Viral Interactions Lead to Decline of Blackberry Plants

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ABSTRACT

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Blackberry yellow vein disease (BYVD) poses a new threat to the blackberry industry in the United States. Blackberry yellow vein associated virus (BYVaV) was originally thought to be the sole cause of this disease. However, BYVaV has been found in several asymptomatic blackberry cultivars. An unusual member of the family *Potyviridae* was identified recently from symptomatic plants and named Blackberry virus Y (BVY). BVY has been shown to spread in the field and cause BYVD when co-infected with BYVaV. Both viruses are asymptomatic in single infections but are readily detectable in asymptomatic plants by reverse transcription–polymerase chain reaction (RT-PCR). However, in mixed infections, the titer of BYVaV is repressed, sometimes to levels undetectable by RT-PCR, while the concentration of BVY is increased several fold. Electron microscopy revealed a variety of viral inclusions in symptomatic leaf samples, but none could be found in single infections with either BVY or BYVaV. Although BYVaV has been consistently associated with BYVD in different geographical regions, the detection of BVY has thus far been restricted to northwest Arkansas. It has been hypothesized that BYVaV is the synergistic determinant of BYVD that causes symptoms in different cultivars at various locations during co-infection with other viruses.

Additional keywords: mixed virus infection, *Rubus*, synergism

Demand for fresh fruit, release of improved cultivars, and relative profitability of the crop are some of the factors that have influenced the popularity of blackberries in the United States (7,21). Blackberry yellow vein disease (BYVD) is a serious and emerging threat to blackberry production in the southern United States in the past few years (15,22,23). The disease results in severe symptoms that are mainly characterized by the yellowing of veins of primocane (first year's growth) leaves with symptomless new leaves (Fig. 1) (22). Symptoms vary and include oak-leaf patterns, irregular chlorosis, and line patterns (23). Floricanes (second year's growth and flowering stem) are severely affected by the disease, resulting in the dieback of canes during fruiting. Few of the 2-year-old 'Chickasaw' blackberry plants showed symptoms in a northwest Arkansas production field in 2003, but during the next 2 years, BYVD spread to the entire planting, resulting in a complete yield loss and plant death.

Analysis of double-stranded RNA (dsRNA) from symptomatic plants and subsequent cloning and sequencing of virus specific cDNA revealed the presence of a member of the genus *Crinivirus* known as Blackberry yellow vein associated virus (BYVaV) (15). All symptomatic plants in the original study were infected with BYVaV (15); however, screening of more plants indicated that BYVaV causes latent infections in several blackberry cultivars. These results and the presence of potyvirus-like inclusion bodies in symptomatic plants led to further investigation by shotgun cloning of dsRNA isolated from infected plants to determine if other viruses were present in the symptomatic plants. The obtained sequences revealed the presence of a member of a new genus in the *Potyviridae*, named Blackberry virus Y (BVY) (24). BVY was shown subsequently to spread from BYVD-affected blackberry plants in the field to healthy 'Chester' sentinel blackberry plants (24), but the BVY-infected sentinel plants did not display symptoms even after two growing seasons under greenhouse conditions.

Infection by unrelated viruses in the same plant may promote synergistic interactions that can manifest more severe symptoms than single infections of the respective viruses (19). It has been demonstrated that co-infection of *Beet mosaic virus*, genus *Potyvirus*, with *Beet yellows virus*, genus *Closterovirus*, or *Beet western yellows virus*, genus *Polerovirus*, in sugar

beet leads to increased symptom expression and enhanced accumulation of all three viruses (27). Often synergistic responses involve titer increase of one or more of the viruses while accumulation of the others does not alter noticeably. The increase in virus concentration could be due to the unrestricted movement of a synergistic partner between different cell types following co-infection with an additional virus (17). Members of the genus *Potyvirus* in synergistic interactions often induce an increase in the titer or enhanced movement of other viruses (5,17,26). Interestingly, in the case of sweet potato severe mosaic disease caused by co-infection of *Sweet potato chlorotic stunt virus* (SPCSV), genus *Crinivirus*, and *Sweet potato feathery mottle virus* (SPFMV), genus *Potyvirus*, the concentration of SPFMV increases up to a thousand-fold while SPCSV concentration decreases dramatically (12,16). Similarly, co-infection of *Soybean mosaic virus*, a potyvirus, with *Bean pod mottle virus*, a comovirus, results in an increase in the potyvirus concentration (13).

