Identification and Host Relations of Turnip ringspot virus, A Novel Comovirus from Ohio

P. Rajakaruna and **S. Khandekar**, Department of Biological Sciences, University of Toledo, Toledo, OH 43606; **T. Meulia**, Molecular and Cellular Imaging Center, The Ohio Agricultural Research and Development Center, Wooster, OH 44691; and **S. M. Leisner**, Department of Biological Sciences, University of Toledo, Toledo, OH 43606

ABSTRACT

Rajakaruna, P., Khandekar, S., Meulia, T., and Leisner, S. M. 2007. Identification and host relations of Turnip ringspot virus, a novel comovirus from Ohio. *Plant Dis.* 91:1212-1220.

Viruslike chlorotic ring spot symptoms and line patterns of unknown origin were observed on a greenhouse-grown turnip plant. The suspected virus was mechanically transmissible to plants in the *Brassicaceae*. Electron microscopic analysis revealed icosahedral particles approximately 28 nm in diameter. Reverse transcriptase–polymerase chain reaction (RT-PCR) analyses suggested that the pathogen is a comovirus, an observation that was confirmed by analysis of portions of the genomic sequence. This virus was provisionally named Turnip ringspot virus (TuRSV). Based on the RNA 1 sequence, TuRSV is most similar to *Radish mosaic virus*, another pathogen that infects members of the *Brassicaceae*. *Arabidopsis thaliana* is susceptible to TuRSV, and 12 out of the 23 ecotypes studied showed symptoms when inoculated with the virus. TuRSV induced a variety of responses on ecotypes from death to no infection. Some ecotypes showed one or two rounds of symptom display followed by recovery when inoculated with TuRSV. About half of the ecotypes (11/23) analyzed showed no symptoms when inoculated with TuRSV. Col-0 plants showed no symptoms, and infectious virus was not recovered from systemic leaves, although it could be detected by RT-PCR. Col-0 plants harboring mutations impairing the ethylene, jasmonic acid, or salicylic acid signaling pathways did not show symptoms when inoculated with TuRSV.

Additional keyword: *Comoviridae*

Comoviruses are positive-sense, single-stranded, RNA viruses that infect a variety of plant species (14,17). These viruses possess bipartite genomes encoding two polyproteins, one on each RNA. The polyproteins are post-translationally processed by a virus-encoded protease to yield the mature forms of each polypeptide. Although comoviruses infect a variety of plant species, relatively few have been reported to cause disease in members of the Brassica family (5,6,9,10,29).

Some comoviruses possess intriguing virus–host relations. For example, *Bean pod mottle virus* exhibits two flushes of symptom induction and recovery when inoculated to soybean (13). However, the mechanism(s) behind this unusual progression of symptoms is unclear. In many cases where recovery from viral infections is

observed, post-transcriptional gene silencing/RNA interference (RNAi) is involved (34,35). This process permits specific degradation of viral RNA. In addition, plants possess other pathways for protection against viral infection. For example, pathways utilizing salicylic acid, ethylene, or jasmonic acid as signaling molecules protect plants against pathogens (12,36). Plants with defects in these signaling pathways are impaired in their ability to resist infection.

In the work described here, a novel Brassica-infecting comovirus is characterized, the host relations of this virus are examined, and the response shown by a particular ecotype of *Arabidopsis thaliana* (Col-0) to this virus is investigated.

MATERIALS AND METHODS

Plant materials. Turnips (*Brassica rapa* cv. Just Right) were inoculated with cell sap from infected turnip plants (21). Sap was prepared by harvesting turnip leaves infected by the virus that was later provisionally named Turnip ringspot virus (TuRSV). The leaves were subsequently homogenized in 3 ml of grinding buffer (GB; 10 mM potassium acetate, pH 7.2) per gram of tissue. Celite (diatomaceous

earth) was added, and the suspension was mixed and rub-inoculated onto leaves 6, 7, and 8 of turnips at the 12-leaf stage. Inoculated plants were grown in a greenhouse at 22°C under natural lighting. Inoculated plants were observed for symptoms until about 30 days postinoculation (DPI). Because more consistent inoculation results were obtained with TuRSV from *A. thaliana*, the virus was maintained in the Aa-0 ecotype by serial passage.

The *A. thaliana* ecotypes used in this study were provided by S. H. Howell (Iowa State University, Ames). In this study, the *pad4* and *npr1* Col-0 mutants in the salicylic acid–signaling pathway (8,37) were used. The *ein2* and *etr1* mutants in the ethylene-signaling pathway (15) were studied. Finally, the *jar1* mutant that is impaired in the jasmonic acid–signaling pathway (30) was examined. All mutants were kindly provided by The *Arabidopsis* Biological Resource Center at the Ohio State University (Columbus).

A. thaliana seeds were planted in moistened Redi Earth potting soil (BFG Supply, Burton, OH). Plants were propagated in a growth chamber under continuous light of 60 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic active radiation (PAR) at 20°C and fertilized with mineral nutrient solution as described previously (1). *A. thaliana* were inoculated with sap from Aa-0 plants prepared by grinding symptomatic leaves in 3 ml of GB per gram of tissue. Celite was added, and the suspension was mixed and rub-inoculated onto rosette leaves 6, 7, 8, and 9 of *A. thaliana* plants at the 10-leaf stage with a plastic spatula (1). Following inoculation, *A. thaliana* plants were propagated in a growth chamber under a 12-h light (at 60 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, PAR), 12-h dark regime at 20°C and fertilized with mineral nutrient solution as described previously. Plants were observed daily for symptom production up to 84 DPI. Each experiment employed 15 to 20 plants of each ecotype and was performed at least three times. To examine Col-0 infection, sap was prepared from wild-type and mutant Col-0 plants inoculated with TuRSV, harvested at 14 DPI, and inoculated to Aa-0 plants as described above.

Corresponding author: S. M. Leisner
E-mail: sleisne@utnet.utoledo.edu

Accession numbers: DQ665367 and EF191015.

Accepted for publication 17 April 2007.

doi:10.1094/PDIS-91-10-1212
© 2007 The American Phytopathological Society

Partial virus purification. TuRSV was partially purified by a combination of differential and sucrose density gradient centrifugation. Symptomatic *A. thaliana* Aa-0 leaves were harvested at approximately 10 DPI. Leaf tissue was ground with a mortar and pestle at 4°C in 3 to 4 ml of GB per gram of tissue, filtered through three layers of cheesecloth, and the filtrate transferred to 50-ml centrifuge tubes. The filtrate was centrifuged at $11,950 \times g$ for 15 min at 4°C. The supernatant was filtered through one layer of Miracloth (Calbiochem, La Jolla, CA) and then centrifuged for 1 h at $145,921 \times g$ at 4°C. The pellets were re-suspended in GB, layered onto 50 ml of 10 to 40% sucrose gradients, and centrifuged in an SW28 rotor for 2 h at $96,467 \times g$ at 4°C. The gradients were fractionated in 5-ml aliquots. Portions of each fraction were diluted 100-fold with sterile distilled water, Celite was added, and the diluted fractions were inoculated to Aa-0 plants. Aa-0 plants inoculated with samples collected near the center-upper portion of the gradient showed symptoms within 5 days and the plants often died. Aa-0 plants inoculated with the other gradient fractions took at least 2 weeks, if at all, to show symptoms. This purification was performed three times with similar results.

