Noel T. Keen

Noel T. Keen—Pioneer Leader in Molecular Plant Pathology

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Abstract

Noel T. Keen (1940–2002) made pioneering contributions to molecular plant pathology during a period when the study of disease mechanisms was transformed by the new tools of molecular genetics. His primary contributions involved race-specific elicitors of plant defenses and bacterial pectic enzymes. In collaboration with Brian J. Staskawicz and Frances Jurnak, respectively, Noel cloned the first avirulence gene and determined that pectate lyase C possessed a novel structural motif, known as the parallel β-helix. Noel received his B.S. and M.S. from Iowa State University in Ames and his Ph.D. from the Department of Plant Pathology at the University of Wisconsin in Madison in 1968. He joined the faculty of the Department of Plant Pathology at the University of California at Riverside the same year and remained there his entire career. He served as Chair of the department from 1983 to 1989 and in 1997 assumed the William and Sue Johnson Endowed Chair in Molecular Plant Pathology. He became a Fellow of the American Phytopathological Society in 1991, a Fellow of the American Association for the Advancement of Science in 1996, a Fellow of the American Academy of Microbiology in 1997, and a member of the National Academy of Sciences in 1997. He was serving as President of the American Phytopathological Society (2001–2002) at the time of his death.

INTRODUCTION

Noel T. Keen was a pioneer who opened large territories of plant pathology research to molecular investigation in the latter part of the twentieth century. Between 1968 and 2002 he strode rapidly through a series of important research areas, leaving a legacy of key discoveries, useful tools, thriving younger investigators, and a transformed research landscape. Much of the history of molecular plant pathology over the past quarter century can be followed through the example of Noel's research as his interests progressed from the biochemistry of defense elicitors and phytoalexins, to the molecular biology of avirulence proteins and pectic enzymes, and eventually to the functional genomics of bacterial virulence. In each case, Noel exploited technological breakthroughs to probe phenomena that had broad explanatory utility in plant pathology.

Noel was bred and trained in the Midwest but spent his entire career at the University of California at Riverside (UCR). As a scientist, he was a citizen of the world and had particularly close relationships with laboratories in Japan. Noel's stature in the field was marked by high honors such as election to the National Academy of Sciences and the presidency of the American Phytopathological Society, but his influence far exceeded these honors. In the following sections we attempt to explain the effect of his work on the field of molecular plant pathology during a period of transformative change in biology. What will be harder to convey is the picture of Noel as a person, with his deep Midwestern drawl of colorful vernacular, his disarming directness, his fundamental kindliness, and his legendary generosity. These attributes doubled the impact of Noel's pioneering work, because in addition to opening many new research territories, he encouraged others to bring their plows, get to work, and join the harvest.

FROM THE FARM TO PRODUCTIVE M.S. RESEARCH IN IOWA

Noel was born in Marshalltown, Iowa, on August 13, 1940. He was raised on a family farm and attended a small rural school where he spent much of his free time hanging around the science labs. His sister, Judith J. Carr, recalls Noel's youth: "Noel was raised on a family farm in central Iowa—an excellent setting to learn many practical and artful skills. On the farm we all learned the hard work and focus needed to get the crops planted between spring rain storms or to harvest the alfalfa before an impending storm. Both of our parents were great organizers and used limited resources to obtain the maximum result in farm business practices, crop rotation, gardening, etc. Certainly, teamwork on the farm was important, and other farmers are always ready to pitch in and help one another in a time of need." Many of Noel's traits can be traced back to his youth on the farm. These include his deep interest in nature and agriculture, his hard work habits, his openness to collaborative teamwork, his rural swagger and speech, and his manner of keeping a down-to-earth egalitarianism while becoming a giant in his profession.