Although poorly understood, experiments involving the multi-functional helper component-proteinase (HC-Pro) have shed new light on the molecular mechanisms of plant viral synergisms involving potyviruses (26). It has been shown that HC-Pro of potyviruses mediates the suppression of host gene silencing and that this suppression plays a role in the synergism in mixed infections by enhancing the titer of other viruses (3).

Observation of viral inclusions in plant cells is often helpful in identifying mixed viral infections (14). In addition, quantification of relative viral accumulation in mixed infections may contribute to the understanding of the roles played by individual viruses. Northern blot and real-time polymerase chain reaction (rt-PCR) are two of the most reliable molecular techniques available for the quantification of viral RNAs in mixed infections (12,16).

The aim of this study was to examine whether mixed infections of BYVaV and BVY lead to the development of BYVD. Cytopathological changes and relative accumulation of BYVaV and BVY RNA associated with mixed infections and single infections were studied. The results indicate an uncommon synergism between BYVaV and BVY where unusual viral inclusions and an increase in BVY and decrease in BYVaV levels were observed.

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*The e-Xtra logo stands for "electronic extra" and indicates that Figure 1 appears in color in the online edition.

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MATERIALS AND METHODS

Plant material and virus isolates. Asymptomatic tissue-culture propagated 'Chickasaw' blackberry plants naturally infected with BYVaV were obtained from a commercial nursery. Reverse transcription–polymerase chain reaction (RT-PCR) amplification and sequencing of a 566-bp region in BYVaV RNA 1 and a 549-bp region in RNA 2 from the asymptomatic tissue-cultured plants confirmed the identity of BYVaV (22). Asymptomatic BVY-infected 'Chester' blackberry plants were obtained by placing healthy 'Chester' plants for a 2-week period amidst the symptomatic 'Chickasaw' blackberry plants in the field (24). RT-PCR amplification and sequencing demonstrated that a 3,000-bp region near the 5' end of BVY from the field-infected 'Chester' and symptomatic 'Chickasaw' plants were identical (24).

Total nucleic acid (TNA) extraction and RT-PCR detection. Blackberry leaves were collected early in the morning as sampling during the day resulted in inconsistent RT-PCR amplification due to poor TNA quality. The TNA extraction followed a modified Hughes and Galau procedure (11,23). RT-PCR was performed as described previously (22), and products were sequenced in an ABI 3100 automated sequencer at the University of Arkansas DNA Resource Center using specific primers (Table 1).

Spread of BVY in the field. In a study to determine when transmission occurred during the growing season, sets of 10 BYVaV-infected (symptomless) 'Chickasaw' plants were placed on pedestals next to BYVD-affected symptomatic field plants at 2-week intervals. Control plants and plants brought in from the field after the transmission access periods were sprayed to control insects and kept in an insect-free greenhouse after return from the field. The plants were overwintered outdoors in a cold frame covered with bark mulch. The following spring, all plants were tested for the presence of BVY and BYVaV by RT-PCR and monitored for the occurrence of BYVD symptoms.

Approach grafting. Viral interactions suspected to be synergistic in nature can be verified by grafting plants that are singly infected with individual viruses (9). One-year-old, asymptomatic 'Chickasaw' and 'Chester' blackberry plants infected singly with BYVaV and BVY were approach grafted to plants infected with the other virus. Stems with a pencil-size thickness were selected in both sets of plants; an incision was made to peel the bark on one side for about 5 cm on both stems. Unions of the stems were ensured by binding both of the cut surfaces together with grafting tape (10). Plants were evaluated 1 month after grafting for symptom expression and movement of BYVaV and BVY through RT-PCR analysis.