Those fractions with the highest infectivity were diluted 10-fold with GB, transferred to 50-ml ultracentrifuge tubes, and centrifuged at $209,406 \times g$ at 4°C for 2 h. The pellets were pooled and resuspended in 0.5% of the total initial volume of 4°C GB. The resuspended pellets were termed virus concentrate. Virus concentrate was subjected to electron microscopy as described previously (27).

Reverse transcriptase-polymerase chain reaction (RT-PCR) amplification, cloning, and sequence analysis of TuRSV genomic fragments. One gram of symptomatic systemically infected Aa-0 rosette leaf tissue was harvested at 14 DPI and sent to Agdia (Elkhart, IN) for RT-PCR analysis. RT-PCR was conducted with proprietary pan-comovirus primers, and the PCR product, approximately 450 bp, was kindly sent back to the authors.

This PCR product was purified by electrophoresis through a 0.8% agarose gel made with TAE (1 mM EDTA, 40 mM Tris-acetate, pH 8.3) buffer. The ~450-bp band was excised and the DNA fragment purified using the Amicon Ultrafree-DA DNA extraction spun column system (Millipore, Bedford, MA). The extracted PCR product was phenol extracted and concentrated by ethanol precipitation (3). The PCR product was then inserted into the pCR2.1 vector by ligation using the TA Cloning Kit by Invitrogen Corporation (Carlsbad, CA) according to the manufacturer's specifications. The ligation mixture was introduced into *Escherichia coli* strain DH5 α by calcium chloride transformation. *E. coli* harboring the pCR2.1 plasmids

with the PCR product were identified by digestion with *EcoRI*. Those pCR2.1 plasmids with inserts were then sent to MWG Biotech (High Point, NC) for sequencing using the M13 universal forward and reverse primers. Three clones were sequenced in both directions and all were identical. This sequence was examined using BLAST (Basic Local Alignment Search Tool) (2). A preliminary alignment was performed on the comoviral sequences to identify conserved regions downstream of those aligning with the TuRSV sequence. From this, the degenerate reverse oligonucleotide TuRSV-2RC (5' GTIGG-CAAACCTCCCCTTCC 3') was designed. A forward oligonucleotide, TuRSV-1F (5' ACTCCTTTTGTGGAGGAGAC 3'), was designed from inside the above PCR fragment.

Preliminary multiple sequence alignment was performed on RNA 2 of the comoviral genomes in GenBank, and this information was used to design two PCR primers: TuRSV2-1F, 5' CATTTAATC-CAAACCTTGT 3', and TuRSV2-2R, 5' ACCAGCACCICACCTATAG 3'. These primers were predicted to amplify a fragment of approximately 900 bp when used in RT-PCR.

Approximately 0.3 g of TuRSV-infected turnip tissue was harvested at 21 DPI, frozen in liquid nitrogen, and ground in a mortar and pestle. Total RNA was isolated from the infected plant tissue using TRI-ZOL Reagent (<http://plantpath.wisc.edu/~amyc/Protocols/protocols%20-%20plant%20viruses.htm>). Approximately 5% of the RNA isolated was mixed with 1 μ g of random hexamer oligonucleotides, heated at 70°C for 10 min, and then plunged into ice for 5 min. Reverse transcriptase reactions were then initiated in a total volume of 20 μ l by the addition of 100 U of MMLV reverse transcriptase (Promega Corporation, Madison, WI), 20 U of Optizyme Recombinant RNase Inhibitor (Promega), and all four deoxynucleotide triphosphates in the buffer provided by Promega according to their specifications. Samples were incubated at 42°C for 1 h. Following incubation, 1 μ l of the reverse transcriptase mixture was used for PCR.

All PCR reaction mixtures contained a final concentration of 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 200 μ M each dNTP, 0.5 U *Taq* DNA polymerase (Promega), as well 200 nm each TuRSV-1F and TuRSV-2RC primers (Integrated DNA Technologies, Inc., Coralville, IA) (for RNA 1) or the TuRSV2-1F and TuRSV2-2R primers (for RNA 2). The PCR program consisted of a 35 cycle amplification program of 1 min at 92°C, 1 min at 45°C, and 1 min at 72°C. The amplifications were performed in 500- μ l thin wall microcentrifuge tubes in an Eppendorf Mastercycler Personal (Brinkman Instruments, Inc., Westbury, NY). PCR products were separated by

electrophoresis through a 2% agarose gel in TBE buffer (3). A TuRSV RNA 1 fragment of approximately 900 bp and a fragment about 800 bp from RNA 2 were amplified by this approach. The sequence for the TuRSV RNA 1 fragment (accession no. DQ665367) and the portion of RNA 2 aligning with other plant viruses (accession no. EF191015) were submitted to GenBank.

To monitor both viral RNAs simultaneously, two new sets of primers were designed. One pair, TuRSV RNA1sp-1F: 5' TCGCAGGCTAGAAGATTGTGA 3' and TuRSV RNA1sp-2R: 5' GGCCCTAAGTGTTCTCTGGTC 3', was used to amplify a 369-bp fragment from TuRSV RNA 1 (nucleotides 705-1073). A second pair, TuRSV RNA2sp-1F: 5' CTGAGCGCC TAAGTGATGTTCT 3' and TuRSV RNA2sp-2R: 5' CTTTACCTCGGATAC CACTGTTGT 3', was used to amplify a 168-bp fragment from TuRSV RNA 2 (nucleotides 23-190). PCR reactions were performed as above but with both sets of primers in the same reaction mixture. To test the specificity of these primers, approximately 0.05 g of dried *Radish mosaic virus* (RaMV)-infected tissue (ATCC number PV-138) was ground in an RNase free 1.5-ml tube on dry ice. RNA was then isolated from the tissue using the PureLink Plant RNA Reagent (Invitrogen) according to the manufacturer's specifications. RT-PCR was carried out as described above.

The sequence of the putative protein segment encoded by the TuRSV PCR products was compared with the deduced translation product encoded by the aligned segment of either RNA 1 or RNA 2 of other plant viruses by performing a BLAST search (2) for related sequences. Alignments with plant viral sequences with BLAST scores greater than 80 when compared with the TuRSV sequences were performed using the ClustalW program contained within the MegAlign program of the DNASTAR Lasergene 7 software package (Madison, WI) using a Gonnet matrix. The pairwise alignment parameters included a gap penalty of 10.0 and a gap length of 0.1. Multiple alignment parameters were set at a gap penalty of 10.0, a gap length penalty of 0.2, and delay divergent sequences of 30%. The phylogenetic relationship between these viruses was calculated from the distance data. Unrooted phylogenetic trees were constructed using maximum parsimony, and branching patterns were tested by bootstrap analysis of 1,000 trials by employing the PAUP program (31).