Noel attended Iowa State University in Ames, obtaining a B.S. in botany in 1963 and an M.S. in plant pathology in 1965. His M.S. thesis research, guided by James C. Horton, produced four papers addressing the regulation of plant cell wall–degrading enzymes in *Phoma* (*Pyrenochaeta*) *terrestris*, the causal agent of pink root of onion (9, 10, 23, 24). The papers demonstrated that pathogen cellulase synthesis is repressible by sugars, that polygalacturonase is the primary fungal factor contributing to maceration activity, and that changes to the plant that increased root sugar content also increased resistance to pink root. The work was hypothesis driven, addressed mechanisms underlying pathogenesis, used a variety of biochemical techniques, involved both the pathogen and the host, and yielded insights potentially useful in disease control. The thoroughness and productivity of this early work, as well as the attention to both mechanism and application, were predictive of the rest of Noel's career.

A "DIAMOND IN THE ROUGH" PH.D. STUDENT AT MADISON

Noel pursued his Ph.D. in the Department of Plant Pathology at the University of Wisconsin, Madison, under the guidance of Paul Williams. He first studied proteolytic activity and histology of *Pseudomonas syringae* pv. *lachrymans* in angular leaf spot of cucumber and then turned to a multifaceted study of the physiology of clubroot of cabbage disease caused by *Plasmodiophora brassicae*. Noel developed a method to isolate intact plasmodia, documented increased uptake of photosynthates into infected hypotcotyls, and studied pathogen-induced changes in lipid and starch metabolism (28, 34, 35, 58). Williams noted that Noel always thought in chemical terms and he had a unique ability to convert the language of chemistry to biology. As with his M.S. research and throughout his career, Noel used a remarkable array of chemical and biological methods in his research. Noel's research generated more than a dozen publications, which were compiled into a thesis in place of a separately written thesis document—a harbinger of the modern Ph.D. thesis in many areas of science.

Noel's strong personality is well remembered by his advisor and his Wisconsin colleagues (43). "Unbounded energy, intensity, and total commitment to the scientific process distinguished Noel as a student. He had no fear of failure, because he was driven more by the process of science and its mechanisms rather than the outcomes. There were no disappointing results if the process in getting the results was pure." Noel is also remembered for his colorful language and as being a "diamond in the rough," beneath whose "gruff exterior was a sensitive and thoughtful person who cared deeply about others' feelings" and was "incredibly generous with his lab mates, spending hours training them and explaining concepts without a trace of arrogance or impatience . . . seemingly unaware of his exceptional intelligence and talent." Noel's lab mate John Mildenhall recalls, "As a foreigner and newcomer to the department, I soon realized that Noel was a man dedicated to his research. One aspect of his student days that made an indelible impression on me was his strict routine—he worked every night until 10 PM and he would be back in the lab before 8 AM the next morning. After writing examinations most of us would recuperate at the local tavern for a few beers, but Noel would quietly return to his lab and continue with his research."

Noel also had a reputation for playing rough in the realms of scientific debate and athletics. John Mildenhall recalls: "He was forthright in his opinions and I recall presenting a seminar and Noel disagreed with some of my conclusions, and the debate that followed was fierce!!" Paul Williams taught Noel to play soccer, where Noel became a "bull in a china shop." One of Noel's idiosyncrasies was that the extraordinary generosity he showed in his research activities did not translate into his athletics. He was a fiercely competitive athlete, and the roster of Noel's soccer opponents who had to be carried off the field with bruised shins even included faculty members like Luis Sequeira. As Paul Williams recalls, some in the department (particularly faculty) had a hard time knowing what to make of Noel, but they eventually recognized him as a giant.

Noel's legendary ability to speak with spell-binding clarity about science—despite the conditions or audience—was also evident at this early stage in his career, as recalled by Michael Stanghellini, a Ph.D. student at Berkeley at the time and later a colleague of Noel's at Riverside. The incident involved the Annual Meeting of the American Phytopathological Society in Washington, DC, in 1967. "We all were graduate students at that time. Several of us, including Noel, were selected to make evening research presentations to the society. It was all rather traumatic. Noel, just before his talk, went to the men's room and placed his slides on a shelf in the restroom. While occupied, someone walked off with his slides and he had to give the presentation 'a capella'. He received a standing ovation." Noel's speaking skill ought not have been so surprising to the audience, but they were unaware that Noel showed his promise much earlier when he won an Iowa state public speaking contest in high school.

As summarized by Paul Williams, "they broke the mold after making Noel Keen." Noel received his Ph.D. in 1968 and went directly to his first (and only) job.