Electron microscopy. BYVD-affected symptomatic blackberry leaves from the

field and plants infected singly with either BYVaV or BVY were used to prepare thin-sections for electron microscopy as described previously (23). Leaf tissue samples (2 mm²) along the chlorotic veins were fixed, and ultra-thin sections were placed on a copper grid. The sections were double-stained for 15 min with a 2% aqueous solution of uranyl acetate (pH 5.0) and for 5 min with lead citrate (pH 12.0). Specimens were examined using a JEOL 100 CX (Tokyo, Japan) transmission electron microscope.

Nucleic acid spot hybridization (NASH). TNA from healthy and symptomatic blackberry leaf samples was extracted, denatured, and dotted on a nylon membrane following a previously described procedure (22). TNA concentrations ranging from 200 to 3.1 ng/μl were prepared by making twofold dilutions of the TNA extract in a final volume of 10 μl for dotting the membrane. BYVaV- and BVY-specific probes were synthesized using virus specific primers (Table 1) as described earlier (22), and hybridization

Fig. 1. Blackberry yellow vein disease symptoms in a leaf from a primocane of 'Chickasaw' infected with Blackberry yellow vein associated virus and Blackberry virus Y.

Table 1. Primers used in reverse transcription–polymerase chain reaction (RT-PCR) detection and real-time PCR quantification of Blackberry yellow vein associated virus (BYVaV) and Blackberry virus Y (BVY)^a

Primer name	Primer sequence (5'-3')
Detection primers	
BYVaV PF	GGGTAAATGAGAGATTGGATG
BYVaV PR	TGGGATTTGTCAAACCTTGGTG
BVY PF	CTCAGCAGAGAGATTGGTG
BVY PR	CCAAGAACATCGGCGAGT
Real-time PCR primers	
BYVaV RF	CGGTGTCCTCAAGATGTTTGT
BYVaV RR	TGGTTGTGTGAAGGCAATGTA
BVY RF	GAATTTGATGCAGAGGCCATA
BVY RR	TGCTTTAAGTGAGCCTTTCCA
Ubiquitin RF (endogenous control)	GTTGATTTTGTCTGGGAAGC
Ubiquitin RR	GATCTTGGCCTTCACGTTGT

^a PF, polymerase forward; PR, polymerase reverse; RF, real-time forward; RR, real-time reverse.

was carried out following standard procedures (20). Prior to hybridization with the BYVaV probe, the probe of the BVY was stripped from the blot. The blots were exposed to X-ray film (Kodak BioMax MS film; Eastman Kodak Company, Rochester, NY) for 4 h and the signal intensities were measured by ImageQuant (Molecular Dynamics, Sunnyvale, CA) software. NASH was performed twice with TNA extractions from two different sets of plants.

Real-time PCR. Tissue samples were collected from the leaflets of the third leaf from the top of primocanes of greenhouse-grown plants. The samples included asymptomatic single infections of BYVaV and BVY and symptomatic and asymptomatic leaves of plants with mixed infections. TNA was extracted from leaf samples, and 0.5 µg TNA from each sample was used for cDNA synthesis using 0.5 µg random primers, 4 µl 10× RT-buffer, 1 µl 10 mM dNTPs, 1 µl 100 mM DTT, and 100 units of MMLV reverse transcriptase (Promega, Madison, WI) in a 20-µl reaction volume for 50 min at 42°C followed by a 10-min incubation at 80°C. The cDNA was then diluted with sterile water to 10 times the original reaction volume. Master mixes consisting of Brilliant SYBR Green QPCR Master Mix (Stratagene, La Jolla, CA) and specific primers (Table 1) for BYVaV, BVY, and the ubiquitin gene (endogenous control for TNA) were prepared. In a 96-well optical plate, 1 µl cDNA of each sample was added in triplicate for each primer combination. The real-time PCR reaction was performed in an Mx3005P QPCR System (Stratagene). The PCR was initiated with a pre-denaturation step of 94°C for 5 min. Subsequently, 40 cycles were performed at 94°C for 20 s for denaturation and 55°C for 30 s for primer annealing and extension. This experiment was repeated twice.

