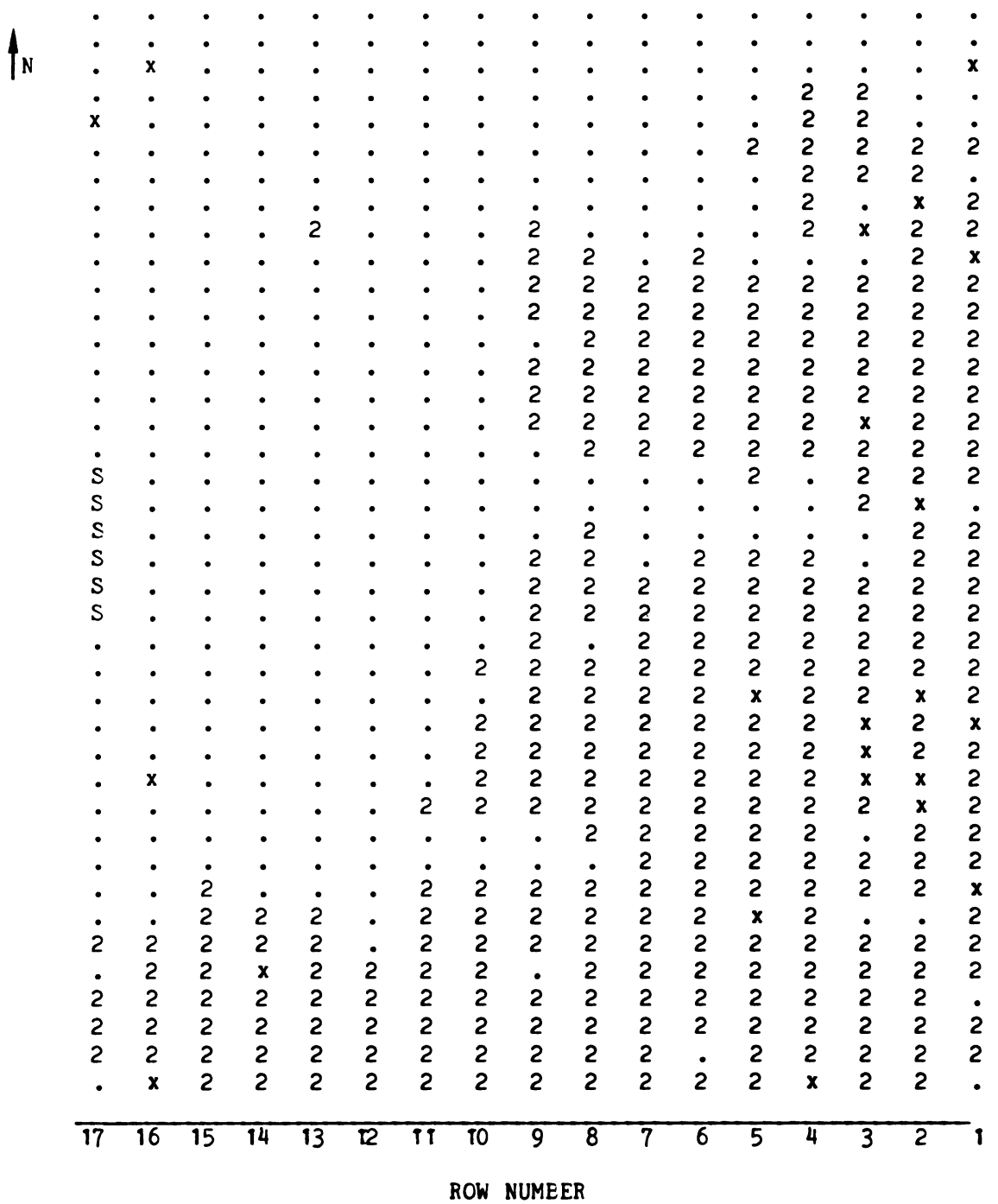

Figure 4A. Pattern of spread of peach rosette mosaic virus (PRMV) in a cv. Concord grape vineyard. . = healthy, x = missing vine, S = space in field and 2 = date vine was positive for PRMV, tested by ELISA in 1982. Mattawan, MI

3. Detection of PRMV in Concord Grape Seeds

Seeds removed from fruit produced on PRMV-infected vines were tested individually by ELISA (Table 17). Fifty-five of the 396 seeds tested (14.0 %) were positive for PRMV. Virus was also detected in 93 of the 114 (82.0 %) seeds surface disinfected with a 0.5 % solution of NaOCl, to inactivate virions on the surface of the seed coat. None of the 100 seeds extracted from berries on healthy vines contained virus. Seed-halves, from virus-infected seed, ground in 0.1 M phosphate buffer pH 7.0 did not produce symptoms when inoculated onto **C. quinoa** plants.

A preliminary experiment was conducted in 1981 to assess whether virus is located in all parts of the seed. The embryo was separated from the seed-halves and assayed for PRMV separately from the remaining seed parts containing the endosperm and seed coat. Each sample was ground in 1 ml virus extraction buffer. Seven of the nine embryos (78.0%) from seed of infected vines were positive for PRMV. Four of the nine (44.4 %) samples consisting of remaining seed parts (endosperm and seed coat) contained virus. Peach rosette mosaic virus was not detected in any of the nine excised embryos or remaining seed parts of seeds from healthy vines.

4. Detection of PRMV in Seeds and Germinated Seedlings Following Heat Processing

Grape seed samples were collected at timed intervals following crushing and heat processing. Half of these seed lots were assayed directly for PRMV, while the other half was stratified and germinated. Seedlings were then tested for PRMV infection.

Table 17. Detection of Peach Rosette Mosaic Virus (PRMV) in Untreated and Surface Disinfected Seed Coats of cv. Concord Grape Seed.

Vine Status of Seed Source	Number of Seeds Assayed	Number of Seeds[c,d] PRMV-Infected	% Seeds Infected
Healthy vines	100	0.0	0.0
Diseased vines[a]	396	55.0	14.0
Diseased vines[b]	114	93.0	82.0

[a] Seeds not treated with in a 0.5 % (v/v) NaOCl solution, prior to ELISA testing.

[b] Seeds treated 3 minutes in a 0.5 %(v/v)NaOCl solution, followed by three-3 minute washes in sterilized distilled water.

[c] Individual seeds were tested for PRMV using enzyme-linked immunosorbent assay (ELISA).

[d] Mean A_{405nm} reading of healthy seeds =0.08; diseased seeds = 0.15-0.78. PRMV-positive values are greater than the mean plus three stardard deviations of the healthy control (A_{405nm} =0.14).

Seeds from eleven sampling times were tested in 1981 (Table 18). Peach rosette mosaic virus was detected in seed lots collected at all time intervals after processing. The highest percentage (16.9 %) of infected seed was detected at 13 min. after processing, with the lowest (0.39 %) after 25 and 31 min. No successive decrease in the number of infected seeds was observed over time. Of seeds collected from fruit heat-treated at 60 C in the laboratory or from untreated fruit, 79.6 % and 70.4 % of the seeds respectively, tested positive for virus by ELISA. The virus was not detected in seed extracted from heat-treated or untreated fruit from healthy control vines.

Between 150 and 180 seeds from each collection time interval were stratified in moist sand and allowed to germinate (Table 19). The percentage of seeds germinated, ranged from as high as 6.11 % to as low as 0.0 %, with a mean germination rate of 1.2 %. Seeds of fruit from healthy vines either untreated or heat-treated under laboratory conditions, germinated at 1.1 % and 4.4 % respectively. Although the number of seeds germinated was low, 60 C heat did not appear to affect the germination of seeds extracted from fruit from diseased vines. While no seeds germinated after heat treatment of fruit in the laboratory, only 3.9 % (7/180) germinated from untreated diseased lots used as controls. None of the germinated seedlings tested positive for PRMV infection by ELISA.

In 1982, seeds were collected at four times: 0,10,16 and 22 minutes after heat processing at the Welch plant (Table 20). The percent of seeds that assayed positive for PRMV were 12.0, 3.7, 3.0 and 1.0 %, for 0, 10, 16 and 22 min., respectively. While more seeds were tested per time-interval lot in 1982, fewer seeds per seed-lot were positive than in 1981. Twenty-nine and 39.0 % of the seeds from diseased vines, heat treated and

Table 18. Detection of Peach Rosette Mosaic Virus (PRMV) in cv. Concord Grape Seed of Fruit From Diseased and Healthy Vines, Mixed During Commercial Heat Processing For Juice. Lawton, MI 1981.

Time Seeds Collected[a] After Processing (min)	Number of Seeds PRMV Infected[d]/ Number of Seeds Tested	% Seeds PRMV-Infected
0	20/260	7.69
5	10/260	3.85
10	11/260	4.23
13	44/260	16.92
16	6/260	2.31
19	41/260	15.77
22	23/260	8.85
25	1/260	0.39
28	21/260	8.08
31	1/260	0.39
35	10/260	3.80
Controls		
Diseased vines[b]	95/135	70.4
Healthy vines [b]	0/132	0.0
Diseased vines[c]	117/147	79.6
Healthy vines [c]	0/145	0.0

[a] Seeds sampled at timed intervals after processing fruit at 60 C for 120 minutes to determine dilution of seeds from PRMV-infected fruit with seeds from healthy fruit. Six tons of fruit from PRMV-infected vines were mixed with 10 tons of fruit from virus-free vines at the hopper.

[b] Seeds from fruit collected in the vineyard, not heat treated. Tested by the enzyme-linked immunosorbent assay (ELISA).

[c] Seeds from fruit collected in the vineyard, heat treated at 60 C for 2 hours. ELISA tested for PRMV.

[d] Mean A_{405nm} reading of healthy seeds =0.08; diseased seeds =0.12-0.48. Positive values are greater than the mean plus three standard deviations of the healthy control (A_{405nm}= 0.12)

Table 19. Detection of Peach Rosette Mosaic Virus (PRMV) in cv. Concord Grape Seedlings, Following Commercial Heat Treatment and Stratification of the Seeds. Lawton, MI 1981.

Time Seeds Collected[a] After Processing (min)	Number Germinated/[b] Number Planted	Percent Germinated	Number Seedlings Diseased[c,f]/ Number Assayed	Percent Infected
0	1/150	0.67	0/1	0.0
5	2/150	1.33	0/2	0.0
10	1/180	0.56	0/1	0.0
13	11/180	6.11	0/11	0.0
16	6/180	3.33	0/6	0.0
19	1/150	0.67	0/1	0.0
22	0/150	0.00	0/0	0.0
25	1/180	0.56	0/1	0.0
28	0/180	0.00	0/0	0.0
31	0/180	0.00	0/0	0.0
35	0/150	0.00	0/0	0.0
Controls				
Diseased vines[d]	7/180	3.89	0/7	0.0
Healthy vines[d]	2/180	1.11	0/2	0.0
Diseased vines[e]	0/90	0.00	0/0	0.0
Healthy vines[e]	4/90	4.44	0/4	0.0

[a] Seeds sampled at timed intervals after processing fruit at 60 C for 120 min. to detect dilution of seeds from PRMV-infected fruit with seeds from healthy fruit. Six tons of fruit from PRMV-infected vines were mixed with 10 tons of fruit from virus-free vines at the hopper.

[b] Seeds stratified in moist sand 3 months at 4 C, then allowed to germinate in a 1:1 (v/v) soil and peat mixture for 2 months.

[c] Number of germinated seedlings tested positive for PRMV by ELISA.

[d] Fruit collected in the vineyard, and not heat treated.

[e] Seeds from fruit collected in the vineyard, and heat treated at 60 C for 2 hr. ELISA tested for PRMV.

[f] Mean A_{405nm} reading of healthy seeds =0.07, diseased seeds =0.74–1.95. PRMV-positive values are greater than the mean plus 3 standard deviations of the healthy control (A^{405nm} =0.74).

Table 20. Detection of Peach Rosette Mosaic Virus (PRMV) in cv. Concord Grape Seed Collected at Timed Intervals Following Commercial Heat Processing of the Fruit. Lawton, MI 1982.

Time Seeds Sampled[a] After Processing	Number Seeds PRMV-Infected/[c,d] Number of Seeds Tested	% Seeds PRMV-Infected
0	61/510	12.0
10	19/510	3.7
16	14/510	3.0
22	6/510	1.0
Controls		
Diseased vines[b]	198/510	39.0
Healthy vines [b]	0/100	0.0
Diseased vines[c]	148/510	29.0
Healthy vines [c]	0/100	0.0

[a] Seeds sampled at timed intervals after processing fruit at 60 C for 120 minutes to detect dilution of seeds from PRMV-infected fruit with seeds from healthy fruit. Six tons of fruit from PRMV-infected vines were mixed with 10 tons of fruit from virus-free vines.

[b] Seeds obtained from fruit collected in the vineyard, not heat-treated. Tested by the enzyme-linked immunosorbent assay (ELISA).

[c] Seeds from fruit collected in the vineyard, heat-treated at 60 C for 2 hours. ELISA assayed for PRMV.

[d] Mean A_{405nm} reading of healthy seeds =0.07, diseased seeds =0.15-0.74. Positive ELISA values are greater than the mean plus three standard deviations of the healthy control (A_{405nm} = 0.15).

nontreated respectively, contained PRMV. Virus was not detected in control seed from healthy vines.

A low percentage of seed germination was again observed in 1982 (Table 21). The percent of germination of seeds selected at 0, 10, 16 and 22 minutes after processing was 0.0, 0.33, 0.58 and 0.25 %, respectively. Virus was not detected in any of the seedlings that germinated by either the ELISA test or by mechanical inoculation onto **C. quinoa.** Seeds from fruit on diseased vines were either untreated or laboratory heat-treated and were used as controls. Only 2.7 % (29/1075) of the untreated control seeds germinated. Four of these seedlings (14.3 %) assayed positive for PRMV infection. None of the seeds from the heat treated control germinated.

Heat-treated or untreated seed of fruit from healthy and diseased vines were germinated without a 3 month stratification. Again a low number of these 2100 seeds planted in all treatments, germinated (Table 22). Fifteen seedlings germinated from seed extracted from nontreated fruit from diseased vines. Only two germinated from heat-treated fruit. Heat-treated and nontreated seed from healthy vines, germinated at levels of 0.1 % and 0.24 % respectively. However, none of these seedlings were positive for PRMV when assayed by ELISA or inoculated onto **C. quinoa** indicator plants.

5. Yield Data and Sugar Content of Fruit from Healthy and PRMV-Infected Grapevines

The entire fruit yield was weighed from 10 healthy and 10 PRMV-infected vines a few days before commercial harvesting in October of 1981 and 1982 (Table 23). In 1981, the yield of berries on infected vines ranged from 11,113.2 gm to 1587.6 gm with a mean yield of 4277.5 gm per

Table 21. Detection of Peach Rosette Mosaic Virus (PRMV) in Germinated cv. Concord Grape Seedlings Following Commercial Heat Processing of the Seeds. Lawton, MI. 1982.

Time Seeds Sampled[a] After Processing	Number Germinated/[b] Number Stratified	Percent Germinated	Number Infected/[c,f] Number Assayed	Percent Infected
0	0/1200	0.00	0/0	0.0
10	4/1200	0.33	0/4	0.0
16	7/1200	0.58	0/7	0.0
22	3/1200	0.25	0/3	0.0
Controls				
Diseasedvines[d]	29/1075	2.70	4/28	14.3
Healthy vines [d]	0/1090	0.00	0/0	0.0
Diseased vines[e]	0/875	0.00	0/0	0.0
Healthy vines [e]	0/1200	0.00	0/0	0.0

[a] Seeds sampled at timed intervals after processing fruit for 120 min at 60 C to detect dilution of seeds from PRMV-infected fruit with seeds from healthy fruit. Six tons of fruit from PRMV-infected vines mixed with 10 tons of fruit from virus-free vines.