Polyacrylamide gel electrophoretic analysis of TuRSV-infected plants. Partially purified virus preparations from the various sucrose gradient fractions were mixed with one-third volume of sample buffer (6% sodium dodecyl sulfate [SDS], 35% glycerol, 15% 2-mercaptoethanol, 0.1% bromophenol blue, 188 mM Tris-

HCl, pH 6.8), and they were incubated for 5 min at 95°C. The heated samples were permitted to cool and subsequently loaded onto a 10% SDS-polyacrylamide gel (Mini-Protean3; BioRad, Hercules, CA) with a 4% stacking gel (3). Gels were run for 1 h at 16 V/cm (95 volts). Polyacrylamide gels were stained overnight in 30% methanol, 10% acetic acid, 0.025% Coomassie Brilliant Blue R250. Gels were destained at room temperature two times for 20 min in 40% methanol, 7% acetic acid. Gels were then destained two additional times for 30 min each in 5% methanol, 7% acetic acid.

Protein bands were excised from the gel by laser and sequenced by the Michigan Proteome Consortium (Ann Arbor, MI). Briefly, the samples were digested with trypsin. The resulting peptides were extracted from the gel plugs with 2% acetonitrile, 0.1% TFA. The extracted peptides were spotted onto the wells of a MALDI target and overlaid with matrix. The analyses were performed using the ABI 4800 MALDI TOF/TOF.

RESULTS

Identification of a virus causing ring spot symptoms on turnips in Ohio. In the spring of 1996, a turnip was discovered in

a greenhouse on the University of Toledo campus that showed chlorotic ring spot symptoms and line patterns along the major veins on leaves. Leaf samples were harvested, dried over anhydrous calcium chloride, and stored for 6 years at 4°C. In 2002, the leaf tissue was removed from storage and inoculated to turnips. The infectious agent induced systemic symptoms on turnips by 8 DPI. Hence, the infectious agent survived in dried leaf tissue for 6 years and was mechanically transmissible. This infectious agent typically did not induce symptoms on the inoculated turnip leaves. The initial symptoms appeared as chlorotic lesions on systemic leaves. By about 25 DPI, older systemic leaves showed chlorotic ring spots and line patterns along veins, while the younger leaves exhibited mainly chlorotic lesions (Fig. 1). In some cases, the line patterns were extensive and outlined leaf regions. Beyond the chlorotic symptoms caused by the virus on noninoculated leaves, infection had few other effects on plant growth. Occasionally, mild stunting was observed on infected turnips. The symptoms appeared to be seasonal, being more prominent in the winter months than during the summer. This pathogen was provisionally termed Turnip ringspot virus (TuRSV).

Fig. 1. Turnip ringspot virus (TuRSV) symptoms on turnip leaves. Turnip leaves systemically infected with TuRSV were photographed at 25 days postinoculation. **A**, Older systemically infected leaf showing ring spots. **B**, Younger systemically infected leaf showing extensive ring spot-line pattern symptoms.

TuRSV is a comovirus. Initial leaf dip electron microscopic analysis of TuRSV-infected plant tissue proved inconclusive, suggesting that the virus was not present at high titers. Therefore, partially purified virus fractions were prepared (see Materials and Methods) and analyzed by negative stain electron microscopy. The virus particles were icosahedral in shape and approximately 28 nm in diameter (Fig. 2A).

RT-PCR assays performed by Agdia using pan-comovirus primers indicated that TuRSV was a comovirus. The RT-PCR product was cloned and sequenced. The PCR product was 429 bp in length (Fig. 3), and it encoded a contiguous open reading frame of 143 amino acids. Based on multiple sequence alignment, the putative polypeptide encoded by the TuRSV RT-PCR product aligned with amino acids 1354–1496 of *Cowpea mosaic virus* (CPMV; accession no. NP_613203) RNA 1 polypeptide (23). CPMV was used for a comparison because it is the type member of the genus *Comovirus* and the complete sequence is known.

To obtain additional nucleotide sequence information from TuRSV, the RNA 1 polypeptide coding sequences for the other comoviruses were aligned, and a conserved region downstream of that aligning with the TuRSV sequences was identified. A degenerate primer based on this conserved region was designed and a larger portion of the TuRSV sequence was amplified by RT-PCR. In toto, the TuRSV RNA 1 sequence reported here is 1,188 nucleotides long and encodes a putative continuous polypeptide of 396 amino acids (Fig. 3) that aligns with CPMV RNA 1 polypeptide amino acids 1354–1750. This region is contained within the portion of the comoviral polypeptide corresponding to the 87-kDa replicase (23). Multiple sequence alignment of the 1,188-bp segment confirms that TuRSV is most similar to the comoviruses. The TuRSV RT-PCR product was most similar to RaMV (accession no. AAY32935) (26). The TuRSV RNA 1 RT-PCR product was 70.1% identical to RaMV at the nucleotide and 78.7% at the amino acid sequence level. Identity to the other comoviruses is between 55 and 65% at the amino acid and 55 and 60% at the nucleotide sequence level. The unrooted phylogenetic tree constructed from the polypeptide multiple sequence alignment data indicated that the comoviruses cluster together and TuRSV was a part of this clade (Fig. 4). However, TuRSV and RaMV formed their own subcluster within that of the genus *Comovirus*.

To obtain sequence information on the other TuRSV genomic segment, RNA 2 sequences for six comoviruses were aligned and two primers were designed to the conserved region spanned by nucleotides 1914–2899 in CPMV (accession no. NC_003550). These primers were used to amplify a 742-bp fragment of RNA 2 from

TuRSV-infected turnip leaves. However, when the sequence of six independent clones (all of which were identical) was translated *in silico*, only a 420-bp portion of this PCR product that encoded 140 amino acids (Fig. 5) aligned with other comovirus RNA 2 sequences. This 140 amino acid segment aligned with amino acids 496–635 of the CPMV RNA 2–encoded polyprotein (GenBank accession no. CAA25314). Multiple sequence alignment showed that the TuRSV RNA 2 sequence identity with other comovirus proteins varied from 35.3 to 47.1% and 47.1 to 51.6% at the RNA level. The unrooted phylogenetic tree derived from the distance data of the polypeptide sequences indicated that TuRSV is distinct from the other comoviruses.

RT-PCR detection of TuRSV. Since sequence data were now available for portions of each TuRSV genomic segment, this information was used to design primers to simultaneously detect both viral RNAs in infected plants by RT-PCR. These primers were also used to test Koch's postulates (17).

RT-PCR products corresponding to both viral RNAs were detected in TuRSV-inoculated leaves of Aa-0 plants, as well as in noninoculated rosette and cauline leaves (Fig. 2B). However, no PCR products were detected in noninoculated Aa-0 control plants. RT-PCR products corresponding to both TuRSV RNAs were detected in partially purified virus preparations. The two TuRSV RT-PCR products were also detected in the leaves of Aa-0 plants inoculated with the partially purified virus preparations, as well as in noninoculated rosette and cauline leaves. This, in addition to the observation that the plants inoculated with the partially purified virus showed symptoms identical to those inoculated with the original virus, fulfilled Koch's postulates.

To determine specificity, the two primer sets were used to attempt to amplify fragments from RaMV, the virus most closely related to TuRSV based on the RNA 1 sequence. Interestingly, only the RT-PCR product corresponding to RNA 2 was amplified with this primer set. The RT-PCR product corresponding to RNA 1 was not detected in these preparations. Hence, the RNA 1 primer pair is specific for TuRSV and further confirmed that this virus is distinct from RaMV.