Fig. 2. Agarose gel electrophoresis of reverse transcription-polymerase chain reaction products for Blackberry yellow vein associated virus (BYVaV) (~500 bp) and Blackberry virus Y (BVY) (~330 bp). Lane A, 1 kb DNA ladder; lanes B and C, healthy blackberry plants; lanes D and E, symptomatic 'Chickasaw' blackberry plant for BYVaV and BVY, respectively; and lane F, water blank.

RESULTS

Spread of BVY and symptoms. Ten of the 110 sentinel plants used in this study developed symptoms of BYVD 1 month after field exposure, and the symptoms reappeared after overwintering. These plants belonged to the four sets that were placed in the field in July and August of 2003. Symptoms in 'Chickasaw' sentinel plants were similar to the symptoms observed in field-grown 'Chickasaw' plants affected with BYVD. Ten BYVD-infected 'Chickasaw' control plants that were maintained in the greenhouse (not placed in the field) did not express symptoms of BYVD. RT-PCR analysis of symptomatic sentinel 'Chickasaw' plants revealed the presence of BYVaV and BVY (Fig. 2). Approach grafting of BYVaV-infected 'Chickasaw' with BVY-infected 'Chester' resulted in the development of BYVD symptoms in the 'Chickasaw' plants 1 month after grafting, and RT-PCR revealed that symptomatic 'Chickasaw' plants were doubly infected with BYVaV and BVY. However, BYVaV could not be detected in the grafted 'Chester' blackberry plants even 2 months after grafting, and no symptoms were observed in the 'Chester' test plants. Co-infection of both the viruses established by grafting in 'Chickasaw' blackberry plants produced symptoms that closely resembled symptoms in BYVD-affected field grown plants.

Viral inclusions. A variety of viral inclusions were observed in cells of BYVD-affected symptomatic blackberry leaves through thin-section electron microscopy (Fig. 3). The predominant inclusions were cylindrical inclusions, laminated aggregates, proliferated nuclear membranes, and pinwheel inclusions. Of particular impor-

tance was the finding of pinwheel inclusions inside the nucleus. Inclusions that resemble vesicles and tubules that are often associated with infection by criniviruses were also found (23). Mesophyll cells of leaves with single infections of either BVY or BYVaV did not exhibit any noticeable viral inclusions or alterations in the cell. Observations of asymptomatic mixed-infected samples for viral inclusions might have been interesting, but these tissues were not examined in this study.

Viral accumulation. Relative comparisons of viral RNA accumulation in single and mixed infections of BYVaV and BVY revealed variations in concentrations through NASH and real-time PCR analysis. Spotting of twofold dilutions of RNA from 2,000 ng to 31 ng produced easily observable signals for both viruses in single and mixed infections for all the dilutions. However, the intensity of the signal consistently was twofold higher for BVY RNA in mixed infections with severe symptoms compared to that of BVY RNA in single infections and in asymptomatic leaves of BYVD-affected plants. BYVaV concentrations on the other hand decreased by 50% in symptomatic leaves from doubly infected plants, while the signal from asymptomatic leaves of the doubly infected plants and BYVaV single infections were equal in intensity (Fig. 4). Estimates of viral RNA concentrations through real-time PCR from the above samples were consistent with NASH but the differences were slightly larger, perhaps due to the higher sensitivity of the real-time PCR technique. In plants doubly infected with BVY and BYVaV, symptomatic leaves had four times higher concentration of BVY-RNA, while asymptomatic leaves had two-