[b] Seeds stratified in moist sand 3 months in a cold frame outside between September 1982 and January 1983. Seeds germinated in a 1:1 (v/v) soil and peat peat mixture for 3 months.

[c] Number of PRMV-infected seedlings assayed by ELISA/ Number of seedlings tested.

[d] Mature fruit collected from vines and pretested for PRMV and not subjected to heat-treatment.

[e] Mature fruit collected from vines and subjected to heat treatment at 60 C for 2 hours. ELISA assayed for PRMV.

[f] Mean A_{405nm} reading of healthy seeds =0.07, diseased seeds =0.75-0.96. Positive ELISA values are greater than the mean plus three standard deviations of the healthy control (A_{405nm} = 0.08).

Table 22. Detection of Peach Rosette Mosaic Virus (PRMV) in Seedlings from Non-Stratified cv. Concord Grape Seeds[a]. 1982.

Vine Status of Seed Source	Number of Seeds[b] Germinated	% Seeds Germinated	% Seedlings[c] PRMV-Infected
Diseased vines[d]	15/2100	0.71	0.0
Healthy vines [d]	5/2100	0.24	0.0
Diseased vines[e]	2/2100	0.10	0.0
Healthy vines [e]	3/2100	0.14	0.0

[a] Mean A405nm reading of healthy seed lots = 0.07, diseased seed lots = 0.15-0.96. Positive values are greater than the mean plus three standard deviations of the healthy control (A_{405nm} = 0.08)

[b] Seeds not stratified, were planted in a 1:1 (v/v) soil/sand mixture. Seeds germinated after 2 months.

[c] Percent of seedlings PRMV-infected. Tested by enzyme-linked immunosorbent assay (ELISA). No samples were positive.

[d] Seeds from fruit that were not heat-treated.

[e] Seeds obtained from fruit that were heat-treated at 60 C for 2 hours.

Table 23. Comparison of Yield and Percent Sugar Content between Mature Healthy and Peach Rosette Mosaic Virus-Infected cv. Concord Grapevines. Lawton, MI [a]

Vine Number	Vine Status	Weight/Vine 1981 (gm)	Percent Soluble[b] Solids (Brix)	Weight/Vine 1982 (gm)
1	Diseased	11,113.2	16.0	4,938.0
2	Diseased	1,587.6	16.0	1,499.0
3	Diseased	5,125.7	18.5	6,217.0
4	Diseased	2,494.8	17.5	1,272.0
5	Diseased	2,857.7	16.0	4,014.0
6	Diseased	4,717.4	16.5	3,302.0
7	Diseased	4,854.3	17.0	1,540.0
8	Diseased	2,177.3	17.0	4,095.0
9	Diseased	3,764.9	17.0	7,074.0
10	Diseased	4,082.4	15.0	2,440.0
Mean Value		4,277.5	16.7	3,640.1
11	Healthy	7,030.8	16.5	11,109.0
12	Healthy	4,853.5	16.5	4,810.0
13	Healthy	7,620.5	16.5	11,918.0
14	Healthy	10,659.6	16.5	6,310.0
15	Healthy	12,700.8	15.0	9,971.0
16	Healthy	8,663.8	16.0	9,785.0
17	Healthy	5,216.4	15.5	4,982.0
18	Healthy	4,263.8	15.0	8,921.5
19	Healthy	6,395.8	17.0	11,980.0
20	Healthy	8,527.7	16.0	13,885.0
Mean Value		7,593.3	16.1	9,367.4

[a] Vines tested for PRMV by enzyme-linked immunosorbent assay (ELISA) in 1981. Mean yield of healthy is significantly different from mean yield of diseased vines ($P=0.05$) by Students' T-test.

[b] Percent sugar content (Brix) of mature grape samples determined by refractive measurements. 1981. Non-significant differences between healthy and diseased vines determined by Students' T-test.

vine. The yield from diseased vines in 1982 was between 7074.0 and 1272.0 gm with a mean weight of 3640.1 gm. In comparison, total fruit yield (total of 10 vines) of healthy vines was between 12,700.8 and 4263.8 gms with a mean of 7593.3 in 1981 and between 13,885.0 and 4810.0 gm with a mean yield of 9367.4 in 1982. In most cases, yield estimates were made from the same vines in 1981 and 1982, except where vines displayed obvious nutrient deficiencies. Significant differences ($\underline{P}$= 0.05) between the mean yield of the 10 healthy and 10 PRMV-infected vines was determined by Students' t-test for unpaired plots data. These values represent a total decrease in yield of 56.3% in 1981 and 38.9% in 1982 of PRMV-infected grapevines, compared to healthy grapevines.

The effects of virus infection on sugar production in mature berries on infected vines was determined in 1981 by refractive measurements of juice from crushed berries (Table 23). The sugar content varied between 15.0 and 18.5 % brix with a mean of 16.7 for samples from diseased vines and 16.1 brix from healthy vines. These values are not significantly different from each other at the 5 % level ($\underline{P}$ = 0.05).

D. DISCUSSION

Indexing of a cv. Concord vineyard infected with PRMV, over a three year period, demonstrated an elliptical pattern of spread characteristic of several nepovirus diseases. From the 165 vines indexed, 32 were positive for PRMV in 1981, 22 new vines or 52 total vines were infected in 1982, and 9 new vines or 61 total vines were diseased in 1983. While most newly infected vines were located near previously diseased vines, isolated vines, away from the main area of infection, were also detected. These vines were located within some of the rows containing a succession of

PRMV-infected vines. Movement of soil, containing viruliferous **X. americanum** or infected roots, down the row during cultivation practices may play a role in establishing new areas of virus infection. For example, a mechanical grapehoe moves the top 5-10 cm. of soil, were grape roots and nematodes may be located. It should be mentioned, however, that chemical weed control has almost completely replaced the grapehoe.

Peach rosette mosaic virus infection in mature cv. Concord grape seeds varied from 0.39 to 82.0 %. This variability in seed infection was observed whether the seeds were from heat-treated or nonheat-treated fruit from infected vines. Virus was detected in both untreated and surface disinfected seed. The virus was not transmitted to **C. quinoa** indicator plants when inoculated with seed-halves of which the other half tested positive for PRMV by ELISA. Seeds from PRMV-infected vines mixed with seeds from virus-free vines were detected at all time intervals sampled from a commercial processing plant in 1981 and 1982. The number of seeds infected varied for each time interval and did not decrease uniformily with increasing time of sampling in 1981. In 1982, the number of sampling times were reduced and the number of PRMV-infected seeds decreased over time. Therefore, PRMV-infected seeds have been found in seed lots from processed grapes and may be spread to vineyards where seeds are used as mulch.

The variation in seed transmission of many viruses may be affected by several factors e.g. host variety and susceptibility, pathogenicity of the virus strain, temperature, and time of infection. Smith and Hewitt (193) reported that the extent of bean common mosaic virus in 51 bean varieties (**Phaseolus vulgaris** L.) varied from 1.0 to 75.0 percent. They also found a correlation between severity of symptoms produced and seed transmission,

with those varieties exhibiting severe symptoms having high seed infection.

In most cases reported (11,193), true seed transmission of plant viruses requires that the virus be capable of infecting and surviving in the embryo of the host. Preliminary results of assays of seed-parts demonstrated that PRMV is found throughout the mature seed. Virus was detected in 78 % of the embryos and 44 % of the remaining seed-parts containing the seed coat and endosperm. Transmission to the developing embryo would most likely occur through systemic infection of the seed-bearing plant. Peach rosette mosaic virus was not detected in **C. quinoa** plants inoculated with pollen from virus-infected cv. Concord grapevines (175).

Grape seeds germinated poorly under the conditions tested. Peach rosette mosaic virus was detected in only 2.7 % of seedlings from seeds heat-treated in the laboratory at 60 C. The germination rate of heat-treated seeds did not appear to differ significantly from that of untreated seeds collected in the field. In addition, no significant difference in the germination rate between healthy and diseased seed, from either stratified or unstratified seed lots, was observed during this study. None of the seedlings that assayed positive for PRMV by ELISA produced symptoms when inoculated onto C. **quinoa**. This is in contrast to the results obtained by Ramsdell and Meyers (175), where 10.5 % (4/38) of the seedlings of seeds from PRMV-infected vines produced symptoms on C. **quinoa**.

Results from these studies indicate that while PRMV is capable of invading the embryo, probably through infected maternal tissue, infectivity may be lost at the stage of seed maturation and dehydration. Southern bean mosaic virus was detected by Cheo (30) and Crowley (45) in a

high percentage of embryos of infected immature bean seeds, but only in the seed coats of mature seeds. Bowers and Goodman (13) observed inactivation of soybean mosaic virus as soybean seeds matured and dried. Porto and Hagedorn (170) showed that viral antigens could be detected after infectivity was lost. In the majority of virus diseases, virus particles are associated with the seed coat or nucellus and only a small number of embryos of infected plants contain virus (45). Several hypotheses have been proposed for the physical or chemical exclusion of virus from the developing embryo. Cheo (30) proposed an inactivating substance present in the soybean seed, acting to exclude soybean mosaic virus from invading the embryo. Caldwell (23) suggested that an acceleration in the growth rate of the development of the embryo and surrounding tissue would exclude transmission of the virus by disrupting protoplasmic connections with the infected maternal tissue. Several plant viruses, including members in the nepovirus group are able to infect meristematic tissue of the host by overcoming the inhibitory activity during protein synthesis. These viruses are capable of competing with the host cell for phosphorylated compounds (23). While PRMV may be capable of invading the embryo, it may not be able to compete with the host cell for ribosomes and substrates necessary for replication. Further experiments on virus activity within the developing embryo would give insight into the mechanisms involved in PRMV inactivation in the seed and developing seedling.

Peach rosette mosaic virus has a marked effect on the development of the fruit bunch on infected vines. Most of these bunches exhibit a condition known as "berry shelling", where berries are small or underdeveloped and may abscise prematurely. Infection by PRMV, results in

more than a 40 % decrease in yield, but does not appear to affect the sugar content of the fruit. Results from these experiments indicate that PRMV is capable of invading the seed of cv. Concord grapes, but does not survive maturation of the seed or germination under laboratory conditions. These results were different than that obtained by Ramsdell (175), where PRMV was found in four out of thirty-eight seedlings tested. It would be of interest to see if seedlings germinated under field conditions, contained infectious virus particles. Heat-treatment does not appear to affect germination of treated seeds although only a low rate of germination was observed. Peach rosette mosaic virus has been detected in seedlings of infected dandelion plants (175) commonly found in vineyards. These weeds may act as the source of hold-over inoculum or primary inoculum for PRMV in cv. Concord vineyards.

CHAPTER IV. DELIMITING AREAS OF VIRUS INFECTION IN VINEYARDS AND BLUEBERRY FIELDS IN MICHIGAN USING REMOTE SENSING.

A. INTRODUCTION

Remote sensing techniques have been employed in various areas of land management (69,133,159,167,185) for the detection of diseases and insect damage (31,36,124,183). Detection by remote sensing of field crop diseases incited by leaf rusts and blights (36,115,124,125) has been most successful. While detection of plants exhibiting visible signs or symptoms of infection has been achieved, limited success has been obtained with previsual detection, i.e. before symptom expression. Manzer and coworkers (141) were successful in detecting late blight of potato incited by **Phytophthora infestans** by aerial photography 1 to 3 days prior to symptom expression. Burns et al (19) detected tomato ringspot virus infection in snapdragon only a day before symptoms were visible.

Plant response to infection or stress is expressed by either a morphological or physiological change, but is usually due to a combination of both. Morphological alterations may be brought about by a change in leaf orientation or shape due to defoliation, growth reduction or dieback of the main branches. Interference with the normal physiological processes of the plant often results in decreased photosynthesis brought about by chloroplast degeneration and or interruption of the translocation of solutes. Both responses may ultimately alter the amount and type of incident solar radiation that is reflected by the plant canopy. Several models have been proposed to measure and predict changes in the spectral reflectance of plant canopies due to disease (172,184,194,195). Safir et al (184) used a field portable spectroradiometer to detect spectral

differences between healthy and blighted **Helminthosporium maydis** corn plants.

The detection of virus diseases of annual crops has been studied recently (8,112,214) while virus diseases of perennial crops, particularly those of small fruit crops has received little attention. Three serious virus diseases, two infecting highbush blueberry and one infecting **Vitis labrusca** L. cv. Concord grape have resulted in considerable economic loss to the fruit growing industry in Michigan. The cultivar Jersey is one of the most susceptible highbush blueberry cultivars to both BBLMV and BBSSV. Fifty-six percent of the commercial blueberry plantings are of the cultivar Jersey. Peach rosette mosaic virus (PRMV) incites a serious disease in cv. Concord grape and has been detected serologically in over 27 vineyards in southwestern Michigan.

All three diseases induce morphological and physiological modifications in their hosts. Symptoms on BBSSV-infected blueberries are most visible as a reddish, strap-like appearance of the leaves (177) (Figure 7). Blueberry leaf mottle virus induces a mottling and deformity of the leaves along with a general dieback of the main branches (181) (Figure 8). In the cv. Concord grape, leaves on PRMV-infected vines appear cupped and leathery, with a noticable flattening of the leaf sinus (174) (Figure 9). Cane growth is crooked and internodes are shortened. Symptoms induced on susceptible hosts by all three causal viruses are not uniformily visible on the plant, but appear to be affected by environmental and cultural practices. Often, plants may be infected but symptoms may not be expressed for several years.

All three of these virus diseases are readily vectored in the field. An increase in the number of infected fields and the resultant economic

Figure 7. Reddish, strap-like leaf symptoms on cv. Jersey highbush blueberry bushes infected with blueberry shoestring virus (BBSSV).

Figure 8. Leaf mottling and deformity of cv. Jersey highbush blueberry bushes infected with blueberry leaf mottle virus (BBLMV).

Figure 7

Figure 8

Figure 9. Symptoms induced on cv. Concord grapevines by peach rosette mosaic virus(PRMV) appear as a flattening of the leaf-sinus and downward curling of the leaf edges.

Figure 9

loss, makes early detection important. Control measures include roguing infected plants and pesticide treatment.

The use of remote sensing in the detection of plant virus diseases is two-fold. It allows for a cost-effective method to rapidly survey entire fields for crop management and it may also delineate areas of infection. Therefore, the development of an inexpensive, rapid and accurate method to differentiate between healthy and virus-infected plants has many benefits. The approach in the following experiments was to detect spectral and geometric alterations due to infection, in plant canopies, using high and low altitude photography and field spectrophotometric recordings at various phenological stages over a two year period.

B. MATERIALS AND METHODS

1. Ground Truth

To verify spectral differences observed using various remote sensing techniques, areas within blueberry fields and vineyards, containing virus infected and healthy plants were selected in southwestern Michigan. A field containing 450 blueberry bushes was ELISA indexed for the presence of blueberry leaf mottle virus infection during 1981 and 1982. Two plots, one containing 1200 (Field A) and the other 714 (Field B) 25-year-old highbush blueberry plants were assayed for BBSSV in Ottawa county. Concord grape vineyards located in Van Buren county were surveyed for PRMV infection. In vineyard A, (near Lawton, MI.) 375 vines were ELISA tested, while 700 vines were tested in vineyard B (near Mattawan, MI.). All plants within experimental plots were indexed in the beginning of the season, prior to surveying and were subject to similar environmental and cultural practices.