AT THE BENCH IN RIVERSIDE

Noel joined the faculty of the Department of Plant Pathology at UCR in 1968 and remained there his entire career. Although his responsibilities at the departmental, national, and international level grew constantly, Noel, remarkably, never abandoned the lab bench. As described by his colleague William Dawson, Noel was almost like Dr. Jekyll and Mr. Hyde in this regard. There was the "professional Noel, immaculately dressed and giving an eloquent talk with no notes to an international audience, and then there was the everyday, lab rat Noel with the old pair of tennis shorts with the pockets hanging out below the cuff and the stained tee shirt with the ballpoint pen stuck in the collar." Noel worked alongside his students and postdocs throughout his career, and his own bench was an epicenter for many of the seismic shifts in technology that transformed the study of plant disease mechanisms from disease physiology to molecular plant pathology.

A glimpse into Noel's life in the lab is provided in the following comments from Ching-Hong Yang, a senior postdoc in the last four years of Noel's life: "The one thing that impressed me the most was the enthusiasm of Professor Keen for science—all science, not just plant sciences. Whenever there was a new discovery in the lab, Noel was excited like a child at Christmas. He would go back and forth from his office, lab and library (luckily, the UCR Science Library was just next to our building), showing us papers we should read, discuss with us how to further approach the project and some alternative ways to confirm the work. Noel was always eager to be back in his lab and office. If he was out of town for a meeting and back at home at 4 PM, at 4:30 PM we would see him in the lab. Noel never gave up his bench work, even while he was Chair of Plant Path, or the Genetics Program—didn't matter how busy he was, he always spent significant time working in the lab. During the four-year period that I worked with him, Noel never failed to show me every result from his research notebook, DNA bands in the gels, proteins he purified, bacterial colonies he got from cloning plasmids; he also included the bench work that failed."

As is described below, Noel always worked at the cutting edge of technology and he switched research topics readily when new tools made an important problem open to rigorous investigation. Noel noted, when reviewing plant pathology's advances in the twentieth century (18), that "a frustration of the years of 'physiological/biochemical' work was that hypotheses could not be tested rigorously." As a major contributor to the era of physiological research, Noel experienced that frustration directly, and he rapidly adopted (and adapted) new generations of molecular genetic techniques that offered more power in discovery and hypothesis testing.

Noel's curiosity was omnivorous and he worked on a surprisingly wide array of topics, while making landmark advances in a few high priority areas. There appear to have been five keys to Noel's success in research. The first was hard work. Not only did he regularly work nights and weekends throughout his career, but as noted by his UCR colleague and collaborator, James Sims, Noel would sometimes take petri dishes of cultures along with him on trips so he could finish experiments in his hotel room. The second key was a near-photographic memory for experimental detail in his own work and in the papers that he had read. The third key was a broad grasp of important and emerging ideas in biology, chemistry, and biochemistry and an ability to apply that knowledge to research in molecular plant pathology. The fourth key was a rapid work pace, which enabled Noel to write and make administrative decisions quickly so that he could return to the lab. The fifth key was a complete openness to collaboration, although, as will be described below, this typically favored the success of his collaborators and everyone in the field as much as Noel.

ELICITORS AND PHYTOALEXINS: THE DISEASE PHYSIOLOGY ERA

Noel's early work at UCR focused on two economically important diseases—Verticillium wilt of cotton caused by the fungus *Verticillium albo-atrum* and stem and root rot of soybean caused by the oomycete *Phytophthora sojae* (then considered a fungus and designated *P. megasperma* var. *sojae*). These were difficult experimental systems, as there were no established virulence factors such as host-specific toxins or pectic enzymes to anchor the research, and the genetics of the interactions were poorly defined and largely intractable. Noel produced an initial flurry of publications with *V. albo-atrum* exploring catabolic enzyme regulation, polygalacturonase, and protein-lipopolysaccharide complexes, the last of which is still an important part of models for the virulence of this pathogen (6, 27). Noel then focused on Phytophthora rot of soybean. With this pathosystem he addressed the fundamental question of how pathogens elicit plant defenses, the chemical nature of those defenses, and the role of elicitors and antimicrobials in controlling host range—questions that Noel continued to address for the rest of his career.