Fig. 3. Electron micrograph of **A**, a mesophyll cell containing cylindrical inclusions (CI) and laminated aggregates (LA); **B**, pinwheel inclusions (PW) and LA; **C**, PW inclusions inside the nucleus and in the cytoplasm; and **D**, PW inclusions inside the nucleus along with proliferation of nuclear membrane (arrow) in blackberry yellow vein disease affected plants. Bar represents 250 nm.

fold lower concentrations of BVY compared to BVY in singly infected plants. BYVaV was barely detectable in symptomatic doubly infected plants and was estimated to be 500 times lower in concentration compared to plants singly infected with BYVaV (Table 2) while asymptomatic leaves had only one-third the concentration of BYVaV compared to plants with single infections.

DISCUSSION

The results of this study support the hypothesis that co-infection of blackberry plants with BYVaV and BVY causes BYVD as a result of a synergistic interaction. Plants infected with both viruses together exhibited severe symptoms, while single infections of these two viruses in the blackberry cultivars used in this study were symptomless. It appears that spread of BVY occurred in the field via an aerial vector as evidenced by the development of symptoms of BYVD in BYVaV-infected 'Chickasaw' sentinel plants that had been placed in the canopy of a BYVD-affected planting for 2 weeks. In two other studies, BVY dissemination to sentinel 'Chester' plants in the field was observed (23), but BYVaV spread was not demonstrated in these studies (22). BYVD is a serious

threat to blackberry producers in the southern United States, as it can cause severe yield reductions and dieback of infected floricanes ultimately resulting in plant death. During the course of this study, death of numerous BYVD-affected 'Chickasaw' plants was observed in the field and in the greenhouse.

It has been shown that BYVaV is widespread in nursery stock, production fields, and among wild blackberry stands in Arkansas (22). Two potential whitefly vector species were found naturally colonizing blackberry plants in Arkansas, but efforts to demonstrate experimental transmission of BYVaV, a member of the genus *Crinivirus*, failed (22). Interestingly, BVY has also been found in symptomatic wild blackberry plants (24) in combination with BYVaV (22) in northwest Arkansas. Phylogenetic analysis of BVY suggests that an eriophyid mite may be the vector for BVY, and observation of symptomatic blackberry leaves for eriophyid mites revealed at least five different species. Transmission attempts of BVY with several potential eriophyid mite vectors and with two species of aphids, *Myzus persicae* and *Ampiphora agathonica*, found naturally occurring in the field, were not successful (*unpublished*). Besides the spread of BYVaV through propagation materials (22), the modes of vector transmission for both BYVaV and BVY are yet to be demonstrated.

Transmission electron microscopy studies were instrumental in identifying the novel virus BVY as a member of the family *Potyviridae* (24). Visualization of the BYVD-affected symptomatic leaf sections revealed numerous inclusion structures. The majority of the types of viral inclusions observed were characteristic of potyviruses. BVY is an unusual virus that produces a variety of viral inclusions including nuclear pinwheels, which is a new finding. Crinivirus-specific vesicles and tubules were also occasionally found

in symptomatic plants (23). Proliferation of the nuclear membrane was another unusual feature observed in this disease. In contrast, recognizable inclusions were not observed with single infections of either of these viruses (23,24). So, it may be speculated that the synergistic interaction between BYVaV and BVY favors multiplication of BVY, particularly along the veins where most cells are packed with potyviral-like inclusions and the dramatic vein yellowing symptoms of BYVD occur.