2. High Altitude Photography

Aerial photographs of experimental plots were obtained of previously mapped areas at various phenological stages during the 1981 and 1982 growing season. Color, black/white infrared (IR) and color infrared (CIR) film (Eastman Kodak Company, Rochester, N Y 14650) were used. Two 500/ELM Hasselblad camera systems (Victor Hasselblad Inc., Fairfield, New Jersey, 07006) were fastened onto the wing in 1981 (Figure 10) and attached to a belly-mount in 1982, of a Cessna Skyhawk aircraft. A 70 mm film format was used with CIR, film type 2443, with an 80 mm lens and Wratten no. 12 (minus blue) filter to enhance color differences in the near infrared (700-900nm) wavelengths. In 1981, 70 mm Ectachrome type 64 color film was placed in the second camera. An 80 mm lens with a skylight 1A clear filter was used to reduce ultraviolet radiation (UV) and improve haze penetration at high altitudes. Black/white IR film type 2424 with a Wratten no. 25 red filter and 80 mm lens was used in the second camera during 1982. Imagery of field plots was acquired at approximately 305 m above ground level (AGL) within one or two hours of solar noon on the particular date. Field plots were sighted and lined-up with the camera lens using a 14.5 cm^2 prism mounted onto one camera. Repeated, overlapping exposures were acquired simultaneously for both cameras. Negatives of aerial photographs were examined for spectral differences by stereoscopic examination using a Bausch & Lomb Zoom 240 (Bausch & Lomb Company, Rochester, NY 14692).

Figure 10. Aerial photographs were taken 305 meters above the field or vineyard using two Hasselblad cameras mounted onto the wing of a Cessna Skyhawk aircraft.

Figure 11. Spectral reflectance of the plant's canopy was recorded and stored in the Spectron SE-390 microprocessor for data analysis.

Figure 10

Figure 11

3. Low Level Imagery

A. Tower Photography

Low level imagery of individual virus-infected and healthy blueberry bushes or grapevines was obtained in 1981 and 1982 using a Minolta 35 mm camera (Minolta Corp.Inc., 101 Williams Drive, Ramsey, N J 07446) mounted from a 4.6 m hydraulic tower. Color (Ektachrome 200), Cir (Ektachrome 100) and multispectral 70 mm (B/W IR, Ektachrome 2424) photographs were taken of paired diseased and healthy bushes or vines. A Wratten No. 12 filter was used for all 35 mm CIR photography. Vertical and angled photographs were taken at various exposure settings during the phenological stages of full bloom, petal fall, fruit set and senescence. Only BBSSV-infected and healthy bushes and PRMV-infected and healthy vines were photographed.

The camera was mounted onto a telescoping aluminum pole, that extended out 3 meters above the center of the plant canopy. An angle finder was attached to the camera to maintain the desired angle. Pairs of infected and healthy plants were pre-selected, based on morphological similarities and subject to the same environmental conditions.

Optical density values were determined after development of exposed film with a color densitometer (Speedmaster, ESCO Company, Cushing OK, 74023). The density of the film dye layers was obtained by passing white light through either a red, green or blue filter. Shaded parts or bright spots were avoided on the film. A mean density of four readings was obtained for each photograph scanned.

B. Spectroradiometric Determinations Using a Field-Portable Spectroradiometer

1. Spectral Reflectance of Virus-Infected Plants

Most plants, growing under normal non-stressed conditions, will reflect more incident radiation in the near-infrared (700-900 nm) than in the visible wavelengths (400-700 nm) (73). Stressed plants or plants altered by disease may respond by a decrease in reflected radiation which may be too subtle for detection by near-infrared sensitive film. To detect small reflective differences between healthy and virus-infected plants directly, radiometric measurements were taken during 1981 and 1982, using a field-portable spectroradiometer. The spectron SE590 (Spectron Engineering Co., Denver, CO. 80204) instrument measures spectral reflectance simultaneously at 256 wavelengths between 400 and 1100 nm and records these spectral readings on a magnetic tape over a 15 second period (Figure 11).

Data collected was stored and analysed with the SE390 microprocessor connected with the spectroradiometer. Spectral readings of the plant canopy were recorded 2.5 m. above and perpendicular to the plant, using a lens with a 10 degree field of view (FOV) (Figure 12). Infrared photographs taken either prior to or following the spectral scan, served to demonstrate the area on the plant that was scanned. Reflectance ratios of electromagnetic radiation, radiating from the subject to the amount of incident solar radiation on the plant subject, were obtained by normalizing readings to a barium sulfate standard reflectance panel (Figure 13).

Figure 12. Spectral recordings were taken with a spectro-radiometer lens attached to a telescoping aluminum pole, mounted onto a tower 3 meters above the plant canopy.

Figure 13. Spectral reflectance readings of the plant canopy were ratioed to the incident solar radiation measured from a barium sulfate ($BaSO_4$) standard reference panel.

Figure 12

Figure 13

2. Barium Sulfate Reference Panel

Construction of a barium sulfate ($BaSO_4$) reference panel was performed to measure the available solar radiation incident upon the plant canopy. The aluminum panel measured 1.22 m. by 1.22 m. by 0.32 cm. thick and was supported with 3.8 by 3.8 cm. welded aluminum angle irons (6061-T6 aluminum). A highly reflective coating of barium sulfate was applied by spraying the panel twice at a 15 degree angle of tilt, according to the methods of Shai et al (190).

Preparation of the reflective $BaSO_4$ coating was divided into two procedures. The first procedure involved the addition of 3 gm polyvinyl alcohol (99% Hydrolyzed, hot water soluble) to 72.0 gm (0.4 %) heated (90 C) distilled water. Polyvinyl alcohol solids were added slowly to the hot water and kept under constant agitation for 15 min. to prevent agglutination and precipitation. Following the complete solvation of the polyvinyl alcohol, the solution was allowed to cool slowly to room temperature. The solution was kept under continual agitation during this process.

In the second procedure, 125 gm of distilled water was mixed with 125 gm (17.2%) of absolute ethyl alcohol. Once the polyvinyl alcohol-water solution reached room temperature, the second portion containing the alcohol-water mixture was slowly added and blended for 2 min. to prevent dissolution of the polyvinly alcohol. It was critical that the mixture was not allowed to become heated. With constant agitation, 400 gm (55.2%) of the barium sulfate powder (Eastman Kodak Company, Rochester, N Y 14650) was added to the mixture. Approximately 15 min. was required to mix the coating solution completely.

Prior to the application of the $BaSO_4$ coating solution, the aluminum panel was primed with an acrylic flat -white paint to prevent the diffusion and reaction of residual bisulfate or sulfuric acid with the aluminum metal or the metal ions diffusing toward the coating.

In the field, the $BaSO_4$ reference panel was mounted to a balanced tripod approximately 1.8 m above the ground, on the top of a pickup truck cab (Figure 13). Incident solar radiation was recorded prior to scanning plant subjects, at the end of each session (1 hour later) and during periods of solar fluctuation due to cloud interference.

C. Results and Discussion

1. Ground Truth

Field maps of plants tested by ELISA were constructed for five commercial fields during the growing seasons in 1981, 1982 and 1983 to be used as ground truth for aerial and tower photographs as well as for the spectral scans. Two highbush blueberry fields containing bushes infected with BBSSV were indexed in Ottawa county. A section in Field A (Figure 5) containing 1200 25-yr-old bushes was photographed. Seventy of these bushes (5.8 %) were infected with BBSSV, 40 (3.3 %) were detected by ELISA in 1981 and 30 (2.5 %) additional bushes in 1982. Generally, infected bushes appeared to be randomly distributed within this section of the field, but several diseased bushes were adjacent to other infected bushes in the row. However, Lesney et al (132) showed the disease to be spread in a non-random manner and primarily down the row by the blueberry aphid, **Illinoia. pepperi** (MacGil.). Therefore, this area may represent a relatively newly infected section of the field where viruliferous wind-blown apterous or alate aphids have fed and infected the bush. Severely infected bushes

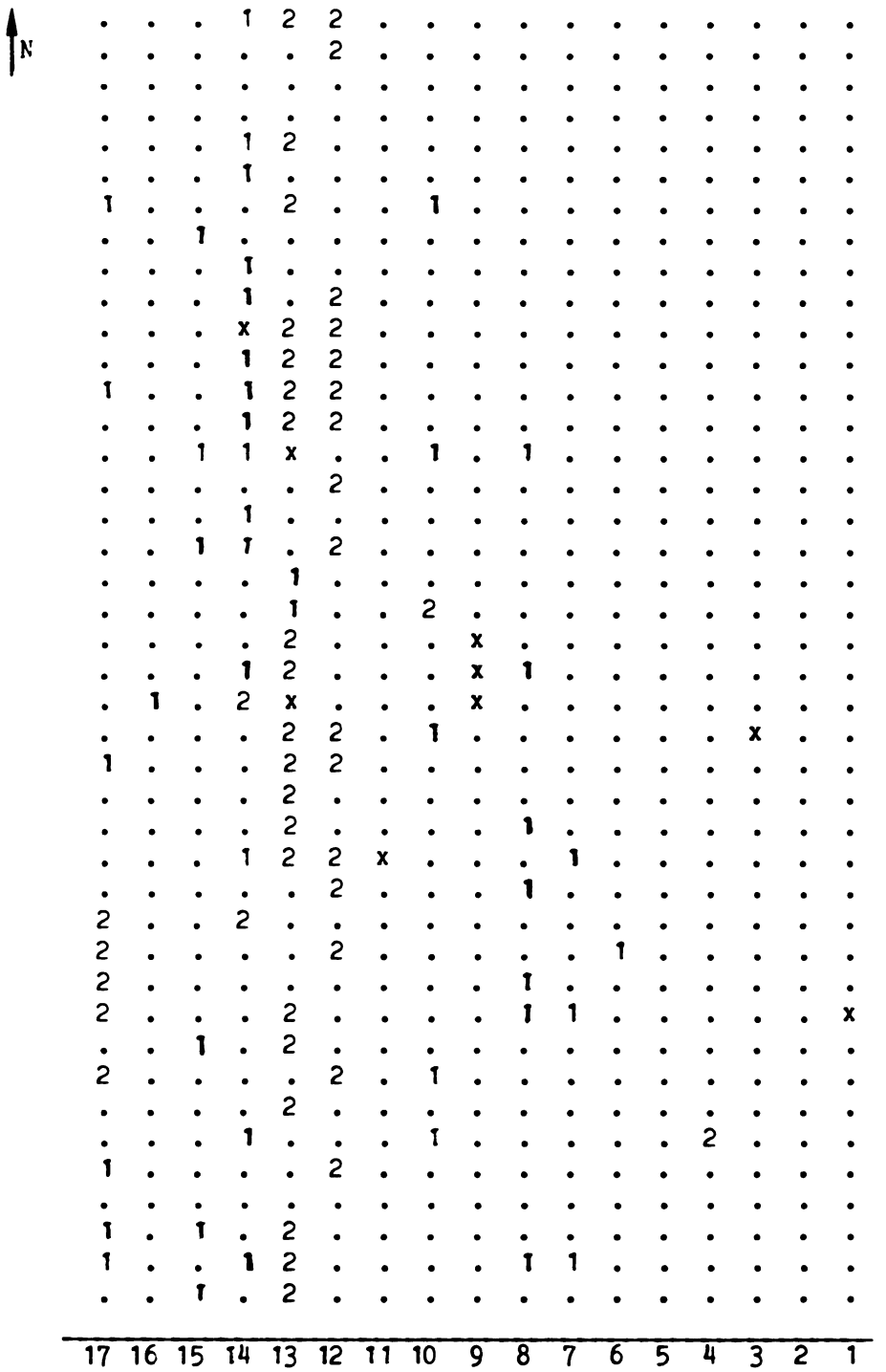

Figure 5. Ground Truth For Remote Sensing and Pattern of Spread of Blueberry Shoestring Virus (EBSSV) in cv. Jersey Blueberry, Indexed Over a Two-Year Period by ELISA. . = healthy, x = missing, 1,2 = indexed positive for BBSSV in 1981 and 1982, respectively. Coopersville, MI.

were stunted, showed decreased vigor and symptoms were noticable on the shoots and leaves. However, symptoms were not uniformily exhibited on these infected bushes.

A section containing 714 cv. Jersey blueberry bushes in Field B (Figure 6), was surrounded by other blueberry cultivars not as susceptible to EBSSV as is Jersey. Within this section, 92 (12.9 %) of the bushes were infected; 46 were detected in 1981 with an additional 46 in 1982. Several BBSSV-infected bushes followed in succession within heavily infected rows. This field typified the bush-to-bush pattern of spread characteristic of the blueberry aphid behavior described by Elsner (61) and Morimoto (153). Unlike field A, where severely infected bushes were obvious, bushes infected in field B did not exhibit reduced vigor, nor were symptoms as noticable on the leaves.

One highbush blueberry field severely infected with BBLMV was indexed in 1982 and 1983 (Figure 2E). Twenty-three bushes (5.1 %) tested positive for BBLMV by ELISA in 1982, while only two additional diseased bushes were detected in 1983. However, at the end of the 1982 growing season, approximately 117 bushes had been cut off by the grower within this area, precluding getting an accurate test of spread thereafter. Several bushes had been cut before field indexing in 1981, and regrew to some extent thus, allowing them to be tested for BBLMV. Many bushes displayed symptoms characteristic of the disease. Infected bushes appeared at random within the field, except in areas of extensive BBLMV-infection where blocks of diseased bushes were observed. Mottling symptoms were more noticable during the early or late parts of the growing season. Infected bushes exhibited the characteristic dieback of the main stems and the canopy appeared more open, displaying more of the ground cover in comparison to healthy bushes.

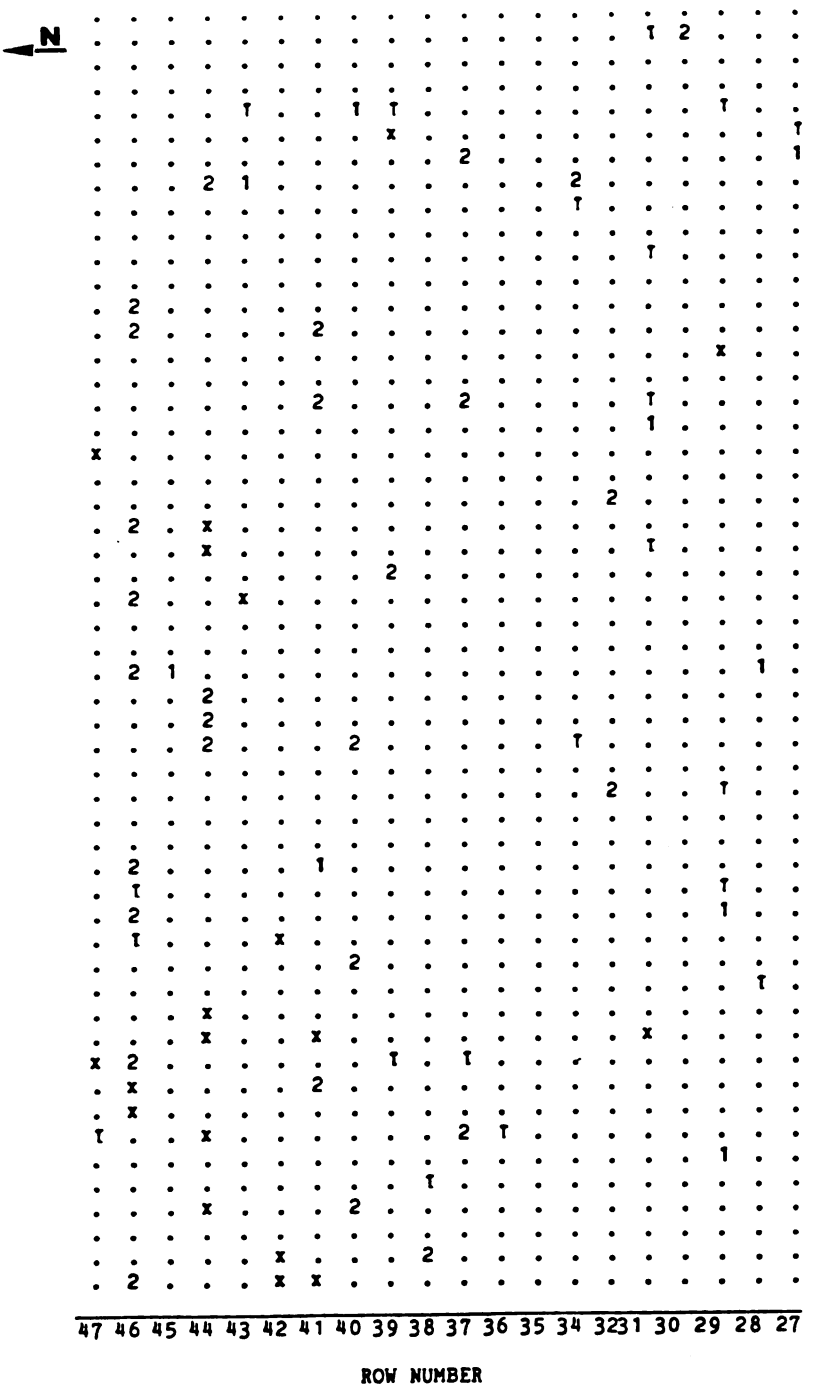

Figure 6. Ground Truth for Remote Sensing and Pattern of Spread of Blueberry Shoestring Virus (BBSSV) Infection in a cv. Jersey Blueberry Field, Indexed Over a Two-Year Period by ELISA. Nuncia, MI. . = healthy, x = missing and 1,2 = indexed positive for BBSSV in 1981 and 1982, respectively.