A key feature of the Phytophthora rot of soybean pathosystem was the availability of resistant cultivars that produced strong defenses and phytoalexins in response to specific races of *P. sojae* (14, 29). Noel obtained evidence implicating surface glycoproteins as defense elicitors (25), and he strengthened the general case for the role of phytoalexins in plant resistance. Phytoalexins were a major focus of research during this era because these secondary metabolites are produced in abundance in tissues expressing defenses and they could be isolated, characterized, and shown to have antimicrobial activity using tools available at the time. Noel also demonstrated a correlation between phytoalexin production and alleles promoting gene-for-gene resistance in the flax-*Melampsora lini* pathosystem and between phytoalexin production and specific elicitor preparations in the race-cultivar-specific soybean-*P. sojae* pathosystem (26). In addition, Noel explored the genetics of *P. sojae* and obtained evidence that the pathogen is diploid and capable of heterokaryosis (39, 40).

Noel was a founding contributor to research on elicitors, and he became a major contributor to the phytoalexin field (19). Two reviews he wrote in the early 1980s give particularly useful insights into the thinking and experimental evidence of that era (15, 16). However, Noel's frustrations with these systems and the limitations of research in this era were well founded (18). For example, *P. sojae* produces multiple elicitors and Noel was unsuccessful in developing an effective genetic system with *P. sojae* that would permit rigorous testing of the role of these elictors. *Melampsora lini* was genetically tractable, but it was recalcitrant to biochemical study because of its inability to be cultured. Furthermore, we now know that in many (but not all) pathosystems, race-cultivar recognition occurs within plant cells, not at the host-pathogen interface, as Noel and others at that time logically expected.

In response to these frustrations, in the early 1980s Noel began exploring the physiological requirements for the hypersensitive response that is elicited in a race-specific manner by another pathogen of soybean—the bacterium *P. syringae* pv. *glycinea* (21). As will be explained next, Noel's shift to this pathosystem prepared him to make founding contributions to the era of molecular plant pathology. Finally, it is worth noting that we now know that the "fungus" *P. sojae* is actually an oomycete, and that Noel's observation of a diploid mycelium was therefore absolutely correct. Furthermore, Noel's early work with *P. sojae* helped establish this organism as a model pathogen that eventually became the first oomycete to be sequenced (57).

GENE-FOR-GENE INTERACTIONS: DAWN OF THE MOLECULAR PLANT PATHOLOGY ERA

Noel's interest in the molecular basis for host specificity, particularly race-cultivar specificity, was likely driven by the agricultural importance of this phenomenon. The breeding of plant resistance through the introduction of single, dominant resistance (*R*) genes began early in the 1900s (1). However, such resistance is often unstable in the field because it can be defeated by new races of the pathogen, and by the 1940s repeated introduction of new *R* genes had produced complex sets of interacting races and cultivars in several economically important pathosystems. Based on genetic studies of the flax-*M. lini* pathosystem, Harold Flor proposed that these cultivar-race interactions were controlled by the interactions of matching plant pathogen avirulence (*avr*) genes and host *R* genes acting in a gene-for-gene manner (5). Although *avr* genes could be defined genetically, connecting them to the world of elicitor biochemistry required knowing what these genes encoded. This required new technology, and as Noel noted in a list of "Twentieth-century milestones in the study of plant-parasite interactions," the construction of the first recombinant plasmid by Cohen and Boyer in 1973 ushered in the era of molecular cloning, which was to have great impact on plant pathology (18). By the early 1980s, the technology had developed sufficiently to be applied to the cloning of the first *avr* gene from *P. syringae* pv. *glycinea*.