Data from NASH and real-time PCR revealed a similar phenomenon in that a fourfold increase in BVY RNA was demonstrated in symptomatic mixed-infected 'Chickasaw' plants compared to asymptomatic single infections of BVY in 'Chester'. This difference in BVY concentration may have been influenced by the cultivars used in this study. However, the increase in BVY accumulation is consistent with the observation of numerous inclusions as revealed by electron microscopy in the mesophyll cells near the chlorotic leaf veins of symptomatic plants. Yellowing symptoms may appear along the veins because BYVaV is a phloem-limited crinivirus and the effect of the synergistic interaction with BVY may be more evident in leaf tissues that support replication of both viruses. It is possible that the crinivirus promotes synergism either by suppressing host defenses against BVY or by complementing a critical protein function that benefits BVY. It has been shown that a molecular chaperone-like heat shock protein homolog (HSP70) (2), produced by members of the family *Closteroviridae* and present in BYVaV (25), is able to complement cell-to-cell movement of other nonrelated plant viruses (1). Expression of HSP70 encoding genes in plants is induced during early stages of closterovirus infections (4), perhaps aiding in the multiplication of BVY by enhanced movement between cells. HC-Pro of potyviruses is a well-known determinant of synergistic

Fig. 4. Nucleic acid spot hybridization (NASH) detection of Blackberry virus Y (BVY) and Blackberry yellow vein associated virus (BYVaV) in blackberry. The blot was initially hybridized with a BVY-specific DNA probe and subsequently with a BYVaV-specific probe after stripping the BVY probe. *symptomatic mixed infection and **asymptomatic mixed infection.

Table 2. Quantification of the amounts of viral RNA by real-time polymerase chain reaction (PCR) in single and mixed infections of Blackberry virus Y (BVY) and Blackberry yellow vein associated virus (BYVaV)

Virus infection ^a	Target virus ^b	$\Delta Ct(T)^c$	$2^{-(\Delta\Delta Ct)^d}$
CSI	BYVaV	-6.53	1.000
MIS	BYVaV	2.41	0.002
MIA	BYVaV	-4.91	0.325
PSI	BYVaV	No Ct	—
CSI	BVY	No Ct	—
MIS	BVY	-14.00	4.316
MIA	BVY	-10.60	0.408
PSI	BVY	-11.89	1.000

^a Virus-infected blackberry plants: CSI, crinivirus single infection; MIS, mixed infection symptomatic; MIA, mixed infection asymptomatic; PSI, potyvirus single infection.

^b Target viruses: BYVaV, (genus *Crinivirus*); BVY, (family *Potyviridae*).

^c Viral RNA amounts relative to endogenous control. $\Delta Ct(T) = Ct(T) - Ct(N)$, where $Ct(T)$ is the replicated average threshold value for viral RNA, and $Ct(N)$ value is the average value for ubiquitin RNA used as normalizer (endogenous control).

^d Relative differences in the viral RNA amounts between single-virus-infected plants and plants infected with both viruses expressing symptoms and asymptomatic. $\Delta\Delta Ct = \Delta Ct(T) - \Delta Ct(Rs)$, where $\Delta Ct(Rs)$ is the ΔCt value for the plant infected with a single virus.

interactions that helps in the cell-to-cell movement of synergistic partner viruses (5,17–19,26). Potyvirus HC-Pro is also a strong suppressor of posttranscriptional gene silencing (3). It is possible that a functionally analogous protein of HC-Pro present in BYVaV might contribute to the suppression of gene silencing against BVY, eliminating a natural plant defense.

A similar synergistic interaction involving a potyvirus and a crinivirus has been described in sweet potato severe mosaic disease, where an increase in the titer of a potyvirus (*Sweet potato feathery mottle virus*) contributes to the development of symptoms, whereas the synergistic determinant, a crinivirus (*Sweet potato chlorotic stunt virus* [SPCSV]), is reduced in concentration (12,16). SPCSV is also known to promote synergism with other unrelated viruses, namely *Sweet potato mild mottle virus* (16) and *Cucumber mosaic virus* (8).