Analysis of putative TuRSV capsid proteins. To detect possible TuRSV capsid proteins in plant tissue samples, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed, followed by Coomassie blue staining. This analysis detected several protein bands that were more prominent in TuRSV-infected than in noninoculated turnips. However, sequence analysis indicated that none of these bands contained viral polypeptides (data not shown). Therefore, TuRSV was

partially purified as above and the virus-containing fractions were pooled, concentrated, and subjected to SDS-PAGE. Three bands were visible in the 55 to 24 kDa range and a prominent band at approximately 17 kDa was observed (data not shown). All four bands were excised and examined by mass spectrometry/mass spectrometry analysis. The largest and smallest bands and a band approximately 35 kDa in size were identified as ribo-

somal proteins. However, the 40-kDa band did not correspond to *A. thaliana* proteins. Instead, two of the peptides derived from the 40-kDa band corresponded to predicted fragments that could be generated from the predicted TuRSV RNA 2–encoded protein (Fig. 5). Hence, the TuRSV capsid protein is about 40 kDa in size.

TuRSV infection of *A. thaliana* ecotypes induces a variety of symptoms. To examine the variety of responses of *A.*

Fig. 2. Characteristics of Turnip ringspot virus (TuRSV). **A**, Transmission electron micrograph at $\times 100,000$ magnification of partially purified TuRSV negatively stained with phosphotungstic acid. Bar indicates 100 nm. **B**, Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis of TuRSV infection in *Arabidopsis thaliana*. Aa-0 and Col-0 plants (indicated) were inoculated with TuRSV, harvested at 16 days postinoculation, and separated into I, inoculated, R, noninoculated rosette, and C, noninoculated cauline, leaves. The appropriate leaves from 15 plants were pooled together and analyzed by RT-PCR using two primer sets in the same reaction. The TuRSV RNA1sp-1F and TuRSV RNA1sp-2R amplify a 369-bp fragment (nucleotides 705–1073) from TuRSV RNA 1 (upper band). The second pair, TuRSV RNA2sp-1F and TuRSV RNA2sp-2R, amplify a 168-bp fragment (nucleotides 23–190) from TuRSV RNA 2 (lower band). P, partially purified TuRSV; PI, inoculated leaves from plant inoculated with partially purified virus; PR, noninoculated rosette leaves from plant inoculated with partially purified virus; PC, noninoculated cauline leaves from plant inoculated with partially purified virus. M, 100-bp markers (Fisher Scientific), sizes indicated on right; Ra, *Radish mosaic virus* (American Type Culture Collection PV 138) dried tissue extracted and then amplified with the same double primer set as above.

thaliana to TuRSV, several ecotypes were inoculated. TuRSV symptoms varied considerably among the 23 ecotypes tested, with 12 ecotypes developing systemic symptoms (Table 1). By 8 DPI, *A. thaliana* ecotypes Nw-4 and Ct-1 exhibited chlorotic

lesions on inoculated leaves, while Aa-0 showed chlorotic lesions with necrotic centers. None of the other ecotypes showed symptoms on inoculated leaves.

As the infection progressed, symptoms were observed on noninoculated rosette

and cauline leaves for many ecotypes. Infected Aa-0, Nd-0, and Nw-4 ecotypes showed chlorotic lesions on noninoculated rosette leaves that eventually became necrotic beginning about 10 DPI (Fig. 6). By 18 DPI, chlorotic lesions were observed on noninoculated rosette and cauline leaves of ecotypes Be-0, Ct-1, Di-0, Est-0, Oy-0, Pla-1, Rsch-4, Sv-0, and Tsu-0. In addition to these symptoms, Est-0 showed vein clearing on cauline leaves. Finally, ecotypes Alu-1, Bur-0, Col-0, Fr-2, Kas-1, Le-0, Mh-0, Np-1, No-0, RLD, Wil-2, and Ws-0 did not develop symptoms during the course of these experiments.

Ct-1 was the only ecotype that died after virus inoculation, and this occurred by about 30 DPI. All of the other ecotypes showing symptoms eventually recovered from the infection and set seed by 84 DPI. TuRSV-infected Aa-0 (Fig. 6) and Nw-4 plants showed an interesting symptom progression. For example, the majority of rosette leaves of infected Aa-0 plants were dead by about 18 DPI. However, lateral meristems were not killed by the virus, and the plants started to recover from the infection, developing new rosettes by about 16 DPI. By 21 DPI, the plants had developed good rosettes, but were also beginning to show another round of viral symptoms on the recovered tissue. However, after this time, the plants recovered a second time, flowered, and set seed by 84 DPI.

Col-0 plants defective in the ethylene, jasmonic acid, and salicylic acid signaling pathways are asymptomatic when inoculated with TuRSV. Col-0 ecotype plants did not show symptoms when inoculated with TuRSV (Fig. 6A). To determine if the Col-0 plants were infected by the virus but asymptomatic, TuRSV-inoculated Col-0 and Aa-0 plants were harvested at 14 DPI, and cell sap derived from this tissue was inoculated to Aa-0 indicator plants. Aa-0 plants inoculated with cell sap from TuRSV-inoculated Col-0 plants remained asymptomatic, while indicator plants inoculated with cell sap from infected Aa-0 plants were highly symptomatic by 14 DPI. Interestingly, both TuRSV RNAs could be detected by RT-PCR in all portions of Col-0 plants tested (Fig. 2B).

Defense pathways in *A. thaliana* often appear to operate via the salicylic acid, ethylene, or jasmonic acid signaling molecules (12,36). Therefore, Col-0 plants harboring mutations in these signaling pathways were predicted to show increased susceptibility to TuRSV. For the salicylic acid pathway, *pad4*, a mutant that is impaired in the accumulation, and *npr1*, a mutant that is insensitive to this signaling molecule (8,37), were tested. For the ethylene pathway, the *ein2* and *etr1* mutants, which are impaired in their responses to this hormone (15), were examined. Finally,