For this work, Noel teamed up with Brian Staskawicz, who had similar interests in race specificity and was then at a pioneering plant biotechnology company, the International Plant Research Institute (IPRI), in San Carlos, CA. Noel spent a sabbatical leave at IPRI in 1980, working with Brian and Douglas Dahlbeck to learn recombinant DNA techniques and to apply them to the cloning of genes encoding bacterial pectic enzymes (for proof-of-principle) and then avirulence genes. Reasoning that an *avr* gene would have a gain-of-function avirulence phenotype if expressed in a normally virulent race, they constructed a cosmid DNA library of race 6 (avirulent on cultivar Harosoy) and conjugated it into race 5 (normally virulent on Harosoy). In so doing, they were able to identify and characterize a single gene, denoted *avrA*, which was responsible for the avirulence of race 6 on Harosoy and other resistant cultivars (53). Data from this classic paper also connected Noel's earlier work involving phytoalexins to *avrA*-dependent defense elicitation.

This finding had seismic repercussions on the field. It provided both molecular confirmation of the most counterintuitive part of Flor's gene-for-gene hypothesis and an elegant method for cloning many *avr* genes from a variety of plant pathogenic bacteria. Furthermore, we now know that these bacterial proteins are delivered into plant cells where they collectively defeat basal plant defenses (unless their presence or activity is detected by cognate R protein sentinels) and that similar events are occurring in the interactions of plants with many fungal, oomycete, and nematode pathogens (13). Thus, the "Avr" proteins are actually double agents with a primary function in virulence, and the cloning of *avrA* was the first step into a vast world of molecular interdiction and subterfuge that is now thought to underlie the host-specific interactions of many pathogens with plants (3, 52).

Having established the role of *avr* genes in the interaction of *P. syringae* pv. *glycinea* races with soybean cultivars through the collaboration with Brian Staskawicz, Noel turned to the role of *avr* genes in higher levels of host specificity—namely, in the specificity of *P. syringae* pathovars for different species of plant hosts. For example, why is pathovar *tomato* not a pathogen of soybean? To address this question, Noel and graduate student Donald Kobayashi constructed a cosmid library of *P. syringae* pv. *tomato* DNA, mobilized it into *P. syringae* pv. *glycinea* race 4 (which is virulent on a wide range of soybean cultivars), and screened the race 4 transconjugants for avirulence phenotypes in several soybean cultivars. Thus, three *avr* genes were identified in pathovar *tomato*, which collectively could account for the avirulence of this pathovar on all cultivars of soybean (37).

Noel made additional key contributions to our understanding of the structure and function of avirulence genes. First, he and his student Stanley Tamaki sequenced the closely related *avrB* and *avrC* genes from *P. syringae* pv. *glycinea* and then performed domain swaps to localize the determinants of race specificity (55, 56). They found that the *avr* genes were flanked by repeated sequences and had unusually low %GC values, which suggested the genes had been recently acquired by *P. syringae*. Swapping domains within the genes revealed that the terminal regions were functionally interchangeable and that specificity was controlled by the central regions of the proteins. We now know that *avr* genes have been acquired by *P. syringae* through horizontal gene transfer events and the N-terminal portions of bacterial Avr proteins carry universal signals that target them to the injector pathway. Thus, Noel's pioneering observations of the cognate DNA sequences and domain structures of the *P. syringae* AvrB and AvrC proteins were predictive of the evolution and modular nature of all of these proteins.

Noel's further investigation of the *P. syringae* pv. *tomato* genes that have avirulence phenotypes in *P. syringae* pv. *glycinea* produced additional important discoveries. For example, Noel and graduate student Jennifer Lorang demonstrated that *avrE* is necessary for the full virulence of *P. syringae* pv. *tomato* PT23 in tomato (42). This was an important observation because it provided the first direct evidence that individual avirulence genes have a role in virulence in *P. syringae*. Subsequent studies by several research groups revealed that *avrE* homologs are universally present and important among bacterial pathogens in the *Pseudomonadaceae* and *Enterobacteriaceae*, which demonstrates again Noel's instinct for finding the most important factors in pathogenesis. Furthermore, Noel's pioneering DNA sequencing efforts with *P. syringae* pv. *tomato* DC3000 revealed that *avrE* is linked to the *hrp* (hypersensitive response and pathogenicity) genes encoding the avirulence protein injector system (41). These sequences provided an early glimpse into the genome of DC3000, which subsequently became the first strain of *P. syringae* to be fully sequenced (2).