BYVaV has been found consistently in BYVD-affected blackberry plants from different parts of the United States (22,23). Although variations in symptoms appear among cultivars and locations, the disease is collectively referred to as BYVD due to the characteristic yellow vein symptoms and the presence of BYVaV. Variations in symptoms such as oak-leaf patterns, irregular chlorosis, and line patterns in blackberry plants from other areas may be caused by co-infection with one or more viruses other than BVY since BVY has not been found in plants from other locations (24). This adds to the complexity and seriousness of this emerging problem. Future studies are required to identify other viruses that may be involved in this disease.

The failure to detect movement of BYVaV to graft-inoculated 'Chester' plants may be due to the restricted occurrence of this virus in phloem tissues. A longer incubation period may be necessary for the phloem-restricted BYVaV to move through grafts between plants. This assumption is partly supported by the finding that approach grafting of 'Chickasaw' and 'Chester' plants singly infected with BYVaV and BVY, respectively, resulted in the movement of BVY to the 'Chickasaw' plants within 4 weeks after grafting but the presence of BYVaV was not detected in the 'Chester' plants 2 months after the approach graft was performed. However, in a different set of experiments, transmission of BYVaV from a BYVD-affected scion to healthy 'Marion' blackberry and 'Meeker' raspberry was detected by RT-PCR assay 4 months after bottle grafting (22). It is likely that in an approach graft, phloem union may not necessarily occur at the graft junction as compared to that of a chip budding technique in which fusion of

phloem vessels ensures success of the grafted scion. Only a cell-to-cell contact between phloem parenchyma can ensure transmission of phloem-limited criniviruses such as BYVaV, while a mere contact between cells is enough to transmit potyviruses such as BVY.

BYVD is a unique and newly described disease that poses a severe threat to the expanding blackberry industry in the southern part of the United States. Emphasis should be given to the identification of virus vectors of BYVaV and BVY, to development of resistant cultivars to replace susceptible ones, and to understanding of the biological interaction of these two viruses in this newly described synergism.