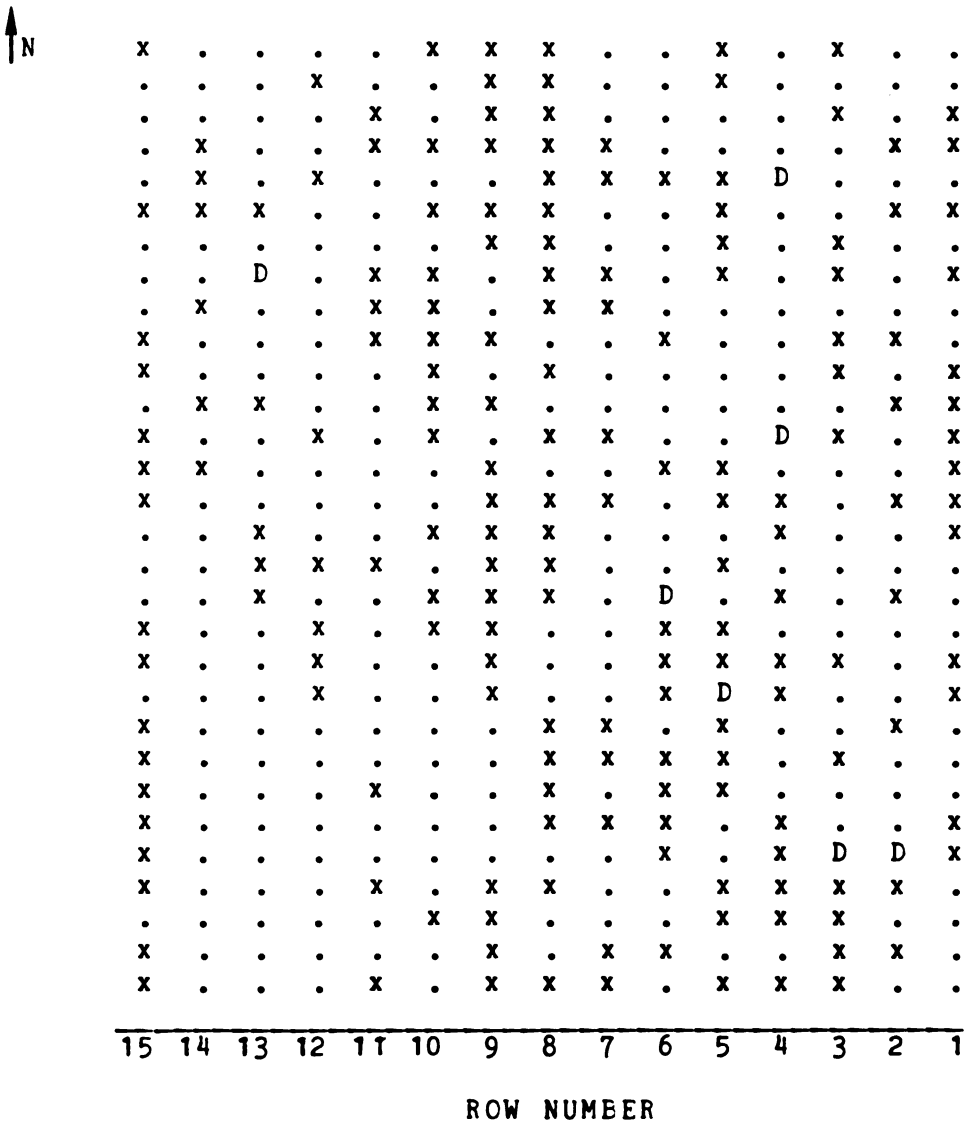

Figure 2E. Sections of a highbush blueberry field ELISA-indexed for ground truth for remote sensing and to determine the spread of blueberry leaf mottle virus (EBLMV), Agnew, Michigan 1983. D=diseased, x = missing, . = healthy.

Peach rosette mosaic virus (PRMV) was detected by ELISA in two cv. Concord grape vineyards located in Van Buren county. In vineyard A, near Lawton, MI. (Figure 3B), thirty-two (8.5 %) of the 375 vines were positive for PRMV-infected in 1981. An additional 23 (14.7 % total) were detected in 1982 and 9 new vines (17.1 % total) in 1983. Spread of PRMV within this field was not random and an elliptical pattern of spread was observed. As stated in chapter III, the pattern of spread was characteristic of the slow progression of a nematode transmitted virus. Newly infected vines appeared in the forefront of the diseased area but isolated PRMV-infected vines were also observed. Vineyard B (Figure 4B) located near Mattawan, MI. was ELISA-indexed in 1982 only. This field was chosen for aerial photography because of the extensive area of continuously infected vines. Of the 700 vines assayed, 312 vines (44.6 %) were infected with PRMV.

2. High Altitude Photography

In 1981, aerial photographs were taken perpendicular to and at a height of 305 m (AGL) above the five field plots on four dates; 1 June, 26 June, 30 July, and 9 October. High altitude photographs were obtained 23 June, 29 July, 25 August and 8 September in 1982. These dates were chosen to approximate the phenological stages of blossom, fruit set, harvest and scenescence within the fields and vineyards.

The standard exposure was 1/500 of a second at an f-stop of 5.6 but varied between 8 and 5.6 without the filter factor. At 305 m, this provided a final scale of approximately 1:3810 (1 mm on the film represents 3.8 m on the ground). After film development, the Color, Cir and B/W IR transparancies were observed stereoscopically using a Bausch

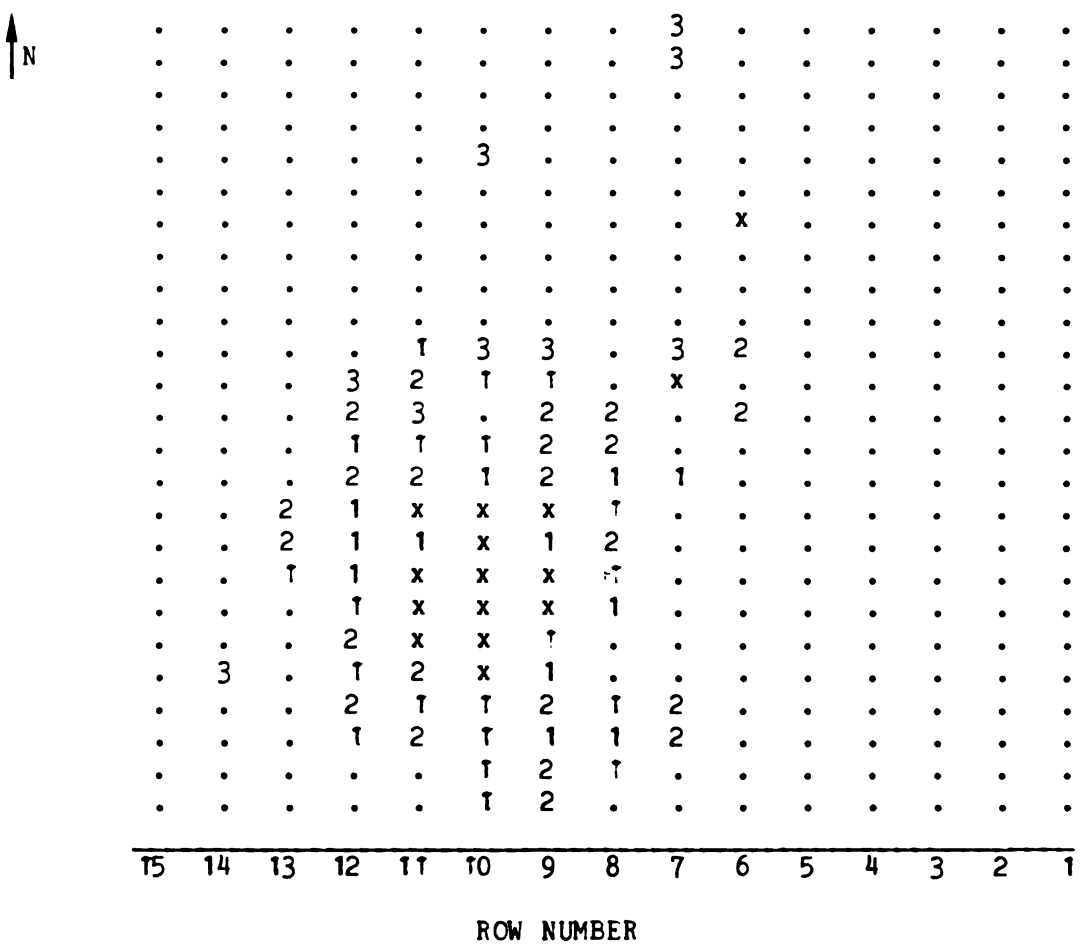

Figure 3B. Sections of a cv. Concord vineyard ELISA-indexed for ground truth for remote sensing and to determine the pattern of spread of peach rosette mosaic virus (PRMV). . = healthy, x = missing and 1,2,3 = date vine was ELISA positive for PRMV in 1981, 1982 and 1983, respectively. Lawton, MI

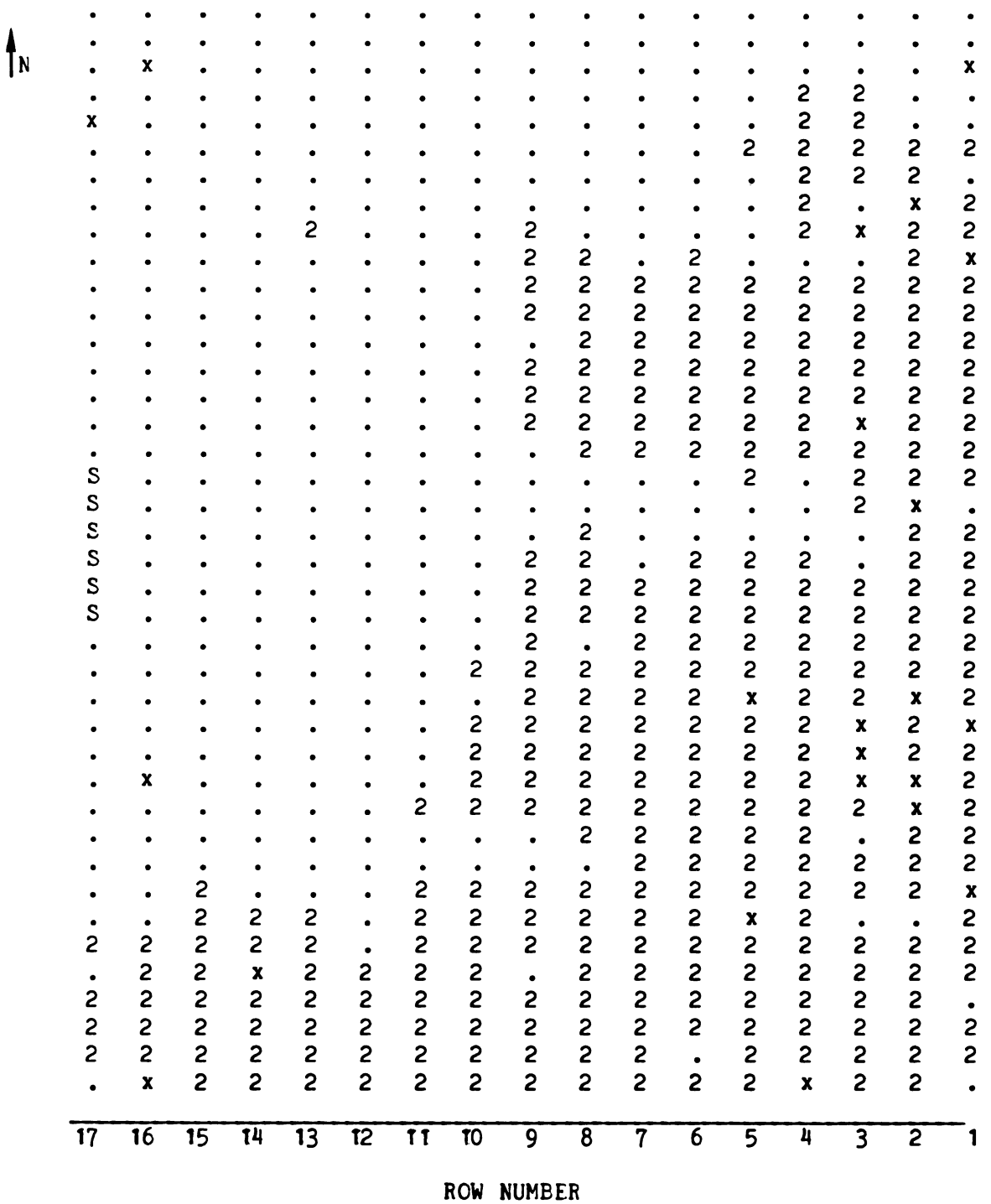

Figure 4B. Sections of a cv. Concord grape vineyard ELISA-indexed for ground truth for remote sensing and to determine the pattern of spread of peach rosette mosaic virus (PRMV). . = healthy, x = missing vine, S = space in field and 2 = date vine was positive for PRMV, tested by ELISA in 1982. Mattawan, MI

and Lomb 240 stereomicroscope with a 10 X lens and mounted onto a Richards light table.

Subtle changes were observed on the Color and CIR transparencies throughout the growing season. Color differences were more obvious on Color film (Figures 14,15,16) than CIR (Figures 17,18,19). However, these variations in reflectance were noticable on healthy as well as virus-infected plants, and probably represent differences in growth stages within and among the plants observed. Unlike field crops where the growth stage within the field is relatively uniform, the stages of growth on small fruit crops represent a non-uniform process, with shoots and leaves developing at different times. Healthy leaves will maintain their green or true color on regular color film but will be reddish-magenta on CIR film. Young leaves reflecting more infrared radiation than older leaves will exhibit a darker magenta color on CIR film. The overall hue of a blueberry bush canopy will therefore appear as a mosaic of light and dark shades of magenta. Dead branches or bushes that have lost their chlorophyll appeared yellow on CIR film, but were not correlated with virus infection.

Variations in the geometry of the plant canopy were more noticable on B/W IR transparencies than on either Color or CIR film. In addition, the interference due to weed or soil reflectance was reduced with this type of film. These geometric differences were particularly noticable in vineyards, early in the season before the canopy had fully expanded. No correlation with virus-infection and a change in canopy geometry was observed.