1																					30	60
GAA	TGT	CCT	AAA	GAT	GAG	AAG	CTG	CCA	TAC	AGA	AAG	ATT	TTT	GAG	AAG	CCT	AAA	ACA	CGT			
E	C	P	K	D	E	K	L	P	Y	R	K	I	F	E	K	P	K	T	R			
1																					10	20
																				90	120	
TGT	TTT	TCT	ATA	CTT	CCG	ATG	GAG	TAC	AAT	CTT	TTG	GTA	CGA	CGC	CGT	TTC	CTA	AAG	TTT			
C	F	S	I	L	P	M	E	Y	N	L	L	V	R	R	R	F	L	K	F			
																				30	40	
																				150	180	
GTT	AGA	TTC	ATT	ATG	AAG	AGA	AGA	GAC	ATT	TTG	CCG	TGC	CAG	GTT	GGT	ATA	AAT	CCG	TAT			
V	R	F	I	M	K	R	R	D	I	L	P	C	Q	V	G	I	N	P	Y			
																				50	60	
																				210	240	
GGA	ATG	GAA	TGG	ACA	GAT	TTG	GCT	GCA	CGC	TTG	AAG	AGC	AAG	GGT	AAT	AAT	GTG	CTT	TGC			
G	M	E	W	T	D	L	A	A	R	L	K	S	K	G	N	N	V	L	C			
																				70	80	
																				270	300	
TGT	GAT	TAC	AGT	AGT	TTT	GAT	GGT	CTA	TTG	TCT	AAA	CAA	GTT	ATG	ACG	GCT	ATG	GCT	AGC			
C	D	Y	S	S	F	D	G	L	L	S	K	Q	V	M	T	A	M	A	S			
																				90	100	
																				330	360	
ATG	ATA	AAC	TCC	TTT	TGT	GGA	GGA	GAC	ACA	AGT	ATC	AAA	AGA	AAA	CGA	GAA	AAT	TTG	TTG			
M	I	N	S	F	C	G	G	D	T	S	I	K	R	K	R	E	N	L	L			
																				110	120	
																				390	420	
ATG	GCA	TGT	TGT	TCT	CGC	TAT	GCC	ATT	TGC	AAG	TCT	GAT	GTC	TGG	CGT	GTT	GAA	TGT	GGG			
M	A	C	C	S	R	Y	A	I	C	K	S	D	V	W	R	V	E	C	G			
																				130	140	
																				450	480	
ATA	CCA	TCT	GGG	TTT	CCA	CTC	ACT	GTG	ATA	TGT	AAT	TCC	ATC	TTC	AAT	GAA	ATT	CTC	ATA			
I	P	S	G	F	P	L	T	V	I	C	N	S	I	F	N	E	I	L	I			
																				150	160	
																				510	540	
CGC	TAC	AGT	TTC	AAA	GCT	ATT	ATG	CGC	AAG	CAG	AAA	GTT	CCA	GGC	CTT	ATT	TCA	ATA	AGC			
R	Y	S	F	K	A	I	M	R	K	Q	K	V	P	G	L	I	S	I	S			
																				170	180	
																				570	600	
TTT	GAC	AAG	TAT	GTT	GGA	ATG	GTT	ACA	TAT	GGG	GAT	GAC	AAC	TTG	CTT	TCT	GTG	AGT	GAA			
F	D	K	Y	V	G	M	V	T	Y	G	D	D	N	L	L	S	V	S	E			
																				190	200	
																				630	660	
GTT	GTT	AAA	CCC	TAT	TTT	GAT	GGA	AAG	AGG	CTG	AAG	GAG	TTT	TTG	GCA	AAG	CAT	AAG	GTT			
V	V	K	P	Y	F	D	G	K	R	L	K	E	F	L	A	K	H	K	V			
																				210	220	
																				690	720	
GTG	ATT	ACA	GAT	GGG	AAA	GAC	AAG	ACC	AGT	CCT	TAC	TTA	CTT	TTT	CGC	AGG	CTA	GAA	GAT			
V	I	T	D	G	K	D	K	T	S	P	Y	L	L	F	R	R	L	E	D			
																				230	240	
																				750	780	
TGT	GAC	TTT	TTG	AAG	CGA	GGT	TTC	AGG	AAG	GAT	AAG	AAT	GGC	ATC	TTT	TGG	AAT	GCT	CCT			
C	D	F	L	K	R	G	F	R	K	D	K	N	G	I	F	W	N	A	P			
																				250	260	
																				810	840	
GAA	GAG	AAA	GAG	AGT	TTG	TGG	GCA	CAG	TTG	CAT	TAT	ATA	AAT	GTT	ACC	AAT	TTA	GAG	CAA			
E	E	K	E	S	L	W	A	Q	L	H	Y	I	N	V	T	N	L	E	Q			
																				270	280	
																				870	900	
CAT	GAG	GCC	TAT	AAA	ACA	AAT	TTG	GTG	AAT	GTG	CTA	CGT	GAG	CTG	TAT	ATG	TGG	GAT	GTT			
H	E	A	Y	K	T	N	L	V	N	V	L	R	E	L	Y	M	W	D	V			
																				290	300	
																				930	960	
AAT	GAG	TGT	TCT	GCT	CTT	AGG	AAG	AAA	GCT	CTA	CAA	CGT	GTG	GAG	TGG	CTG	GTG	CCA	TCA			
N	E	C	S	A	L	R	K	K	A	L	Q	R	V	E	W	L	V	P	S			
																				310	320	
																				990	1020	
GAT	CTC	CCT	ACA	GTT	GCC	CAA	ATA	GAA	GAG	TGG	TAT	GCT	ACA	TGT	CGA	GGA	AGA	TAT	ATG			
D	L	P	T	V	A	Q	I	E	E	W	Y	A	T	C	R	G	R	Y	M			
																				330	340	
																				1050	1080	
CCA	GAT	TCA	GCG	GAT	TCT	ATC	AAC	TTC	CTC	TTA	GAC	CAG	GAA	CAC	TTA	GGG	CCT	TTG	TTG			
P	D	S	A	D	S	I	N	F	L	L	D	Q	E	H	L	G	P	L	L			
																				350	360	
																				1110	1140	
GCA	CCA	CAG	GGT	GTA	CAG	AGA	GGA	GTG	CGT	TTA	ACT	GAC	CAA	GTC	CGT	ACT	GTT	AAT	CTT			
A	P	Q	G	V	Q	R	G	V	R	L	T	D	Q	V	R	T	V	N	L			
																				370	380	
																				1170	1188	
GCA	CAT	GAG	AAC	CAC	ACC	ACA	CGA	AAA	CCA	GGA	GAG	TTG	TGG	ATA	CTG							
A	H	E	N	H	T	T	R	K	P	G	E	L	W	I	L							
																				390	396	

Fig. 3. Nucleotide and predicted protein sequence of a portion of Turnip ringspot virus (TuRSV) RNA 1. Numbers indicate nucleotide (above nucleotide sequence) and amino acid (below amino acid sequence) positions. Underlined, sequence of the initial 429-bp reverse transcriptase-polymerase chain reaction (RT-PCR) product (nucleotides 1–429); double underline, TuRSV-1F primer.

the *jar1* mutant that is impaired in its jasmonic acid responses (30) was studied. While Aa-0 control plants inoculated with TuRSV showed typical symptoms, all of the Col-0 mutants were asymptomatic. Cell sap derived from harvested pooled tissue from TuRSV-infected wild-type or mutant Col-0 plants, subsequently inoculated onto Aa-0, induced no obvious symptoms.

DISCUSSION

Based on a survey of the Virus Identification Database Exchange (5,6,9,10), few viruses infecting turnip show ring spot symptoms. Therefore, when a virus inducing ring spot symptoms on turnips was discovered, further characterization of the pathogen was performed. Electron microscopy of enriched virus preparations showed icosahedral particles approxi-

mately 28 nm in diameter. This virus was provisionally termed Turnip ringspot virus (TuRSV). RT-PCR testing suggested that TuRSV was a comovirus. This was confirmed when the sequences were determined for segments of RNA 1 and RNA 2. Both the RNA 1 and RNA 2 RT-PCR products were most similar to members of the genus *Comovirus* based on BLAST analyses.

