Richard S. Nelson, Ph.D.

Scientist, Plant Biology Division
Ph.D., 1985, Biology, University of Illinois, Urbana-Champaign
Adjunct Associate Professor, Department of Plant Pathology, Oklahoma State University, Stillwater

Research emphasis: plant virology

Interactions between viral and plant factors are vital for viruses to accumulate and move in their hosts. Research in Nelson's laboratory is directed toward understanding these interactions. Additionally, Nelson and his researchers are utilizing this information to develop tools to study plant developmental processes. Collaborative research with multiple laboratories having similar interests has been extremely fruitful during the last two years.

The researchers have determined that *Tobacco mosaic virus* (TMV) contains a suppressor of gene silencing, the 126 kDa protein, that protects both viral and non-viral target RNA. They are investigating how this suppressor of RNA silencing functions. Their working hypothesis is that the 126 kDa protein creates a safe haven for target RNA within a protein complex refractory to host silencing enzymes. They also have determined that *Nicotiana benthamiana* is a natural mutant for an RNA-dependent RNA polymerase (RDR), RDR1, normally responsible for controlling tobamovirus accumulation through RNA silencing (see selected publication below). The inserted nucleotide sequence responsible for the premature termination of RDR1 in *N. benthamiana* was unique within the *Nicotiana* species they have tested and correlates perfectly with the unique systemic necrosis phenotype observed in this plant after TMV infection. Nelson's lab is investigating whether the necrosis is due to the loss of the RNA silencing system (i.e., allowing over-accumulation of host proteins induced during virus infection) or the over-accumulation of virus leading to the induction of other host defense systems responsible for necrosis.

Viruses must move both within and between cells to cause the familiar systemic symptoms observed in hosts. Researchers have determined that the 126 kDa protein from TMV, previously shown by others to be a cell-to-cell movement protein, can, in the absence of other viral proteins, co-align with microfilaments (MFs) in living plant cells. The 126 kDa protein aligns with the MFs as a complex containing many 126 kDa proteins and was observed to move along the MFs. TMV viral replication complexes also trafficked along MFs. They are continuing studies in this area to determine whether the 126 kDa protein or the previously characterized 30 kDa movement protein from TMV drives this trafficking. The results from their studies allow a mechanistic explanation for the requirement of the 126 kDa protein for cell-to-cell movement of the virus. The host factors involved in both TMV intracellular movement and RNA silencing are being studied through multiple methods.

Richard S. Nelson, Ph.D.

One method to study the function of plant proteins necessary for virus infection or plant development is through virus-induced gene silencing (VIGS). Sequences in and around those of cloned viruses are modified to allow the expression of a foreign gene insert. The foreign gene insert is targeted, along with the rest of the invading virus sequence, for destruction by the host RNA silencing system. In this manner, the function of specific host genes can be monitored due to the knock-down of their mRNA and protein accumulation. Nelson and his researchers have characterized a *Brome mosaic virus* (BMV) isolate that infects native tall fescue and rice in collaboration with Dr. Rouf Mian, formerly in the Noble Foundation's Forage Improvement Division. The RNA genomic components within the virus that allow infection of rice have been identified. The cloned virus has been modified for use in reverse genetic screens to determine host gene function. External funding has been obtained to identify the best rice cultivars and optimize the vector for VIGS studies.

In addition to the internally and externally-supported research to characterize and improve the BMV-mediated VIGS system, Nelson's lab has entered into productive interactions with other laboratories studying virus accumulation and movement. They interact with Dr. Yi Li at Peking University to study *Rice dwarf virus* (RDV) movement. In work spearheaded by Dr. Li, the cell-to-cell movement protein, Pns6, encoded within the 12-segment genome of RDV was identified. Li is now using the Nelson lab's BMV vector to further study the movement and accumulation functions of various viral and host factors in rice as they continue their collaboration. Through collaborative work with Dr. Jeanmarie Verchot-Lubicz at Oklahoma State University, the mechanism of cell-to-cell movement by *Potato virus X* (PVX), a member of a large group of viruses that utilize a triple-block of gene products for this function, has been studied. This work has provided both laboratories information allowing model building for virus movement. A third interaction yielding important information has utilized Nelson's laboratory's experience in protein localization studies through cell biological methods. In an interaction with Drs. Ning-Hui Cheng and Kendal Hirschi at the Baylor College of Medicine, Houston, Texas, researchers have determined the subcellular location of a host protein, CXIP4, which interacts with a proton/calcium antiporter. CXIP4 resides predominantly in the nucleus, but with some expression in the cytoplasm, and this information suggests an interesting regulation system for the calcium transport system.

Through work within and between laboratories in

Richard S. Nelson, Ph.D.

the United States and internationally, Nelson and his researchers continue to explore the mechanism of virus accumulation and movement and apply this information for practical benefit.