From the aerial data collected, it would appear that differences in spectral reflectance or geometric changes in these five plots were more a

Figure 14. Aerial Color photograph taken 305 meters above a blueberry shoestring virus (BBSSV)-infected cv. Jersey blueberry field. Coopersville, MI 1981.

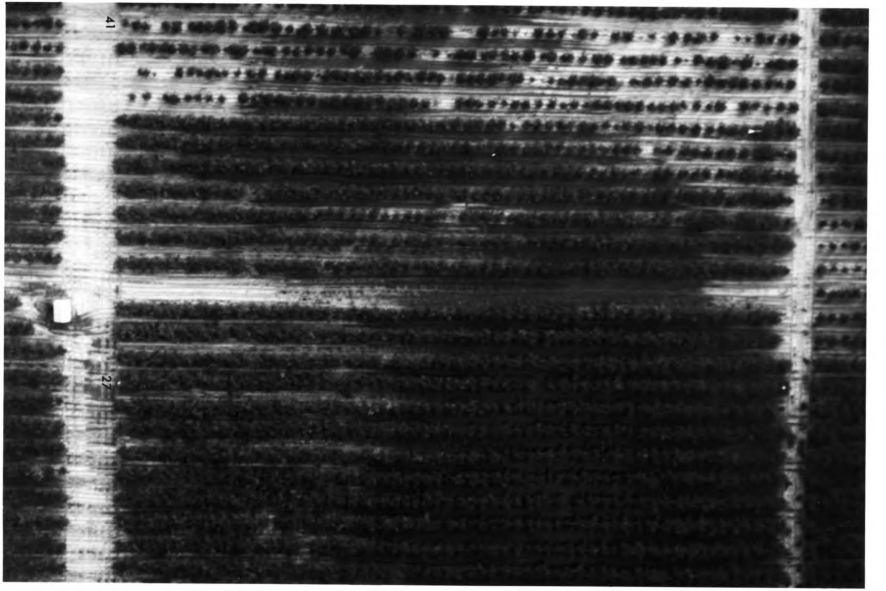
41
12
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Figure 15. Aerial Color photograph taken 305 meters above a blueberry leaf mottle virus (BBLMV)-infected cv. Jersey highbush blueberry field. Agnew, MI 1982


N

Figure 16. Aerial Color photograph taken 305 meters above a peach rosette mosaic virus (PRMV)- infected cv. Concord grape vineyard. Lawton, MI 1981.


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Figure 17. Aerial Color infrared (CIR) photograph taken 305 meters (AGL) above a blueberry shoestring virus (BBSSV)-infected cv. Jersey blueberry field. Coopersville, MI 1981.

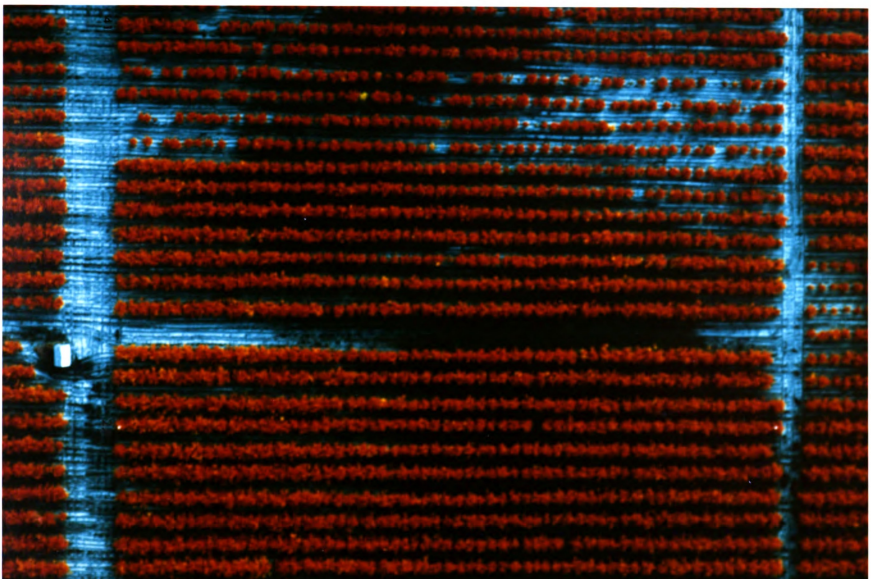
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Figure 18. Aerial Color infrared (CIR) photographs taken 305 meters above a blueberry leaf mottle virus (BBLMV)-infected cv. Jersey blueberry field. Agnew, MI 1982.

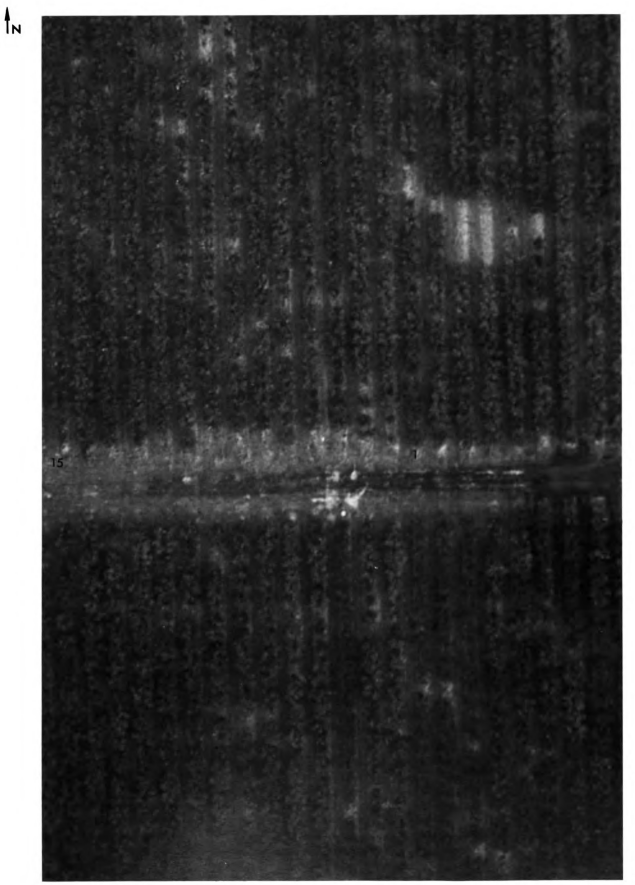
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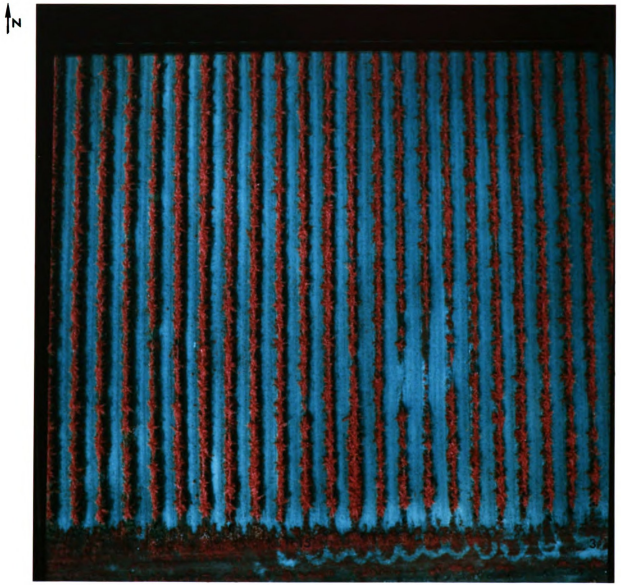
Figure 19. Color infrared (CIR) photograph taken 305 meters above a peach rosette mosaic virus (PRMV)-infected cv. Concord vineyard. Lawton, MI 1981.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result of environmental conditions (soil type, nutrients, etc.) and genotypic variation than virus infection. While these three diseases cause considerable economic loss, the symptoms are not uniform and infection does not result in rapid plant decline. Small reflective changes may occur, but are too subtle to be detected by high altitude photography. Manzer and Cooper (141) reported that symptoms of potato plants diseased with late blight, were more detectable by remote sensing techniques when observations were made within 10 meters of the subject. They concluded that while leaf curl symptoms were obvious within these 10 meters, beyond this distance, infected leaves were hidden by healthy leaf tissue in the foreground. Symptoms on highbush blueberry, resulting from infection by either BBLMV or BBSSV are more noticable on the more succulent shoots, located well within the crown of the bush. In many cases, diagnosis in the field is made only after close ground observations.

3. Tower Photography

Optical density values were obtained from Color and CIR film transparencies following their development. A density value was obtained for each dye layer; yellow, magenta and cyan, by passing white light through a blue, green or red filter, respectively. The filter allows the passage of only one particular wavelength of light through the transparency. The density value represents the amount of light transmitted through the dye layer. Density is the logarithm of opacity and the inverse of reflected radiation. The color densitometer measures values from 0.0 to 3.0 and has no units of measurement since it is a logarithmic value.

For a healthy green plant, most of the reflected light is in the green and infrared wavelengths, while most of the light in the blue and red region is absorbed by plant pigments. Therefore, most of the magenta dye forming layer (sensitive to green light) on color film would be exposed and would absorb most of the green light passed through the transparency. More of the blue and red light would be transmitted through the film since these layers would be less exposed. As a result, the density of the magenta layer would vary around zero depending on the amount of green light reflected by the plant. The densities for the yellow and cyan dye layers would be high and the resultant image on the photograph would appear green (blue plus red). A CIR photograph of a normal green plant would result in partial exposure of the yellow-forming dye layer (sensitive to green), smaller exposure of the magenta layer (sensitive to red) and much higher amount of exposure to the cyan dye layer (sensitive to infrared radiation). Due to the reversal process of CIR film, the resultant dye layers will be formed inversely to the exposure. Therefore the photograph of a green plant will appear red or reddish-magenta because more of the yellow and magenta dye formed (blue plus red). The yellow dye layer would have a medium density value, the magenta a high density value and cyan the lowest value. A stressed plant, exhibiting reduced infrared reflectance would appear darker magenta and the density would increase in the cyan dye layer. In contrast, increases in infrared reflectance would be observed as a lower density value in the cyan dye forming layer.

Density values were obtained from Color and Color-infrared transparencies of two healthy and two PRMV-infected cv. Concord grapevines on 29 July and 18 September. These dates represent approximately the phenological stages of late fruit set and maturity, respectively. Optical

density values were obtained using a 2 mm aperature size on the densitometer. Table twenty-four represents density values of the three dye layers for healthy and infected grapevines. Healthy control values were assigned a zero value or baseline value, as suggested by Murtha (157). Density values of diseased plants represent a positive or negative deviation (Delta "D") from the control value. These density values were obtained from transparencies with the most optimum exposure setting (1/250 sec., f. 2.5 to 5.6) and taken perpendicular to the vine or bush.

a. Peach rosette mosaic virus-infected grapevines

Density values for each dye layer varied from vine to vine on both types of film. Diseased vine number 1 exhibited a small negative deviation (Table 24) from the normal control vine in all dye layers on Color film, indicating an increase in reflectance (in all wavelengths). However, a positive deviation was observed for the other vine (number 2), indicating a decrease in reflectance. Reflectance for vine number 1 was higher in July than September, while reflectance for vine number 2 was the opposite, higher in September than July. Most density variations were observed in the red filter optical density layer (600-700 nm) on Color film. Again, in both July and September, vine 1 exhibited higher reflectance in this region (-0.32 and -0.05), while the opposite or decreased reflectance (0.58 and 0.41) was observed for vine 2.

On CIR film (Table 25), very little deviation from the healthy control in all dye layers was observed for PRMV-infected vine 1. Vine 2 exhibited a more positive deviation or decreased reflectance from the control. Little variation in reflected radiation for vine 1 was observed from 29 July to 18 Sept. Vine 2 reflected less radiation in July than in

Table 24. Density Values of Photographic Dye Layers from Color Photographs of PRMV-Infected and Healthy cv. Concord Grapevines. Lawton, MI. 1981.

Status[a] of Vine	Date[b] (1981)	Density of Photographic Dye Layers[c]		
		Yellow	Magenta	Cyan
1 Healthy	29 July	1.04 +/- 0.14	1.08 +/- 0.15	1.27 +/- 0.20
	18 Sept.	1.32 +/- 0.11	1.16 +/- 0.07	1.33 +/- 0.07
1 Diseased	29 July	0.81 +/- 0.10	0.78 +/- 0.08	0.95 +/- 0.12
	18 Sept.	1.28 +/- 0.32	1.16 +/- 0.40	1.28 +/- 0.33
D[d]	29 July	- 0.23	- 0.30	- 0.32
	18 Sept.	- 0.04	0.00	- 0.05
2 Healthy	29 July	1.22 +/- 0.04	1.15 +/- 0.03	1.36 +/- 0.03
	18 Sept.	1.44 +/- 0.09	0.97 +/- 0.14	1.15 +/- 0.15
2 Diseased	29 July	1.66 +/- 0.29	1.64 +/- 0.42	1.29 +/- 0.51
	18 Sept.	1.44 +/- 0.24	1.35 +/- 0.29	1.56 +/- 0.30
D[d]	29 July	0.44	0.49	0.58
	18 Sept.	0.30	0.38	0.41

[a] Leaf tissue was sampled at six locations on each vine (0.5gm) and tested for peach rosette mosaic virus (PRMV) by enzyme-linked immunosorbent assay (ELISA).

[b] Color photographs were taken 3 m above the vine canopy from a portable-tower, at fruit set (29 July) and fruit maturity (18 September).

[c] Density values of the yellow, magenta and cyan dye layers were obtained by passing white light through a blue, green or red filter respectively. Transmitted light was measured by a color densitometer with a 2 mm aperature. Density values represent the mean of four readings plus or minus the standard deviation.

[d] "D" represents the deviation of density values per dye layer of the diseased value from the healthy (0 base-line) values. Positive values represent a decreased reflectance from healthy, while negative values represent an increase in reflectance in the blue (300-400 nm), green (400-500 nm) and red (600-700nm) wavelengths.

Table 25. Density Values of Photographic Dye Layers from Color Infrared (CIR) Photographs of PRMV-Infected and Healthy cv. Concord Grapevines. Lawton, MI. 1981.

Status[a] of Vine	Date[b] (1981)	Density of Photographic Dye Layers[c]		
		Yellow	Magenta	Cyan
1 Healthy	29 July	1.02 +/- 0.08	1.20 +/- 0.08	0.81 +/- 0.08
	18 Sept.	0.91 +/- 0.15	1.10 +/- 0.16	0.81 +/- 0.11
1 Diseased	29 July	1.09 +/- 0.21	1.22 +/- 0.21	0.91 +/- 0.19
	18 Sept.	0.98 +/- 0.18	1.22 +/- 0.21	0.85 +/- 0.12
D[d]	29 July	0.07	0.02	0.10
	18 Sept.	0.07	0.12	0.04
2 Healthy	29 July	0.88 +/- 0.19	1.12 +/- 0.27	0.67 +/- 0.10
	18 Sept.	1.23 +/- 0.18	1.68 +/- 0.21	1.15 +/- 0.11
2 Diseased	29 July	1.16 +/- 0.46	1.42 +/- 0.63	0.99 +/- 0.37
	18 Sept.	1.23 +/- 0.19	1.53 +/- 0.29	1.04 +/- 0.18
D[d]	29 July	0.28	0.30	0.32
	18 Sept.	0.00	- 0.15	- 0.11

[a] Leaf tissue was sampled at six locations on each vine (0.05 gm) and tested for peach rosette mosaic virus (PRMV) by enzyme-linked immunosorbent assay (ELISA).

[b] Color infrared (CIR) photographs were taken 3 m above the vine canopy from a portable-tower at fruit set (29 July) and fruit maturity (18 September).

[c] Density values of the yellow, magenta and cyan dye layers were obtained by passing white light through a blue, green or red filter respectively. Transmitted light was measured by a color densitometer with a 2mm aperature. Density values represent the mean of four readings plus or minus the standard deviation.