Fig. 4. Unrooted phylogenetic tree indicating relationship of the predicted Turnip ringspot virus (TuRSV) RNA 1 polypeptide segment to the aligned region of other plant viruses. Length of branches corresponds to number of amino acid differences as indicated by the scale bar; numbers at nodes indicate percent bootstrap scores; asterisks denote bootstrap values below 50%. Shaded region indicates comoviruses; bold italics indicates TuRSV. Virus abbreviations and accessions (in parentheses below) are: ALSV (NP_620568), *Apple latent spherical virus*; APMoV (Q02941), *Andean potato mottle virus*; ArMV (AAQ73821), *Arabidopsis mosaic virus*; AYRSV (CAJ33468), *Artichoke yellow ringspot virus*; BBWV-1 (NP_945134), *Broad bean wilt virus 1*; BBWV-2 (AAD38152), *Broad bean wilt virus 2*; BRAV (NP_612604), *Blackcurrent reversion virus*; BPMV (AAM73715), *Bean pod mottle virus*; CLRV (CAE83562), *Cherry leafroll virus*; CNSV (NP_734017), *Cycas necrotic stunt virus*; CPMV (NP_613283), *Cowpea mosaic virus*; CPSMV (NP_619518), *Cowpea severe mosaic virus*; CRLV (AAW92113), *Cherry rasp leaf virus*; GCMV (CAA33405), *Grapevine chrome mosaic virus*; GeMV (BAD99001), *Gentian mosaic virus*; GFLV (NP_619689), *Grapevine fanleaf virus*; MCDV (AAV86083), *Maize chlorotic dwarf virus*; NIMV (BAA74537), *Naval orange infectious mottling virus*; PBRV (CAE83563), *Potato black ringspot virus*; PatMMV (NP_647592), *Patchouli mild mosaic virus*; PRMV (AAB69867), *Peach rosette mosaic virus*; PYFV (NP_619734), *Parsnip yellow fleck virus*; RaMV (AAY32935), *Radish mosaic virus*; RCMV (NP_734030), *Red clover mottle virus*; RpRSV (AAQ77238), *Raspberry ringspot virus*; RTSV (NP_042507), *Rice tungro spherical virus*; SDV (NP_620566), *Satsuma dwarf virus*; SLRSV (YP_227376), *Strawberry latent ringspot virus*; SMOV (NP_599086), *Strawberry mottle virus*; StPV (AAZ76594), *Stocky prune virus*; SqMV (NP_620657), *Squash mosaic virus*; TBRV (AAN72830), *Tomato black ring virus*; ToRSV (NP_620765), *Tomato ringspot virus*; TRSV (NP_919040), *Tobacco ringspot virus*; TuRSV (DQ665367), *Turnip ringspot virus*.

The conserved domain database scan (24) performed while the BLAST search was being conducted indicated that amino acids 1–335 of the TuRSV RNA 1–derived RT-PCR product were analogous to an RNA-dependent RNA-polymerase domain (pfam00680). In the comoviruses, this polymerase is approximately 84 kDa in size and is located at the C-terminus of the RNA 1–derived polyprotein. This sequence

contains the GDD sequence that plays a role in catalyzing the polymerase reaction (17).

Phylogenetic analysis based on the sequence of a portion of the 84-kDa polymerase polypeptide sequence encoded by RNA 1 indicates that the comoviruses cluster together into one major clade that includes TuRSV. TuRSV is most similar to RaMV, and the two viruses form their own

subclade within the comoviruses. This is interesting since both viruses are capable of infecting plants within the *Brassicaceae* causing ring spot symptoms (see 5,6,9,10,29 for data regarding RaMV). The TuRSV polypeptide is less than 80% identical at the amino acid sequence level to the orthologous RaMV protein segment. This degree of sequence identity indicates that the TuRSV sequence is different from that of RaMV. Likewise, RT-PCR reactions employing TuRSV RNA 1 primers failed to generate a product from a RaMV template. Taken together, these data indicate that TuRSV is distinct from RaMV.

The portion of RNA 2 sequence reported here (Fig. 5) encoded a deduced polypeptide orthologous to the N-terminal-proximal segment of the CPMV large capsid protein (amino acids 496–635; GenBank accession no. CAA25314). The phylogenetic tree for the TuRSV RNA 2 sequence was much smaller than that for RNA 1 because it showed limited amino acid sequence identity (35 to 47%) with only the comoviruses (data not shown). These data confirm that TuRSV is a distinct member of the genus *Comovirus*.

SDS-PAGE performed on infected plant tissue extracts was not sensitive enough to detect viral polypeptides (data not shown). In addition, virus particles were detected by electron microscopy only after partial purification. Taken together, these data suggest that TuRSV accumulated to a relatively low titer in infected plants. Interestingly, when virus was partially purified, several ribosomal proteins were found along with the virus capsid protein. The size of the TuRSV coat protein (~40 kDa) is in the correct range for a comovirus (14,16). However, comoviruses typically encode two coat proteins: a large one about 40 kDa and a smaller one about 20 kDa. No 20-kDa protein band was observed on the gels of enriched virus fractions. This suggests at least three possibilities. First, the TuRSV genome may not encode an ~20-kDa protein. Second, the TuRSV “small” coat protein may be the same size as the larger one and might co-migrate on the gel. Third, the ~20-kDa protein is there but below the detection limit by SDS-PAGE.

TuRSV was mechanically transmissible to another member of the *Brassicaceae*. Examination of TuRSV infection in *A. thaliana* indicated that ecotypes vary considerably in their responses to the pathogen. In fact, these data suggest that *A. thaliana* ecotypes exhibit at least four types of responses to TuRSV. First, the Ct-1 ecotype is killed by TuRSV. Second, Aa-0 and Nw-4 go through two rounds of symptom display and recovery. Third, most of the ecotypes initially showing symptoms (e.g., Est-0) recover from the infection. Finally, many of the ecotypes tested, including Col-0, did not show symptoms when inoculated with TuRSV.

Fig. 5. Nucleotide and predicted protein sequence of a portion of Turnip ringspot virus (TuRSV) RNA 2. Numbers indicate nucleotide (above nucleotide sequence) and amino acid (below amino acid sequence) positions. Underlined amino acids correspond to peptides derived from the 40-kDa protein identified by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of purified virus preparations.

Table 1. Turnip ringspot virus symptoms on *Arabidopsis* ecotypes

Ecotype	Inoculated leaves ^a	Systemic leaves ^b	Final result ^c	Development rate ^d
Aa-0	CL/NL	CL/VC	I/R/I/R	S
Alu-0			NS	F
Be-0		CL	I/R	S
Bur-0			NS	F
Col-0			NS	M
Ct-1	CL	CL/VC	I/D	M
Di-0		CL	I/R	F
Fr-2			NS	S
Est-0		CL/VC	I/R	M
Kas-1			NS	M
Le-0			NS	F
Mh-0			NS	S
Nd-0		CL	I/R	F
No-0			NS	M
Np-1			NS	F
Nw-4	CL	CL/VC	I/R/I/R	M
Oy-0		CL/VC	I/R	M
Pla-1		CL	I/R	S
RLD			NS	M
Rsch-4		CL	I/R	S
Sv-0		CL	I/R	S
Tsu-0		CL	I/R	S
Wil-2			NS	F
Ws-0			NS	F

^a Symptoms on inoculated leaves: CL, chlorotic lesions; NL, necrotic lesions.