Ongoing Projects

Protein aggregate formation during plant virus infection and its relationship with disease and RNA silencing

Identification and functional analysis of host and viral factors involved in RNA silencing and suppression of silencing

Isolation of host proteins involved in cell-to-cell movement of viruses

Development and use of virus vectors for gene knockout through virus-induced gene silencing

Lab Staff 2003-2005

Shelly A. Carter – research associate
Xin Shun Ding – senior research associate II
Shu-Jun Yang – postdoctoral fellow
Matthew Cross – summer hourly student
Jian-Zhong Liu – postdoctoral fellow
Anthony B. Cole – postdoctoral fellow
Shuirong Zhang – intern
Nobumitsu Sasaki – postdoctoral fellow
Palanichelvam Karuppaiah – postdoctoral fellow
Ying Chen – intern
Srinivasa Chaluvadi – postdoctoral fellow
Kim Ballard – research assistant
Yi Li – visiting scientist (on Rockefeller Grant; Professor, Peking University)

Selected Publication

Yang, S.-J., Carter, S.A., Cole, A.B., Cheng, N.-H. and Nelson, R.S. (2004). A natural variant of a host RNA-dependent RNA polymerase is associated with increased susceptibility to viruses by *Nicotiana benthamiana*. *Proceedings of the National Academy of Sciences, USA* 101: 6297-6302.

Nicotiana benthamiana displays more intense symptoms during infection with members of the genus Tobamovirus and is a host for a broad range of RNA and DNA viruses. The cause of these unusual disease and susceptibility phenotypes has been a mystery to plant virologists for nearly a century. Work in a multitude of laboratories has shown that RNA silencing is an important host defense response to virus challenge. In this publication, researchers show that *N. benthamiana*

Richard S. Nelson, Ph.D.

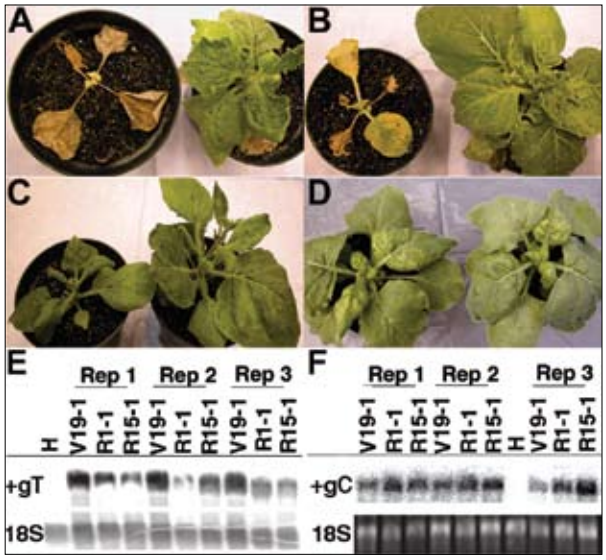

Figure 1. Symptom suppression and decreased viral RNA accumulation displayed by T1 progeny of *N. benthamiana* expressing *MtRdRP1* after challenge with *Tobacco mosaic virus, Sunn hemp mosaic virus, Turnip vein clearing virus, or Cucumber mosaic virus* TMV, SHMV, TVCV or CMV. For A–D (images), the plant on the left did not contain *MtRdRP1* (line V19-1), and the plant on the right expressed *MtRdRP1* (line R1-1 for TMV, SHMV, or CMV and R15-1 for TVCV). (A) Symptoms at 21 dpi with TMV U1. (B) Symptoms at 21 dpi with SHMV. (C) Symptoms at 21 dpi with TVCV. (D) Symptoms at 14 dpi with CMV. (E) Accumulation of TVCV RNA in plants inoculated with TVCV. Leaves were harvested at 10 dpi, and total RNA from *MtRdRP1*-expressing (lines R1-1 and R15-1), vector control (line V19-1) and healthy (H) plants was hybridized with a probe complementary to the 3' end of the TVCV genome (nucleotides 5455–6311; +gT). The same membrane was probed for 18S rRNA (18S). Each group of three lanes represents results from developmentally matched plants. (F) Accumulation of CMV RNA in plants inoculated with CMV. Leaves were harvested at 13 dpi, and total RNA from *MtRdRP1*-expressin, vector control and healthy plants was hybridized with a probe complementary to CMV RNA 2 (nucleotides 367–840;+gC). Ethidium-bromide-stained 18S rRNA (18S) was visualized on the membrane. Each group of three lanes represents results from developmentally matched plants.

is a natural mutant in the RNA silencing pathway that controls the accumulation of tobamoviruses in other species. The altered gene encodes a member within the host RNA-dependent RNA polymerase family, RDR1. Through transformation of *N. benthamiana* with a functional salicylic acid-inducible ortholog of the RDR1 from *Medicago truncatula*, they were able to increase the resistance of *N. benthamiana* against a range of tobamoviruses (*Sunn hemp mosaic virus*, *Turnip vein clearing virus*, *Tobacco mosaic virus*; Figure 1). However, they did not increase resistance against other RNA viruses such as *Cucumber mosaic virus* and *Potato virus X*. The results indicate that *N. benthamiana* lacks a fully active RDR1 leading to the intense symptoms exhibited by tobamoviruses. These results also likely explain why TMV expressing an attenuated 126 kDa suppressor protein of RNA silencing can infect *N. benthamiana* well. However, the lack of increased resistance against other plant viruses indicates that other plant factors, functioning *in trans* or *in cis*, must control

Richard S. Nelson, Ph.D.

the range of viruses that can infect *N. benthamiana*. The researchers are investigating whether the absence of protection against these other viruses is due to inactivity of other host-mediated protection pathways or due to improper expression (either amount or location) of complementing, active RDR1 from *Medicago truncatula*.