[d] "D" represents the deviation of density values per dye layer of the diseased from the healthy value (0 base-line). Positive values represent a decrease in reflectance, while negative values represent an increase in reflectance in the green (400-500 nm), red (500-600 nm) and infrared (700-900 nm) wavelengths.

September in contrast, particularly in the red and infrared wavelengths. Again the most noticable deviation from the normal was observed in the cyan dye layer, with a higher density value representing a decrease in near-infrared radiation in June, but increasing in September. However, these trends were not consistently observed and may not represent changes brought about by infection.

The increase in N-IR reflectance in September, for vine 2, may be a result of moisture loss in the leaf mesophyll. This phenomenon has been observed on photographs comparing the reflectance of broadleaf and coniferous trees (D. Lusch, personal communication). An increase in reflected infrared radiation was observed late in the season on the broadleaf trees that had reduced moisture content in response to dormancy, resulting in a darker magenta color than the conifers. One would expect the opposite since conifers usually have a much lower moisture content than broadleafs.

b.Blueberry shoestring virus-infected blueberry bushes

Optical density values were determined for two virus-free and BBSSV-infected blueberry bushes. Values of transmitted light were obtained using a 4 mm diameter aperature, at three phenological stages: bloom (3 June), fruit maturity (31 July) and scenescence (18 September). Results of color photographs (Table 26) indicate a variation in spectral reflectance between bushes. Diseased bush number 1, exhibited a negative deviation from the healthy control in all dye layers, indicating decreased reflectance in the wavelength region 300 to 700 nm. A greater deviation is again obvious in the cyan dye layer, sensitive to reflected radiation in the 600 to 700 nm wavelenghs. A shift or increase in reflected red light would give these results. Also, an increase in reflected radiation

Table 26. Density Values of Photographic Dye Layers from Color Photographs of BBSSV-Infected and Healthy cv. Jersey Highbush Blueberry. Coopersville, MI. 1981.

Status[a] of Bush	Date[b] (1981)	Density of Photographic Dye Layer[c]		
		Yellow	Magenta	Cyan
1 Healthy	3 June	1.10 +/- 0.07	0.88 +/- 0.06	1.32 +/- 0.09
	31 July	1.29 +/- 0.09	1.17 +/- 0.09	1.31 +/- 0.11
	18 Sept.	1.25 +/- 0.13	1.14 +/- 0.17	1.23 +/- 0.13
1 Diseased	3 June	0.76 +/- 0.13	0.65 +/- 0.16	0.69 +/- 0.11
	31 July	1.15 +/- 0.22	1.09 +/- 0.22	1.15 +/- 0.23
	18 Sept.	0.92 +/- 0.13	0.92 +/- 0.12	0.99 +/- 0.11
D[d]	3 June	- 0.34	- 0.23	- 0.63
	31 July	- 0.14	- 0.08	- 0.16
	18 Sept.	- 0.33	- 0.22	- 0.24
2 Healthy	3 June	0.86 +/- 0.09	0.81 +/- 0.09	0.83 +/- 0.09
	31 July	1.10 +/- 0.08	1.00 +/- 0.10	1.12 +/- 0.10
	18 Sept.	0.94 +/- 0.16	0.84 +/- 0.15	0.89 +/- 0.16
2 Diseased	3 June	1.00 +/- 0.10	0.88 +/- 0.08	0.95 +/- 0.10
	31 July	1.04 +/- 0.14	0.97 +/- 0.16	1.06 +/- 0.15
	18 Sept.	0.97 +/- 0.12	0.87 +/- 0.13	0.94 +/- 0.12
D[d]	3 June	0.14	0.07	0.12
	31 July	- 0.06	- 0.03	- 0.06
	18 Sept.	0.03	0.03	0.05

[a] Leaf tissue was sampled from blueberry shoestring virus (BBSSV)-infected bushes at six locations on each bush (0.05 gm) and tested by enzyme-linked immunosorbent assay (ELISA).

[b] Color photographs were taken 3 m above the bush canopy from a portable-tower at bloom (3 June), fruit maturity (31 July) and senescence (18 September).

[c] Density values of the yellow, magenta and cyan dye layers were obtained by passing white light through a blue, green or red filter respectively. Transmitted light was measured by a color densitometer with a 4 mm aperature. Density values represent the mean of four readings plus or minus the standard deviation.

[d] "D" represents the deviation of density values per dye layer of the diseased from the healthy value (0 base-line). Positive values represent a decrease in radiation, while negative values represent an increase in reflectance in the blue (300-400 nm), green (400-500 nm) and red (600-700 nm) wavelengths.

in the blue region of the spectrum is indicated by lower density values for the same plant. As the reflected radiation increases in the 400 to 500 nm and 600 to 700 nm ranges the normal reflectance curve tends to flatten out. These results may demonstrate a change in the amount or function of the plants pigments and ultimately photosynthesis. The plant might appear light magenta in comparison to darker reddish-magenta of a healthy plant although this was not observed.

The variability in reflected radiation in all wavelengths and particularly the cyan dye layer was most noticable on the CIR transparencies of the two BBSSV-infected bushes (Table 27). In contrast to results obtained on the color film, bush number 1 exhibited little change from the healthy control. However, it did show increased reflectance over the healthy bush in September. The reverse was observed for bush number 2 on color infrared film. An increase in reflectance (more negative departure from healthy) in all dye layers occured early in the season, with little deviation from the healthy control as the season progressed.

In summary, reflective differences from healthy plants were observed for both PRMV-infected grapevines and BBSSV-infected blueberry bushes on Color as well as CIR photographs. The greatest deviation, whether positive or negative appeared in the early part of the growing season and in particular on the cyan dye forming layer of the film. These results would indicate changes in reflectivities in the 600 to 700 nm (red) and 700 to 900 nm (infrared) regions of the electromagnetic spectrum. Generally, a negative departure or lower density values was found, indicating an increase in reflectance. However, the greatest variability was observed between plants, and no consistent trend can be determined without a larger sample size.

Table 27. Density Values of Photographic Dye Layers from Color Infrared (CIR) Photographs of BBSSV-Infected and Healthy cv. Jersey Highbush Elueberry. Coopersvillé, MI. 1981.

Status[a] of Bush	Date[b] (1981)	Density of Photographic Dye Layer[c]		
		Yellow	Magenta	Cyan
1 Healthy	3 June	0.50 +/- 0.01	0.49 +/- 0.04	0.63 +/- 0.03
	31 July	1.03 +/- 0.19	0.73 +/- 0.06	1.24 +/- 0.26
	18 Sept.	1.20 +/- 0.29	0.94 +/- 0.23	1.36 +/- 0.30
1 Diseased	3 June	0.53 +/- 0.11	0.62 +/- 0.14	0.60 +/- 0.09
	31 July	1.07 +/- 0.21	0.88 +/- 0.24	1.30 +/- 0.18
	18 Sept.	1.05 +/- 0.10	0.83 +/- 0.04	1.21 +/- 0.15
D[d]	3 June	0.03	0.13	- 0.03
	31 July	0.04	0.15	0.06
	18 Sept.	- 0.15	- 0.11	- 0.15
2 Healthy	3 June	1.06 +/- 0.09	1.23 +/- 0.09	1.27 +/- 0.10
	31 July	1.13 +/- 0.29	0.90 +/- 0.17	1.35 +/- 0.33
	18 Sept.	1.00 +/- 0.24	0.73 +/- 0.20	1.18 +/- 0.27
2 Diseased	3 June	0.68 +/- 0.07	0.74 +/- 0.05	0.81 +/- 0.09
	31 July	1.22 +/- 0.25	0.84 +/- 0.20	1.48 +/- 0.25
	18 Sept.	1.08 +/- 0.10	0.79 +/- 0.05	1.20 +/- 0.09
D[d]	3 June	- 0.38	- 0.49	- 0.46
	31 July	0.09	- 0.06	0.13
	18 Sept.	0.08	0.06	0.02

[a] Leaf tissue was sampled at six locations on each bush (0.5 gm) and tested for blueberry shoestring virus (BBSSV) by enzyme-linked innunosorbent assay (ELISA).

[b] Color infrared photographs were taken 3 m above the bush canopy from a portable-tower at bloom (3 June), fruit maturity (31 July) and scenescence (18 September).

[c] Density values of the yellow, magenta and cyan dye layers were obtained by passing white light through a blue, green or red filter respectively. Transmitted light was measured by a color densitometer with a 4 mm aperature. Density values represent the mean of four readings plus or minus the standard deviation.

[d] "D" represents the deviation of density values per dye layer of the diseased from the healthy values (0 base-line). Positive values represent a decrease in reflectance, while negative represents an increase in reflectance in the green (400-500), red (600-700) and infrared (700-900) wavelengths.

The use of low level imagery in the detection of BBSSV-infected blueberry bushes and PRMV-infected grapevines appears to have limitations due to uncontrolled variables. Fritz (68) commented on the short exposure latitude of near-infrared film for obtaining optimum exposures. He concluded that bracketing one-half exposure stop on either side of the best exposure may result in either over or under exposed film. Other variability may be found from one roll of film to the next. Some film may have higher densities to begin with. However, this may be controlled partially by purchasing film with the same lot numbers. Processing temperatures and chemicals may also affect the thickness of the dye layers and ultimately the amount of transmitted light. One of the most important variations to consider when analysing photographic imagery of commercial crops is the inherent variation of the plants themselves. Spectral deviations from the expected results may represent a normal condition for that particular plant and may not represent a diseased state. While most cropping systems appear uniform, slight variations in fertilizer, water, and other environmental and cultural practices as well as diseases, other than the ones being investigated, will affect the normal spectral signature of a particular plant. These similar results were observed on spectral scans of individual healthy and virus-infected leaves from blueberry bushes and grapevines as well as on photographs taken of individual healthy and symptomatic leaves at different phenological stages (A. Childress, D. Ramsdell and G. Safir, unpublished data).

4. Spectral Reflectance Curves of Virus-Infected and Virus-Free Blueberries and Grapevines Obtained With a Field-Portable Spectroradiometer

The reflectance value of a small replica of the barium sulfate panel, constructed under the same conditions and at the same time as the larger

panel was measured in a laboratory spectroradiometer (Environmental Research Institute of Michigan (ERIM) Ann Arbor, MI 48107). The panel reflected 85 % of the incident radiation.

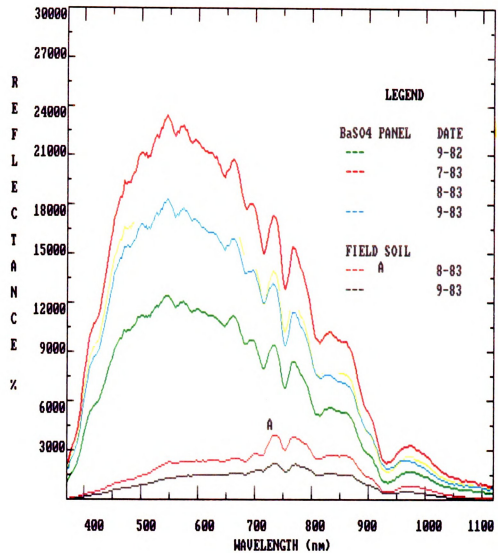
A mean reflectance value was obtained from two readings for each plant target during the field experiments. Data stored in the SE-590 microprocessor was transfered to an IBM XT Personal Computer (International Business Machines Corp., P.C Box 1328-C, Boca Raton, FL 33432) for analysis. Spectral curves of reflected radiation for the barium sulfate panel, field soil and tested plants were plotted using a plotting package developed for the IBM. (Spectra by W. Inslin and A. Childress). Paired diseased and healthy blueberry bushes or grapevines were tested for significant differences using a two-tailed T-test for paired observations (Dr. Tracy L. Gustafson, M D, 7705 Gattis School Rd., Round Rock, TX 78664). Reflective differences in the visible portion (350 to 640 nm) of the electromagnetic spectrum between healthy and diseased plants were either small or inconsistent and only the results observed in the infrared region (700-1100 nm) will be discussed. Fox (65) found that leaves containing sooty mold reduced reflectance more in the infrared region than the visible. His results showed that leaves with visible signs of mold, reflected very little while healthy leaves reflected five times more infrared radiation. Deviations between healthy and diseased plants were small (5-7 % reduction) in the visible wavelengths with the onset of disease. However, diseased plants exhibited between 10 to 40 % reduced infrared reflectance than did the healthy plant.

a. Peach Rosette Mosaic Virus (PRMV)

Reflectance curves of incident electromagnetic radiation on the $BaSO_4$ panel (Figure 20), demonstrated that more radiation was available and striking the vineyard in the early part of the season (15-20 %), decreasing as the season progressed, with the least in September. This pattern would be expected since it follows the natural progression of the solar equinox. Reflectance of the vineyard soil was approximately 3 % higher in August than in September (Figure 20).

Four PRMV-infected grapevines were each paired with a healthy control vine on 10 September of 1982. From the spectral data obtained, three of the four diseased vines exhibited lower infrared reflectance than their healthy counterparts (Figure 21). One diseased bush reflected a higher amount of IR radiation than the control. In 1983, seven diseased vines were each paired with adjacent or nearby healthy vines of similar morphology and location in the vineyard. Spectral reflectance was measured between 12:00 and 2:00 p.m. at three times during the growing season, 11 July (fruit set), 29 August (initial fruit ripening) and 13 September (early fruit maturity). In July, four of these diseased bushes reflected significantly higher (Figure 22), while two reflected lower infrared radiation than healthy controls. Reflective differences ranged between 10 to 40 % with a mean of 27 %. One pair was not tested in July. During the time the berries were ripening in August, three of the seven PRMV-infected vines reflected 28 % higher, while two reflected 16 % less radiation in the 700 to 900 nm wavelength region of the spectrum (Figure 22). One pair of vines reflected the same amount of infrared radiation. In September, three diseased vines reflected 25 % more while three

Figure 20. Reflectance curves of incident radiation reflected from a $BaSO_4$ panel and soil reflectance, obtained using a field-portable spectroradiometer in a cv. Concord grape vineyard. Lawton, MI 1982 and 1983.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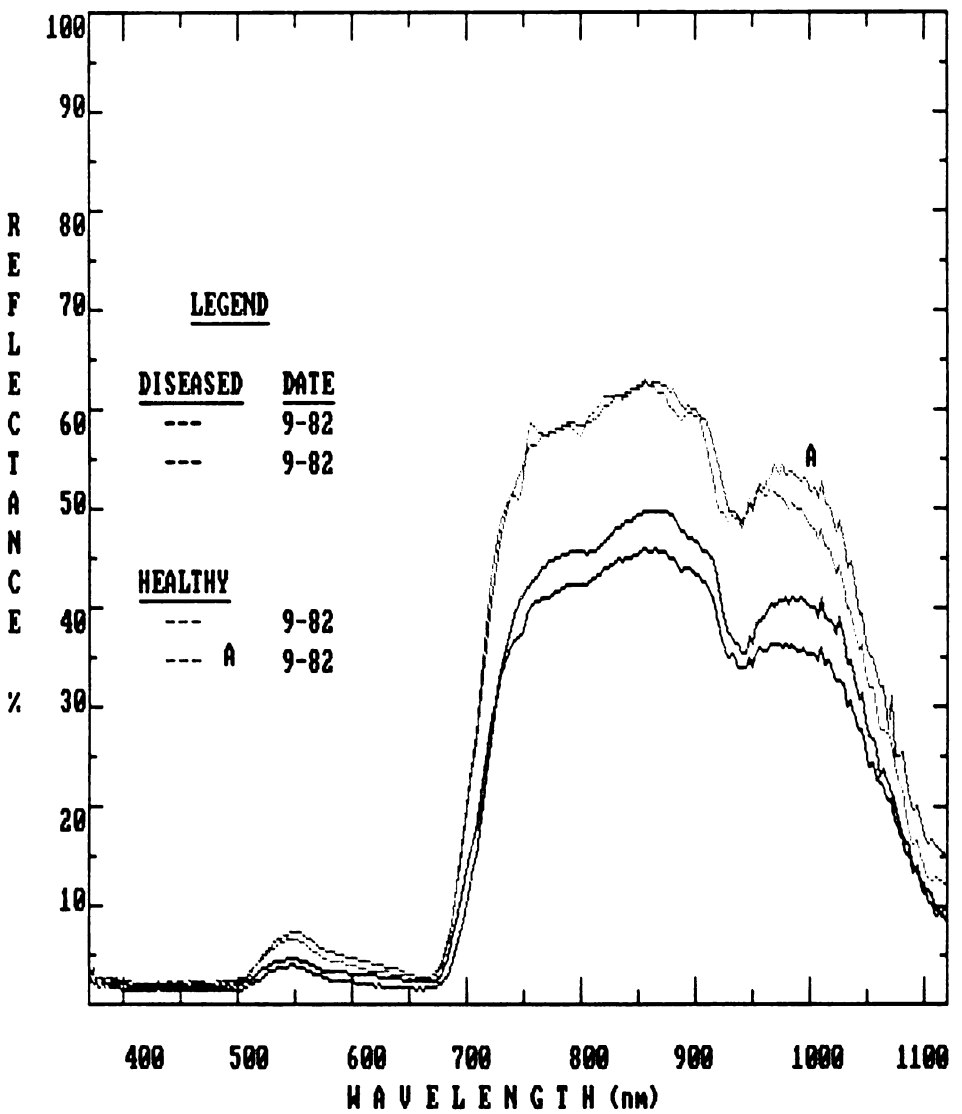
WAVELENGTH (nm)