SYRINGOLIDES: FROM AVIRULENCE GENES TO BIOCHEMISTRY

In an intriguing, final chapter to his study of race-specific elicitors, Noel discovered that the avirulence gene *avrD*, which is present in several pathovars of *P. syringae*, directs the production of a family of low-molecular-weight elicitors of the hypersensitive response, known as syringolides (32). Syringolides can be isolated from bacteria expressing *avrD*, and they elicit the hypersensitive response only in soybean cultivars carrying the *Rpg4* resistance gene. The first *avrD* gene was cloned from *P. syringae* pv. *tomato* on the basis of its ability to confer avirulence to *P. syringae* pv. *glycinea* race 4 (37). Noel collaborated with the Staskawicz lab in discovering that *avrD* directed the production of a race-specific elicitor (32), and the subsequent elucidation of the structure of the elicitor was done through collaboration with UCR colleague James Sims (44), with whom Noel collaborated beginning in 1969 on projects involving the biochemistry of fungal respiratory enzymes, soybean phytoalexins, host-specific toxins, and antimicrobial compounds. Substantial progress has been made in characterizing the ability of several *P. syringae* avirulence proteins to trigger the hypersensitive response through modification of defense-related proteins within plant cells (8), but the avirulence activity of AvrD remains unique in involving a low-molecular-weight, nonpeptide elicitor that can be produced in bacteria.

Noel tried to unravel the AvrD mystery by identifying functional polymorphisms in alleles from different *P. syringae* strains and by identifying a syringolide-binding protein in *Rpg4* soybean using radiolabeled syringolide 1 (11, 12). Surprisingly, the 34-kDa binding protein was also present in *rpg4* soybean cultivars. In search of the *Rpg4* product, Noel sought soybean proteins that interact with the P34 syringolide receptor. Okinaka et al. (45) reported that the receptor interacts with a soybean photorespiration enzyme, NADH-dependent hydroxypyruvate reductase, and that the inhibition of this activity by syringolide probably induces the hypersensitive response. However, the reductase does not appear to be a product of *Rpg4*, and the mystery is further heightened by remarkable similarities between the molecular behaviors of the bacterial syringolides and the fungal host-specific toxin victorin (45). Solving this mystery is now left as a challenge for the next generation.

PECTIC ENZYMES: FROM GENE SEQUENCES TO PROTEIN STRUCTURES

Noel and the IPRI team reported the cloning of the first pathogen pectic enzyme genes the same year they published the cloning of *avrA* (20). Pectic enzymes had been implicated for decades as important virulence factors in the soft rot diseases caused by *Erwinia carotovora* and *Erwinia chrysanthemi* (recently reclassified as *Pectobacterium carotovorum* and *Dickeya dadantii*, respectively) but their function had not been genetically investigated. Noel inaugurated the molecular genetic era of *Erwinia* pectic enzyme research by being the first of several researchers in France, England, Belgium, and the United States to report the cloning of *pel* (pectate lyase) genes (20). While other groups focused on mutagenesis of the multiple *pel* genes in each *E. chrysanthemi* strain and identification of regulatory proteins controlling *pel* expression, Noel investigated the structure and function of the four Pel isozymes produced by *E. chrysanthemi* EC16. Noel and his students were pioneers in sequencing *pel* genes, characterizing their promoters, and then overexpressing the genes with strong heterologous promoters (7, 31). The *pel* genes strongly expressed in *Escherichia coli* were used to demonstrate that pectate lyase production is sufficient for bacterial maceration of plant tissues (31), to characterize depolymerization mechanisms of the different isozymes (48), and, most importantly, to obtain crystals of the purified pectate lyases.

The availability of purified pectate lyase C enabled a collaboration with Frances Jurnak, who was then a structural biologist in the Department of Biochemistry at UCR. As reported in a 1993 paper in *Science* by Yoder et al. (59), solving the pectate lyase C crystal structure revealed a new domain motif known as the parallel β-helix, which is formed by the parallel association of β-strands in the protein. The team went on to determine the structure of pectate lyase C complexed with a plant cell wall fragment and to determine that pectate lyase E has a similar parallel β-helix structure (38, 50). Diverse other proteins were subsequently shown to have the parallel β-helix structure. This work represents a landmark collaborative contribution of molecular plant pathology research to fundamental biology.