^b Symptoms on systemic leaves: CL, chlorotic lesions; VC, vein clearing.

^c Progression of infection: I, infection; R, recovery; D, death; NS, no symptoms.

^d Developmental rate based on time to bolting following germination: F, fast (bolts 15.5 to 18 days postgermination); M, moderate (bolts 19 to 31 days postgermination); and S, slow (bolts 32 to 41 days postgermination) (22).

Perhaps the most interesting ecotypes were those such as Est-0 and Aa-0, which pass through one and two rounds of symptom display and recovery, respectively. This type of host–virus system is unique to our experience with *A. thaliana*. However, the two rounds of symptom display and recovery exhibited by the Aa-0 and Nw-4 ecotypes are reminiscent of the situation with the *Comovirus* sp. *Bean pod mottle virus* (BPMV) in soybean (13). Because recovery of plants is often associated with RNA interference (RNAi) (33–36), this may provide an explanation for the phenomena observed with TuRSV infection of these *A. thaliana* ecotypes. These data are intriguing because at least one comovirus protein/gene has been proposed to suppress RNAi in the host (7). Because most of the ecotypes showing symptoms when inoculated with TuRSV recover from infection, these data would suggest that TuRSV cannot completely suppress RNAi. Possibly repression of RNAi is a characteristic either that TuRSV has lost, or that is not effective in certain *A. thaliana* ecotypes.

As observed previously with other viruses (20–22), ecotypes that flower rapidly appear more resistant to TuRSV than plants that flower more slowly. Some of this is likely due to “developmental resistance (22).” Developmental resistance was proposed to be due to the pattern of virus transport through the plant and could explain why most of the rapidly flowering ecotypes (e.g., Alu-1, Bur-0, Le-0, Np-1, Wil-2, and Ws-0) are asymptomatic when inoculated with TuRSV. In contrast, the majority of the slow-flowering ecotypes (e.g., Aa-0, Be-0, Pla-1, Rsch-4, Sv-0, and Tsu-0) show symptoms when infected by TuRSV, even though they eventually recover. This is, of course, not an absolute rule, because there are fast-flowering ecotypes (e.g., Di-0 and Nd-0) that show symptoms when inoculated with TuRSV, and there are asymptomatic slow-flowering varieties (e.g., Fr-2 and Mh-0). The ecotypes that flower at a moderate rate are more variable, containing an equal number of symptomatic (e.g., Ct-1, Est-0, Nw-4, and Oy-0) and asymptomatic (e.g., Col-0, Kas-1, No-0, and RLD) plants when inoculated with TuRSV.

As appears to be true of other plant viruses, the symptoms induced by TuRSV on *A. thaliana* ecotypes are virus-specific. For example, both Di-0 and Tsu-0 are susceptible to TuRSV, but resistant to *Turnip crinkle virus* and to most isolates of *Cauliflower mosaic virus* (CaMV), respectively (1,4,11,21,25,28). The Aa-0 ecotype is susceptible to both CaMV and *Beet curly top virus*, but recovery of the plants after infection in either case was not reported (19,20). A similar observation is true for Est-0 plants: they are susceptible to CaMV and *Cucumber mosaic virus* but recovery was not observed

(20,32). Est-0 is infected with lethal results by another member of the *Comoviridae*, *Tobacco ringspot virus* (TRSV) (18). Hence, different members of a virus family can induce different symptoms on the same ecotype.

Finally, Col-0 is tolerant to TuRSV infection because the plants were asymptomatic, yet virus could still be detected throughout the plant (16). This is intriguing because Col-0 is also tolerant of TRSV infection (18). Impairment of the ethylene, jasmonic acid, or salicylic acid signaling pathways in Col-0 did not compromise the response of these plants to TuRSV. Hence, the ethylene, jasmonic acid, or salicylic acid signaling pathways are not required to inhibit symptom formation; otherwise these plants would be symptomatic. Alternatively, these signal-

ing pathways may be functionally redundant.

It is intriguing that infectious virus was not recovered from TuRSV-infected *A. thaliana*. Our prior data show that TuRSV accumulated to low titers in infected plants. Perhaps TuRSV accumulated to lower levels in Col-0 than in the other ecotypes. Under these circumstances, TuRSV could be detected by RT-PCR but may not be present at a high enough quantity to initiate new infections when inoculated to indicator plants.

In summary, we report the characterization of a comovirus discovered in Ohio, provisionally named TuRSV, that infects plants in the *Brassicaceae*. TuRSV infects *A. thaliana* ecotypes with a range of consequences. Thus, we can now employ the power of *A. thaliana* molecular genetics to study Comovirus–host relations.

Fig. 6. Responses of *Arabidopsis thaliana* ecotypes to Turnip ringspot virus (TuRSV). **A**, Aa-0 (left) and Col-0 (right) infected with TuRSV and photographed at 14 days postinoculation. **B**, Recovery and development of systemic symptoms on Aa-0 plants by TuRSV. Aa-0 plants at 21 days postinoculation show the development of new (recovered) rosette leaves and are surrounded by a halo of dead leaves. **C**, Enlargement of boxed area of B showing reinfection by TuRSV. Arrow indicates a second round of infection on recovered tissue.

ACKNOWLEDGMENTS

We thank John Gray, Stephen Goldman, Lirim Shemshedini (The University of Toledo, Toledo, OH), and the University of Toledo Plant Science Research Center for their assistance. We also thank Stephen H. Howell (Iowa State University, Ames) for the *Arabidopsis* ecotypes and The *Arabidopsis* Biological Resource Center at the Ohio State University (Columbus) for the mutants used in this study. Finally, we thank Agdia (Elkhart, IN) for their kind assistance in providing the TuRSV RT-PCR product. This work was supported in part by NIH grant no. 1R15AI50641-01, USDA grant no. 96-3503-3284, USDA SCA 58-3607-1-193, and the University of Toledo deARCE Memorial Endowment Fund in Support of Medical Research and Development, and the Ohio Plant Biotechnology Consortium, administered through the Ohio Agricultural Research and Development Center.