Figure 20

Figure 21. Spectral reflectance of PRMV-infected and healthy cv. Concord grapevines under field conditions, using a field-portable spectroradiometer at 3 meters height above the plant canopy, referenced to a $BaSO_4$ panel. Lawton, MI 1982.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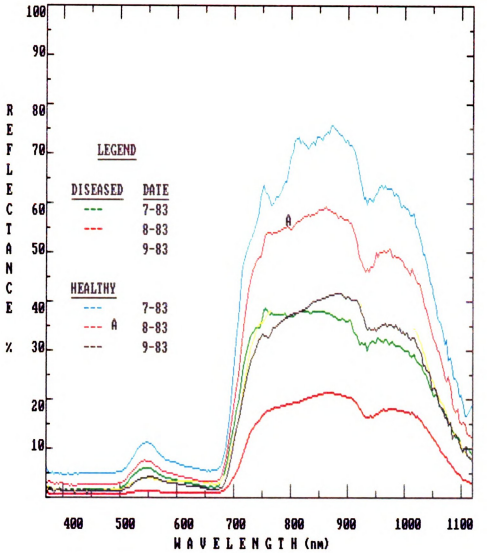
WAVELENGTH (nm)

Figure 21

Figure 22. Spectral reflectance of PRMV-infected and healthy cv. Concord grapevines, using a field-portable spectroradiometer at 3 meters height above the plant canopy, referenced to a $BaSO_4$ panel. Lawton, MI 1983.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WAVELENGTH (nm)

Figure 22

reflected 23 % less than the paired healthy vine. Little difference in relected IR was observed between one pair of diseased and healthy vines.

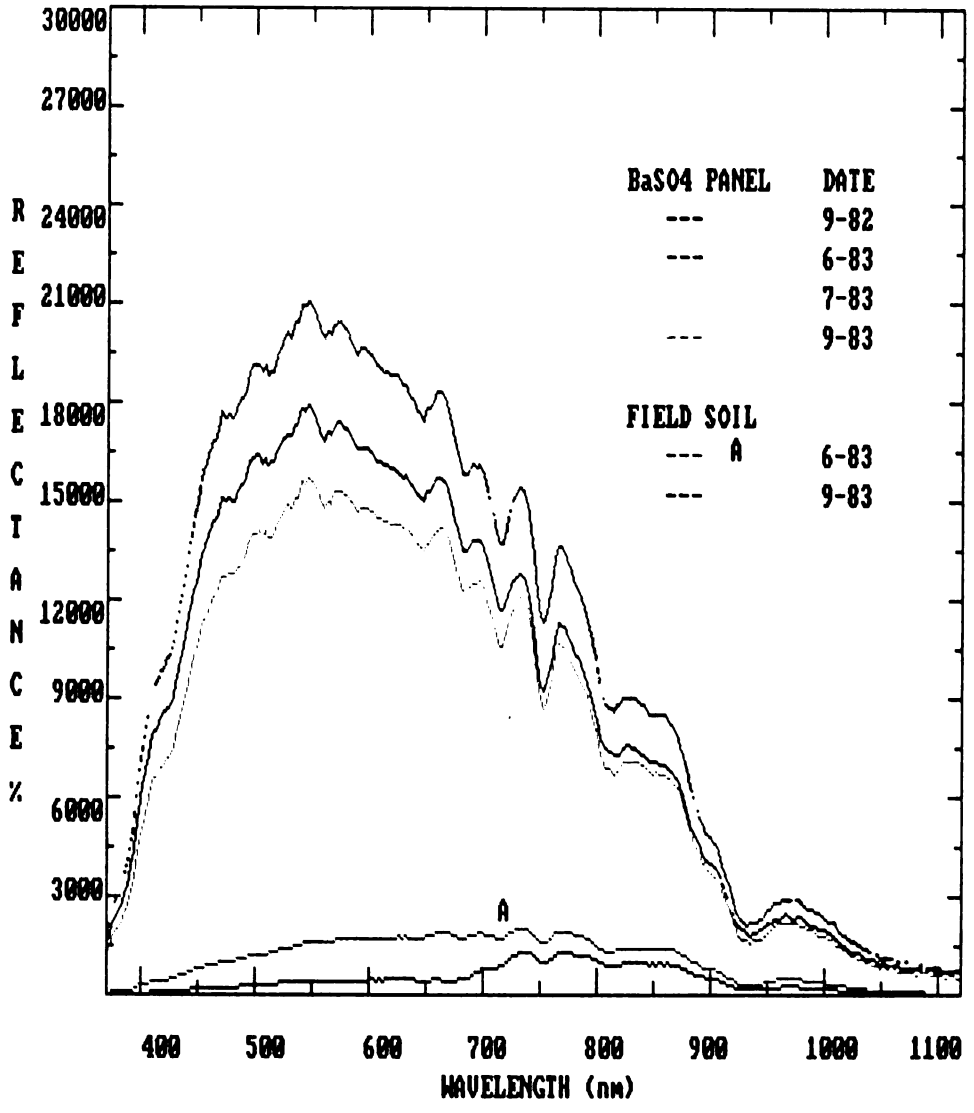
In general, great variability in the amount of reflected radiation in the infrared region was observed among diseased and among healthy plants. Cultural and environmental factors may influence the spectral signature of each plant, without showing obvious visual differences. Significant differences were observed between PRMV-infected and paired virus-free vines as regards the amount of reflected infrared radiation.

b. Blueberry Leaf Mottle Virus (BBLMV)

Spectral Reflectance curves were obtained in a highbush blueberry field containing BBLMV-infected bushes on 9 September 1982, and at fruit set (24 June), harvest (22 July) and scenescence (27 September) in 1983. Results of incident radiation reflected by the $BaSO_4$ panel (Figure 23) at these dates indicate that a higher amount of visible radiation was available early in the season, increasing slightly in July and decreasing by approximately 20 % in September. Smaller differences were observed in the infrared region, but followed the same trend. Field soil reflectivities (Figure 23) varied by 5 % in the visible region between June and September, but were similar in the infrared region.

Five BBLMV-infected bushes and their healthy counterpart bushes were tested in 1982. Two of these diseased bushes exhibited a slightly higher (3-5 %) reflectance (Figure 24), two reflected less (15-35 %) and one the same amount of infrared radiation than their healthy counterpart. In June of 1983, two of the seven BBLMV-infected bushes had a slightly higher infrared reflectance (3-5 %) (Figure 25), four a lower reflectance (15-35 %) (Figure 26) and one the same reflectance as its healthy paired bush.

Figure 23. Reflectance curves of incident spectral radiation reflected from a $BaSO_4$ panel and field soil, using a field spectroradiometer at 3 meters height above the plant canopy. Agnew, MI 1982 and 1983.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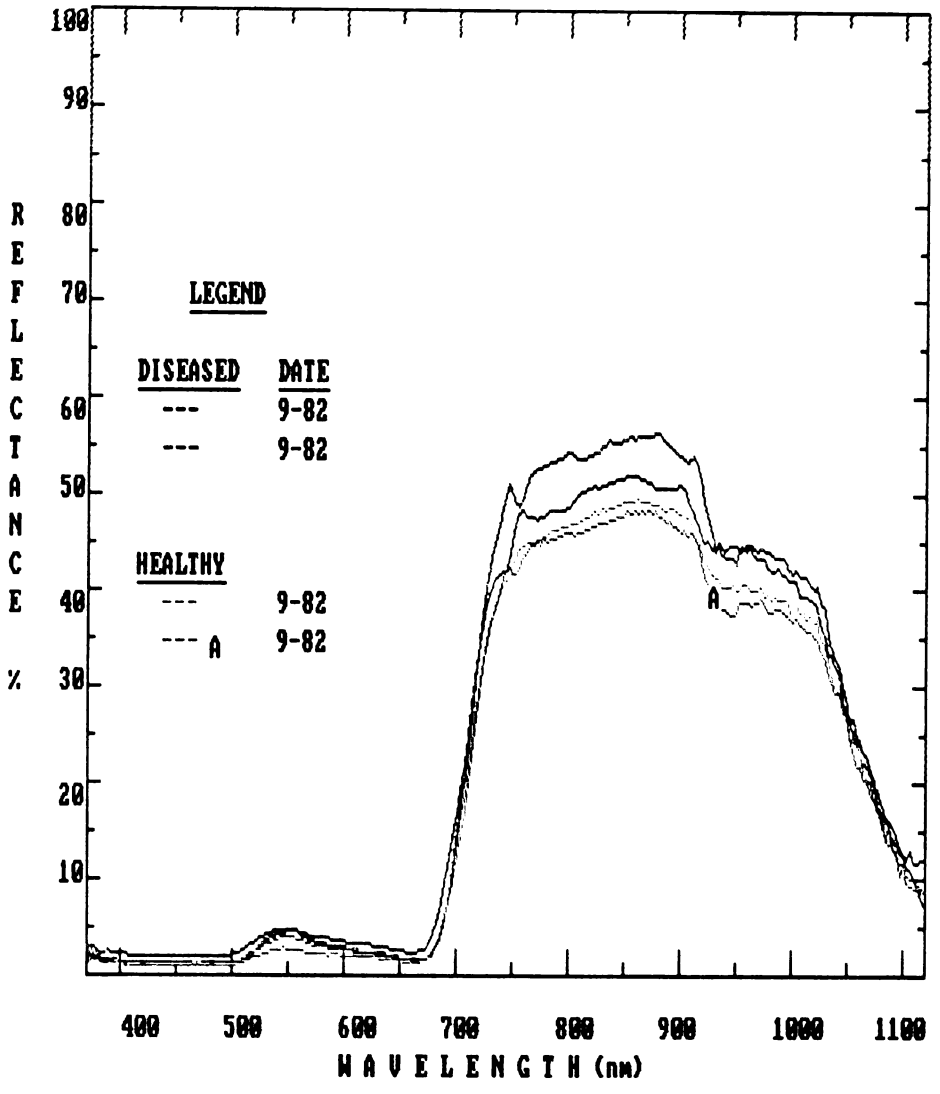
WAVELENGTH (nm)

Figure 23

Figure 24. Spectral reflectance of BBLMV-infected and healthy cv. Jersey highbush blueberry bushes, using a field-portable spectroradiometer at 3 meters above the plant canopy, referenced to a $BaSO_4$ panel. Agnew, MI 1982.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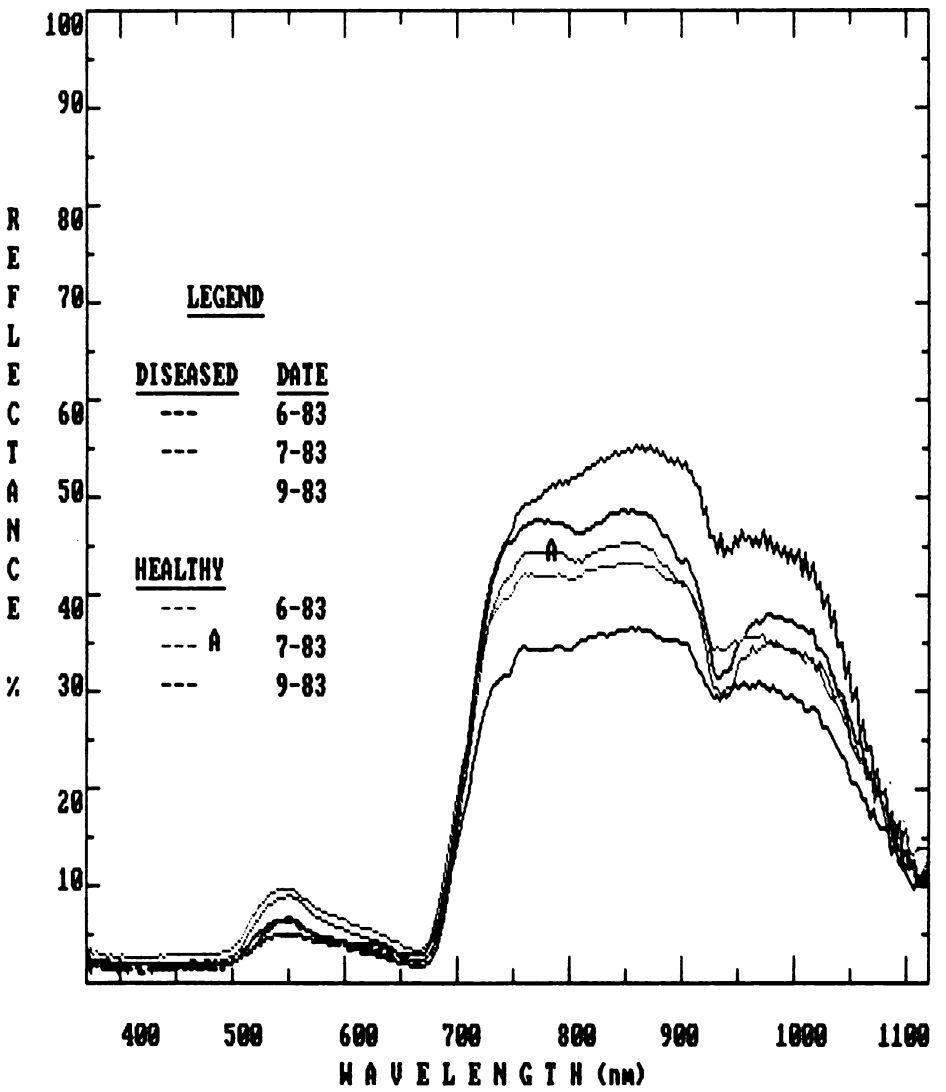
WAVELENGTH (nm)