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LITERATURE CITED

- Agranovsky, A., Filimonov, A. S., Filimonova, S. Y., Morozov, S. Y., Schiemann, J., Lese-mann, D., and Atabekov, J. G. 1998. Beet yellows closterovirus HSP70-like protein mediates the cell-to-cell movement of a potexvirus transport-deficient mutant and hordeivirus-based chimeric virus. *J. Gen. Virol.* 79:889-895.
- Alicai, T., Fenby, N. S., Gibson, R. W., Adipala, E., Vetten, H. J., Foster, G. D., and Seal, S. E. 1999. Occurrence of two serotypes of sweet potato chlorotic stunt virus in East Africa and their associated differences in coat protein and HSP70 homologue gene sequences. *Plant Pathol.* 48:718-726.
- Anandalakshmi, R., Pruss, G. J., Ge, X., Marathe, R., Mallory, A. C., Smith, T. H., and Vance, V. B. 1998. A viral suppressor of gene silencing in plants. *Proc. Natl. Acad. Sci. U.S.A.* 95:13079-13084.
- Aranda, M. A., Escaler, M., Wang, D., and Maule, A. J. 1996. Induction of HSP70 and polyubiquitin expression associated with plant virus replication. *Proc. Natl. Acad. Sci. U.S.A.* 93:15289-15293.
- Barker, H. 1987. Invasion of non-phloem tissue in *Nicotiana clelandii* by potato leafroll luteovirus is enhanced in plants also infected with potato Y potyvirus. *J. Gen. Virol.* 68:1223-1227.
- Brunner, A. M., Yakovlev, I. A., and Strauss, S. H. 2004. Validating internal controls for quantitative plant gene expression studies. *BMC Plant Biol.* 4:14.
- Clark, J. R. 1999. University of Arkansas Fruit Varieties: Sources of Blackberries, Grapes, and Blueberries. Cooperative Extension Service, University of Arkansas. MP 418.
- Cohen, J., and Loebenstein, G. 1991. Role of a whitefly-transmitted agent in infection of sweet potato by cucumber mosaic virus. *Plant Dis.* 75:291-292.
- Converse, R. H. 1987. *Virus Diseases of Small Fruits*. U.S. Dep. Agric. Handb. 631.
- Garner, R. J. 1979. *The Grafters' Handbook*. Oxford University Press, New York.
- Hughes, D. W., and Galau, G. 1988. Preparation of RNA from cotton leaves and pollen. *Plant Mol. Biol. Rep.* 6:253-257.
- Karyeija, R. F., Kreuze, J. F., Gibson, R. W., and Valkonen, J. P. T. 2000. Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. *Virology* 269:26-36.
- Lee, Y. S., and Ross, J. P. 1972. Top necrosis and cellular changes in soybean doubly infected by soybean mosaic and bean pod mottle viruses. *Phytopathology* 62:839-845.
- Martin, E. M., Cho, J. D., Kim, J. S., Goeke, S. C., Kim, K. S., and Gergerich, R. C. 2004. Novel cytopathological structures induced by mixed infection of unrelated plant viruses. *Phytopathology* 94:111-119.
- Martin, R. R., Tzanetakis, I. E., Gergerich, R., Fernandez, G., and Pesic, Z. 2004. Blackberry yellow vein associated virus: A new crinivirus found in blackberry. *Acta Hort.* 656:137-142.
- Mukasa, S. B., Rubaihayo, P. R., and Valkonen, J. P. T. 2006. Interactions between a crinivirus, an ipomovirus and a potyvirus in coinfecting sweetpotato plants. *Plant Pathol.* 55:458-467.
- Poolpol, P., and Inouye, T. 1986. Enhancement of cucumber mosaic virus multiplication by zucchini yellow mosaic virus in doubly infected cucumber plants. *Ann. Phytopathol. Soc. Jpn.* 52:22-30.
- Pruss, G., Ge, X., Shi, M. X., Carrington, J. C., and Vance, V. B. 1997. Plant viral synergism: The potyviral genome encodes a broad-range pathogenicity enhancer that transactivates replication of heterologous viruses. *Plant Cell* 9:859-868.
- Rochow, W. F., and Ross, A. F. 1955. Virus multiplication in plants doubly infected by potato viruses X and Y. *Virology* 1:10-27.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Spiers, J. M., Pittman, P. O., and Braswell, J. H. 1998. An overview of the leading small fruit crops grown in the United States. World Conference on Horticultural Research, 17-20 June, Rome, Italy.
- Susaimuthu, J., Gergerich, R. C., Bray, M. M., Clay, K. A., Clark, J. R., Tzanetakis, I. E., and Martin, R. R. 2007. Incidence and ecology of Blackberry yellow vein associated virus. *Plant Dis.* 91:809-813.
- Susaimuthu, J., Tzanetakis, I. E., Gergerich, R. C., and Martin, R. R. 2006. Yellow vein-affected blackberries and the presence of a novel *Crinivirus*. *Plant Pathol.* 55:607-613.
- Susaimuthu, J., Tzanetakis, I. E., Gergerich, R. C., and Martin, R. R. 2008. A member of a new genus in the *Potyviridae* infects *Rubus*. *Virus Res.* 131:145-151.
- Tzanetakis, I. E., Susaimuthu, J., Gergerich, R., and Martin, R. R. 2006. Nucleotide sequence of Blackberry yellow vein associated virus, a novel member of the *Closteroviridae*. *Virus Res.* 116:196-200.
- Vance, V. B., Berger, P. H., Carrington, J. C., Hunt, A. G., and Shi, X. M. 1995. 5' proximal potyvirus sequences mediate potato virus X/potyviral synergistic disease in transgenic tobacco. *Virology* 206:583-590.
- Wintermantel, W. M. 2005. Co-infection of *Beet mosaic virus* with beet yellowing viruses leads to increased symptom expression on sugar beet. *Plant Dis.* 89:325-331.