Scientific Community Service

Editorial board, *Molecular Plant Pathology*

Reviewed manuscripts for the journals *Journal of General Virology, Journal of Virology, Journal of Virological Methods, Molecular Plant-Microbe Interactions, Plant Journal, Proceedings National Academy of Sciences, Virology*

Ad hoc reviewer for Binational Agriculture Research and Development (BARD) Fund, National Science Foundation Grant Program, United States Department of Agriculture Competitive Research Grants Office

Co-organizer, Plasmodesmata 2004, 5th International Conference on Plasmodesmata and Function, 2004

Organizer, Noble Foundation Virology Mini-Retreat, 2003

Guest lecturer in Plant Pathology on long-distance virus movement, Oklahoma State University, 2004

Graduate student committee member, Department of Entomology/Plant Pathology, Oklahoma State University

Invited Talks

Ohio State University, Columbus, 2003

Phloem 2003, International Conference on Phloem Transport, Bayreuth, Germany

University of Wisconsin, Madison, 2004

Plasmodesmata 2004: International Conference on Plasmodesmal Function, Pacific Grove, Calif.

John Innes Centre, Norwich, U.K., 2004

University of York, Centre for Novel Agricultural Products, York, U.K,, 2004

Rice Functional Genomics Meeting, Tucson, Ariz., 2004

Publications 2003-April 2005

Mitra, R., Krishnamurthy, K., Blancaflor, E., Payton, M., Nelson, R.S. and Verchot-Lubicz, J. (2003). The *Potato virus X* TGBp2 protein association with the endoplasmic reticulum plays a role in but is not sufficient for viral cell-to-cell movement. *Virology* 312: 35-48.

Nelson, R.S., Ding, X.S. and Carter, S.A. (2004). Virus movement in plants. p. 1280-1283. In Encyclopedia of Plant and Crop Science ed R.M. Goodman, Marcel Dekker, New York

Richard S. Nelson, Ph.D.

Yang, S.-J., Carter, S.A., Cole, A.B., Cheng, N.-H. and Nelson, R.S. (2004). A natural variant of a host RNA-dependent RNA polymerase is associated with increased susceptibility to viruses by *Nicotiana benthamiana. Proceedings of the National Academy of Sciences, USA* 101: 6297-6302

Cheng, N.-H., Liu, J.-Z., Nelson, R.S. and Hirschi, K. D. (2004). Characterization of CXIP4, a novel *Arabidopsis* protein that activates the H+/Ca2+ antiporter, CAX1. *FEBS Letters* 559: 99-106.

Li, Y., Bao, Y. M., Wei, C. H., Kang, Z.S., Zhong, Y.W., Mao, P., Gang, W., Chen, Z.L., Schiemann, J. and Nelson, R.S. (2004). Rice dwarf phytoreovirus segment S6-encoded nonstructural protein has a cell-to-cell movement function. *Journal of Virology* 78: 5382-5389.

Ding, X.S., Liu, J., Cheng, N.-H., Folimonov, A., Hou, Y.-M., Bao, Y., Katagi, C., Carter, S.A. and Nelson, R.S. (2004). The *Tobacco mosaic virus* 126kDa protein associated with virus replication and movement suppresses RNA silencing. *Molecular Plant-Microbe Interactions* 17: 583-592.

Ding, X.S. and Nelson, R.S. (2004). Sources of virus inoculum under natural conditions. p. 109-111. In Viruses and Virus diseases of *Poaceae (Gramineae)*. eds H. Lapierre and P.-A. Signoret, INRA editions, Versailles, France.

Mian, M.A.R., Zwonitzer, J.C., Hopkins, A. A., Ding, X.S. and Nelson, R.S. (2005). Response of tall fescue genotypes to a new strain of *Brome mosaic virus. Plant Disease* 89: 224-227.

Nelson, R.S. (2005). Movement of viruses to and through plasmodesmata. p. 188-211. In Plasmodesmata. ed K.J. Oparka, Blackwell Publishing, Oxford, U.K.

Liu, Jian-Zhong, Blancaflor, E.B. and Nelson, R.S. (2005) The *Tobacco mosaic virus* 126-kDa protein, a constituent of the virus replication complex, alone or within the complex aligns with and traffics along microfilaments. *Plant Physiology* (in press).