Figure 24

Figure 25. Spectral reflectance of BBLMV-infected and healthy cv. Jersey highbush blueberry bushes, using a field-portable spectroradiometer at 3 meters height above the plant canopy, referenced to a $BaSO_4$ panel. Agnew, MI 1983.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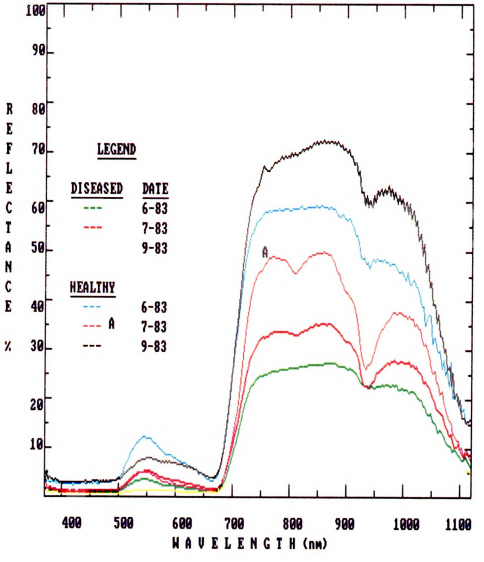
WAVELENGTH (nm)

Figure 25

Figure 26. Spectral reflectance of BBLMV-infected and healthy cv. Jersey highbush blueberry bushes, using a field-portable spectroradiometer at 3 meters height above the plant canopy, referenced to a $BaSO_4$ panel. Agnew, MI 1983.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WAVELENGTH (nm)

Figure 26

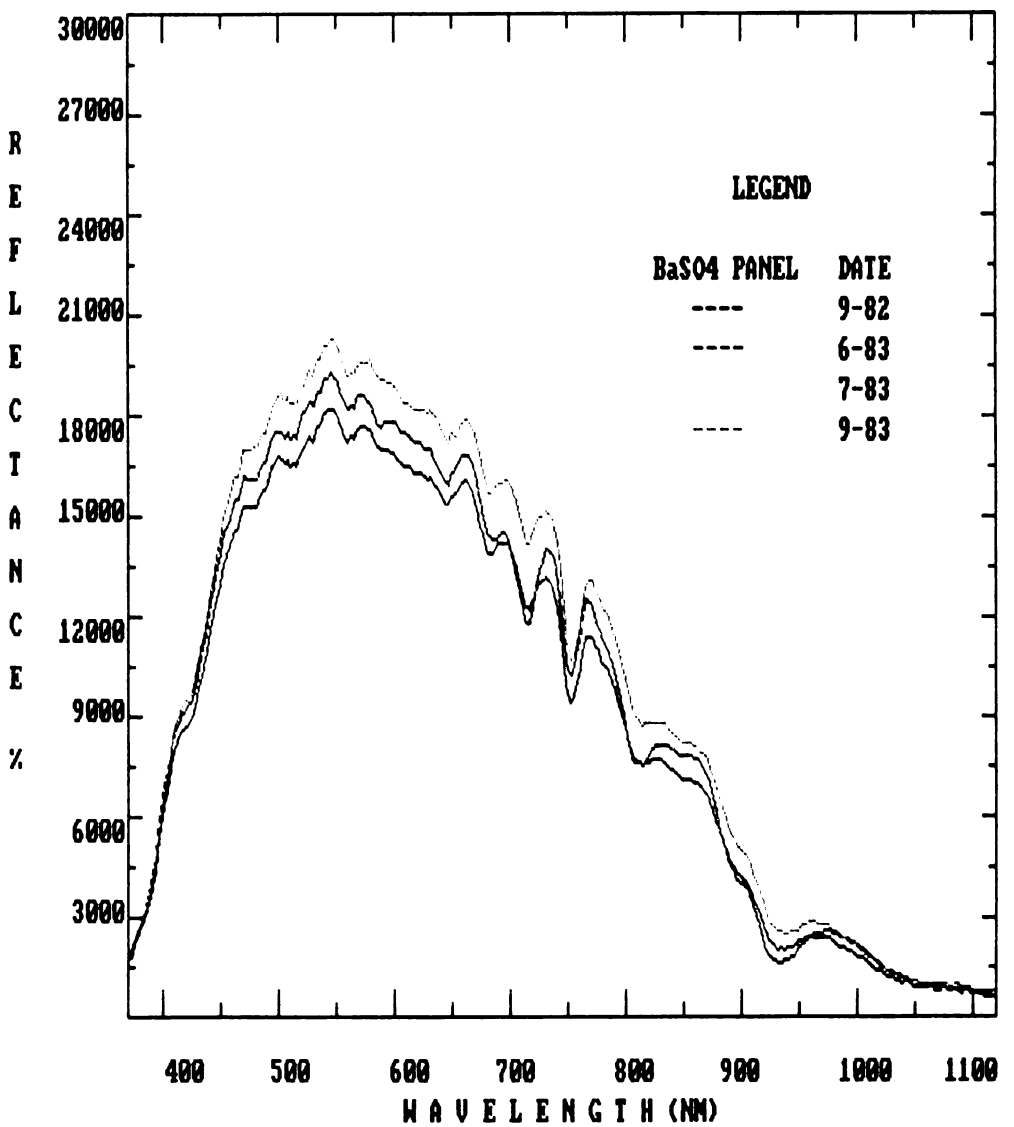
Three diseased bushes reflected 23 % higher infrared radiation than the healthy bush in July. The other four diseased bushes reflected 20 % less infrared radiation. The range in differences of reflected infrared radiation was between 3 and 35 percent. At senescence, four diseased bushes exhibited a mean increase of 17 % reflected infrared radiation than healthy, while three exhibited a 28 % decrease.

Generally, a decrease in reflected infrared radiation was observed for diseased bushes in comparison to their healthy paired bush in 1982 and 1983. Variability occurred not only among diseased bushes but among healthy as well. The same diseased bush did not consistently reflect more or less during the season in comparison to the healthy. However, a decrease in reflectance occurred on more diseased bushes in the early part of the growing season. Interestingly, the decrease in reflected infrared radiation due to the water absorbtion at 950 nm was more noticable in July than either June or September, possibly indicating more hydration of the leaves at this time of year. Both diseased and healthy bushes displayed this response.

c. Blueberry Shoestring Virus (BBSSV)

Figure 27 represents reflectance curves of incident solar radiation reflected from a $BaSO_4$ panel located in a highbush blueberry field containg BBSSV-infected bushes. Ratios of available radiation to reflected radiation by infected and healthy bushes were determined on 9 September 1982 and at three phenological stages during 1983; fruit set (22 June), initial fruit ripening (21 July) and senescence (23 September). Results indicated that more radiation was incident in the field in July and September than in June for the visible part of the spectrum, with less difference observed in the 700 to 900 nm or infrared wavelengths. Soil

Figure 27. Spectral reflectance curves of incident solar radiation reflected from a $BaSO_4$ panel at 3 meters above the highbush blueberry plant canopy. Coopersville, MI 1982 and 1983.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WAVELENGTH (NM)

Figure 27

reflectance (Figure 28) varied slightly for all three dates in 1983, but were much lower than the soil reflectance values observed in September of 1982. This deviation may be a result of a drier or lighter area of the soil observed in the field in 1982. A decrease in soil reflectance was observed in the 950 nm range in July but not June or September. The same occurance was observed in the blueberry field with BBLMV-infected bushes. As discussed previously, the field soil may be more hydrated at this time of the season, resulting in reduced infrared radiation being reflected due to water absorption.

Spectral reflectance curves of five pairs of diseased and healthy bushes were obtained in 1982. Three of these diseased bushes reflected between 15 to 30 % more infrared radiation than did their healthy partner (Figure 29). One diseased bush reflected 3 to 5 % less infrared radiation, while the other reflected the same as its paired healthy bush. Early in the 1983 growing season (3 June), seven of the eight diseased bushes observed, reflected between 15 to 35 % more infrared radiation than their healthy partner. No difference between healthy and diseased was observed for one pair. Five of these eight bushes reflected between 15 to 35 % less than healthy in July (Figure 30), while two reflected 26 % more infrared radiation. One pair of diseased and healthy bushes did not significantly differ in the amount of reflected radiation in the 700 to 900 nm wavelength range. A similar trend was observed in August when the plants were approaching dormancy. Five of the seven BBSSV-infected bushes showed a significant ($\underline{P}$ =0.05) (5 to 40 %) decrease in reflected infrared, while two reflected between 10 and 30 % more.

Six of the eight pairs of diseased and healthy bushes exhibited a marked decrease (20 %) in reflected infrared radiation at the 950 nm water

Figure 28. Blueberry field soil reflectance curves ratioed to a $BaSO_4$ reference panel at various times during the growing season. Coopersville, MI 1982 and 1983.

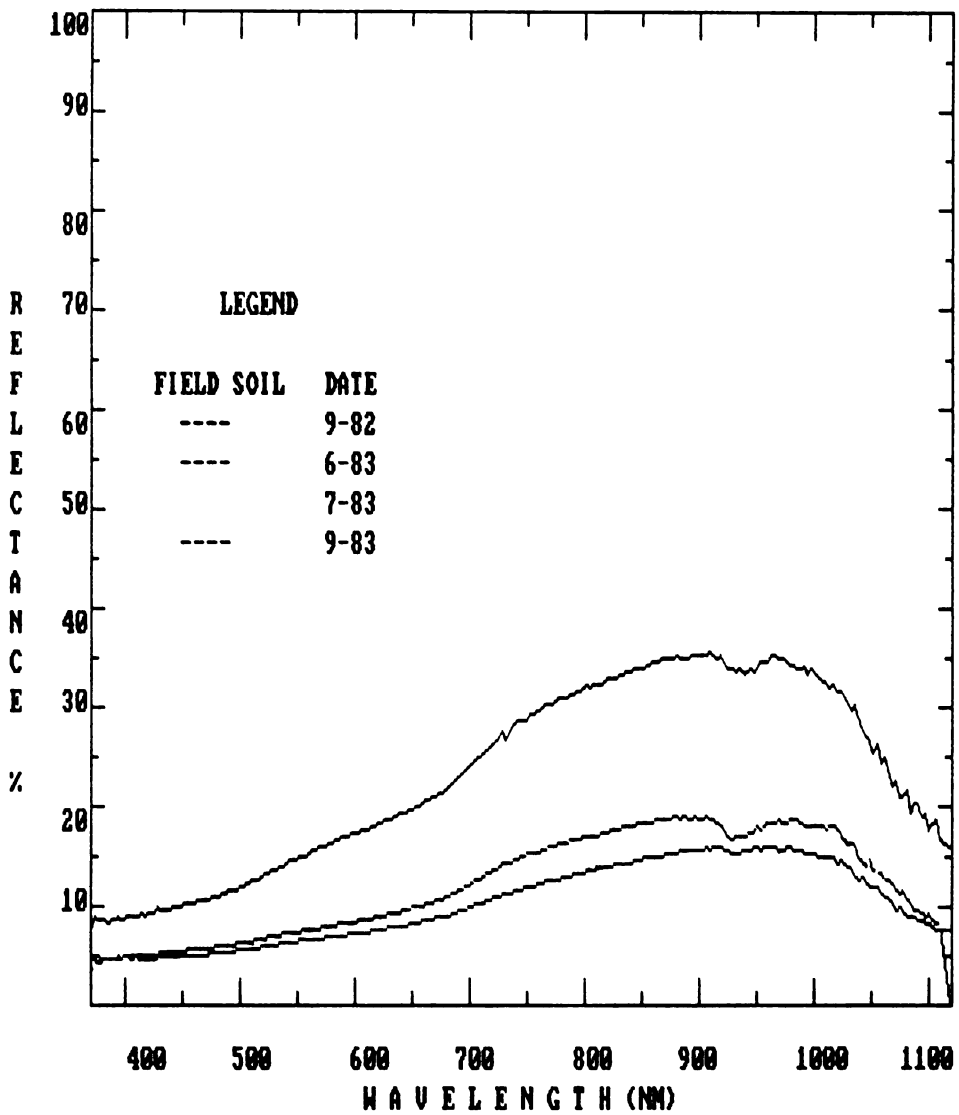
100
90
80
70
60
50
40
30
20
10
R
E
F
L
E
C
T
A
N
C
E
%
LEGEND
FIELD SOIL DATE
---- 9-82
---- 6-83
7-83
---- 9-83
400
500
600
700
800
900
1000
1100
W A V E L E N G T H (NM)

Figure 28

Figure 29. Spectral reflectance of BBSSV-infected and healthy cv. Jersey highbush blueberry bushes, using a field-portable spectroradiometer at 3 meters height above the plant canopy, referenced to a $BaSO_4$ panel. Coopersville, MI 1982.

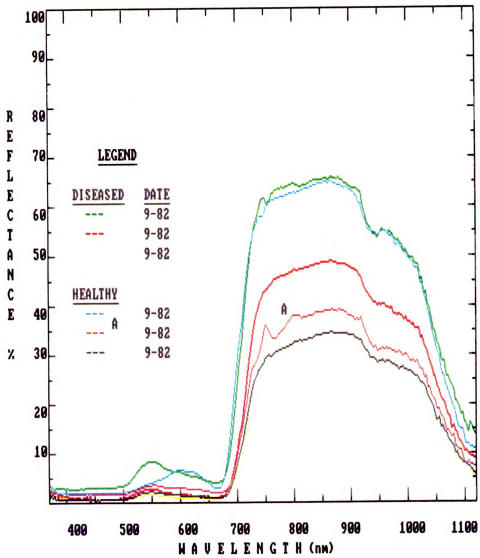
REFLECTANCE %
100
90
80
70
60
50
40
30
20
10
LEGEND
DISEASED DATE
9-82
9-82
9-82
HEALTHY
A
9-82
9-82
9-82
A
400
500
600
700
800
900
1000
1100
WAVELENGTH (nm)

Figure 29

Figure 30. Spectral reflectance of BBSSV-infected and healthy highbush blueberry bushes, using a field-portable spectroradiometer at 3 meters height above the plant canopy, referenced to a $BaSO_4$ panel. Coopersville, MI 1983.

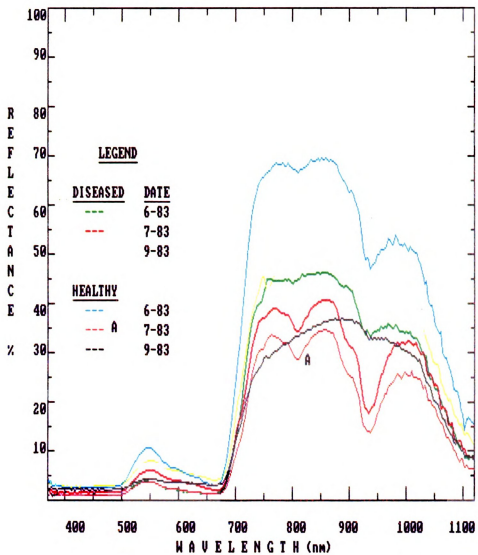
REFLECTANCE %
100
90
80
70
60
50
40
30
20
10
LEGEND
DISEASED DATE
6-83
7-83
9-83
HEALTHY
6-83
A 7-83
9-83
A
400
500
600
700
800
900
1000
1100
WAVELENGTH (nm)

Figure 30

absorption band in July, but not in either June or August. This supports the results found for the field soil at the same time of the season. Two pairs also exhibited this response in June as well as in July. This response was more consistently found within this field than the blueberry field with BBLMV-infected bushes. Similar to that observed in the BBLMV-infected field, the largest deviations in reflected infrared radiation occurred early in the growing season. Similar conclusions were made from Color and Color-infrared photographs taken of PRMV-infected and BBSSV-infected plants at the same height above the canopy. Most deviations in density values on the developed film were observed on photographs taken early in the growing season and were most noticable in the cyan dye-forming layer or the infrared region of the electromagnetic spectrum (700 to 900 nm).

A larger sample size might be necessary to determine a consistent trend of either increased or decreased infrared reflectance for virus-infected grapevines or highbush blueberry bushes. Considerable variability was observed not only due to environmental conditions but also amoung plants in a commercial setting. Therefore, detection of these virus diseases by remote sensing techniques appears to afford limited usefulness as a routine method of assessing disease in the field.

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