LITERATURE CITED

- Agama, K., Beach, S., Schoelz, J., and Leisner, S. M. 2002. The 5' third of *Cauliflower mosaic virus* gene VI conditions resistance breakage in *Arabidopsis* ecotype Tsu-0. *Phytopathology* 92:190-196.
- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 25:3389-3402.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. 1993. *Current Protocols in Molecular Biology*. Greene Publishing Associates and John Wiley & Sons, Cambridge, MA.
- Balazs, E., and Lebeurier, G. 1981. *Arabidopsis* is a host of *Cauliflower mosaic virus*. *Arabidopsis Inf. Serv.* 18:130-134.
- Boswell, K. F., Dallwitz, M. J., Gibbs, A. J., and Watson, L. 1986. The VIDE (Virus Identification Data Exchange) project: A database for plant viruses. *Rev. Plant Pathol.* 65:221-231.
- Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L., and Zurcher, E. J. Plant Viruses Online: Descriptions and Lists from the VIDE Database (20) August, 1996. <http://image.fs.uidaho.edu/vid/>
- Canizares, M. C., Taylor, K. M., and Lomonosoff, G. P. 2004. Surface-exposed C-terminal amino acids of the small coat protein of *Cowpea mosaic virus* are required for suppression of silencing. *J. Gen. Virol.* 85:3431-3435.
- Cao, H., Bowling, S. A., Gordon, A. S., and Dong, X. 1994. Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. *Plant Cell* 6:1583-1592.
- Dallwitz, M. J. 1980. A general system for coding taxonomic descriptions. *Taxon* 29:41-47.
- Dallwitz, M. J., Paine, T. A., and Zurcher, E. J. 1993. User's Guide to the DELTA System: A general system for processing taxonomic descriptions. 4th ed. CSIRO Division of Entomology, Canberra.
- Dempsey, D. A., Wobbe, K. K., and Klessig, D. F. 1993. Resistance and susceptible responses of *Arabidopsis thaliana* to *Turnip crinkle virus*. *Phytopathology* 83:1021-1029.
- Feys, B. J., and Parker, J. E. 2000. Interplay of signaling pathways in plant disease resistance. *Trends Genet.* 16:449-455.
- Gillaspie, A. G., and Bancroft, J. B. 1965. The rate of accumulation, specific infectivity, and electrophoretic characteristics of *Bean pod mottle virus* in bean and soybean. *Phytopathology* 55:906-908.
- Goldbach, R. W., and Wellink, J. 1996. Comoviruses: Molecular biology and replication. Chapter 3 in: *The Plant Viruses: Polyhedral Virions and Bipartite Rna Genomes*. B. D. Harrison and A. F. Murant, eds. Plenum Press, New York.
- Guzman, P., and Ecker, J. R. 1990. Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. *Plant Cell* 2:513-523.
- Heitefuss, R. 1997. General principles of host-parasite interactions. Pages 19-32 in: *Resistance of Crop Plants Against Fungi*. H. Hartleb, R. Heitefuss, and H.-H. Hoppe, eds. Gustav Fischer, Stuttgart.
- Hull, R. 2002. *Matthews' Plant Virology*. 4th ed. Academic Press, San Diego.
- Lee, J. M., Hartman, G. L., Domier, L. L., and Bent, A. F. 1996. Identification and map location of *TTR1*, a single locus in *Arabidopsis thaliana* that confers tolerance to *Tobacco ringspot nepovirus*. *Mol. Plant-Microbe Interact.* 9:729-735.
- Lee, S., Stenger, D. C., Bisaro, D. M., and Davis, K. R. 1994. Identification of loci in *Arabidopsis* that confer resistance to geminivirus infection. *Plant J.* 6:525-535.
- Leisner, S. M., and Howell S. H. 1992. Symptom variation in different *Arabidopsis thaliana* ecotypes produced by *Cauliflower mosaic virus*. *Phytopathology* 82:1042-1046.
- Leisner, S. M., Turgeon, R., and Howell, S. H. 1992. Long distance movement of *Cauliflower mosaic virus* in infected turnip plants. *Mol. Plant-Microbe Interact.* 5:41-47.
- Leisner, S. M., Turgeon, R., and Howell, S. H. 1993. Effects of host plant development and genetic determinants on the long-distance movement of *Cauliflower mosaic virus* in *Arabidopsis*. *Plant Cell* 5:191-202.
- Lomonosoff, G. P., and Shanks, M. 1983. The nucleotide sequence of cowpea mosaic virus B RNA. *EMBO J.* 2:2253-2258.
- Marchler-Bauer, A., Anderson, J. B., Cherukuri, P. F., DeWeese-Scott, C., Geer, L. Y., Gwadz, M., He, S., Hurwitz, D. I., Jackson, J. D., Ke, Z., Lanczycki, C. J., Liebert, C. A., Liu, C., Marchler, G. H., Mullokandov, M., Shoemaker, B. A., Simonyan, V., Song, J. S., Thiessen, P. A., Yamashita, R. A., Yin, J. J., Shang, D., and Bryant, S. H. 2005. CDD: A conserved domain database for protein classification. *Nucleic Acids Res.* 33:D192-196.
- Melcher, U. 1989. Symptoms of *Cauliflower mosaic virus* infection in *Arabidopsis thaliana* and turnip. *Bot. Gaz.* 150:139-147.
- Petrzik, K., Hola, M., and Spak, J. 2005. Complete nucleotide sequence of Radish mosaic virus RNA polymerase gene and phylogenetic relationships in the genus Comovirus. *Acta Virol.* 49:271-275.
- Redinbaugh, M. G., Seifers, D. L., Meulia, T., Abt, J. J., Anderson, R. J., Styer, W. E., Ackerman, J., Salomon, R., Houghton, W., Creamer, R., Gordon, D. T., and Hogenhout, S. A. 2002. *Maize fine streak virus*, a new leafhopper-transmitted rhabdovirus. *Phytopathology* 92:1167-1174.
- Simon, A. E., Li, X. H., Lew, J. E., Stange, R., Zhang, C., Polacco, M., and Carpenter, C. D. 1992. Susceptibility and resistance of *Arabidopsis thaliana* to *Turnip crinkle virus*. *Mol. Plant-Microbe Interact.* 5:496-503.
- Spak, J. 1992. Characterization, purification and serology of the Czechoslovak isolate of *Radish mosaic virus*. *Acta Virol.* 36:191-197.
- Staswick, P. E., Su, W., and Howell, S. H. 1992. Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. *Proc. Natl. Acad. Sci. USA* 89:6837-6840.
- Swofford, D. L. 2000. PAUP*: Phylogenetic Analysis Using Parsimony and Other Methods (software). Sinauer Associates, Sunderland, MA.
- Takahashi, H., Goto, N., and Ehara, Y. 1994. Hypersensitive response in *Cucumber mosaic virus*-inoculated *Arabidopsis thaliana*. *Plant J.* 6:369-377.
- van Regenmortel, M. H. V., Fauquet, C. M., Bishop, D. H. L., Carstens, E. B., Estes, M. K., Lemon, S. M., Maniloff, J., Mayo, M. A., McGeoch, D. J., Pringle, C. R., and Wickner, R. B. 2000. *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*. Academic Press, San Diego.
- Voinnet, O. 2001. RNA silencing as a plant immune system against viruses. *Trends Genet.* 17:449-459.
- Voinnet, O., Pinto, Y. M., and Baulcombe, D. C. 1999. Suppression of gene silencing: A general strategy used by diverse DNA and RNA viruses of plants. *Proc. Natl. Acad. Sci. USA* 96:14147-14152.
- Whitham, S. A., and Dinesh-Kumar, S. P. 2002. Signalling in plant-virus interactions. In: *Plant Signal Transduction*. D. Scheel and C. Wasternack, eds. Oxford University Press, Oxford.
- Zhou, N., Tootle, T. L., Tsui, F., Klessig, D. F., and Glazebrook, J. 1998. *PAD4* functions upstream from salicylic acid to control defense responses in *Arabidopsis*. *Plant Cell* 10:1021-1030.