GENOMICS: AN AUSPICIOUS BEGINNING ON A NEW FRONTIER

Genomics was the final research frontier that Noel explored. In a 1999 commentary entitled "Functional genomics: Plant-microbe interactions gingerly puts a foot in the water" (36), Noel and Ching-Hong Yang decried the slow progress in this area by asking "why has plant-microbe interactions, a natural field for functional genomics approaches, been so slow adopting them?" They went on to say that "our field has historically been cautious to invoke new technologies and philosophies" and "a more serious problem has been the rather myopic failure of monetary sources to recognize and fund the power of the new technology." Noel did three things to remedy the situation. He lobbied federal agencies to support competitive grant programs that would be open to plant pathogen genome projects. He wrote, with Nicole Perna at the University of Wisconsin, a successful USDA/NSF grant proposal for sequencing the genome of *E. chrysanthemi* 3937. And, characteristic of Noel, he jump-started a genomewide analysis of *E. chrysanthemi* gene expression in planta by using a microarray comprised of random clones. Because there were no 3937 genome sequence data yet available, interesting clones that were differentially expressed in planta were identified by individual sequencing reactions. This first microarray analysis of a plant pathogen in infected tissue was a technical achievement, and the report by Okinaka et al. (46) revealed a new world of metabolic adaptations by the bacterium to life in plants. Occurring near the end of his life, it represents a landmark paper at the dawn of yet another era in molecular plant pathology research.

PROMOTING MOLECULAR PLANT PATHOLOGY THROUGH NEW TOOLS

Through keeping his hands on the tools of molecular biology, Noel saw ways to improve them, and he generated a variety of resources for the molecular plant pathology research community. Probably the most important of these tools was a set of improved broad-host-range vectors for DNA cloning in Gram-negative bacteria (33). One of these vectors, pRK415, is still in wide use nearly 20 years later because of the unique set of desirable features Noel had the foresight to engineer. The paper describing these vectors has garnered more than 900 citations thus far. Other tools that Noel developed or improved include a Tn7-*lux* system for studying bacterial gene expression (51), a helium flow device for particle bombardment transformation of plants (54), and a set of plasmids expressing *E. chrysanthemi* pectic enzymes at very high levels (31). These tools and the many virulence-related genes that Noel cloned were freely available to anyone who asked. In fact, the experience that all requesters seem to share is that of receiving the material so quickly that it had to have been put in the mail within minutes of Noel hanging up the phone. And along with the requested plasmid usually came a scribbled note with a few cryptic restriction sites and antibiotic markers identified, the significance of which often only became clear after the requestor began working with the construct and realized that Noel was way ahead in anticipating what would be needed. Like a modern-day Johnny Appleseed, Noel freely spread his insights, techniques, tools, and clones so that the field could flourish.

IN THE DEPARTMENT AND AT HOME IN RIVERSIDE

Noel was a force in the Department of Plant Pathology at UCR, beginning with his arrival in 1968, when he reorganized, modernized, and successfully taught the department's graduate course in Physiological Plant Pathology. Although Noel's primary activity was always research, he served as Chair of the department from 1983 to 1989 and as Chair of the UCR Genetics Graduate Program from 1994 to 1997, as Acting Director of the UCR Biotechnology Center from 1997 to 2001, and as a member of the Board of Directors of Connecting Research and Economic Development for the 21st Century (CORE21), a consortium of universities bringing technology to the marketplace, from 1998 to 2002. Noel's commitment to science education at all levels is further revealed by the major role he played in establishing the Alpha Center at UCR, which coordinates math and science outreach to K-12 students. Noel was also Chair of the Chancellor's Advisory Committee, which was responsible for a major overhaul of biological sciences teaching in the College of Natural and Agricultural Sciences. Moreover, among faculty associated with the Citrus Experiment Station, he was a leader in contributing to the biology curriculum by teaching undergraduate molecular biology and advising biological sciences undergraduates. Noel was also an active member of multiple graduate programs, including Plant Pathology, Genetics, Microbiology, and Biochemistry and Molecular Biology.

As a department chair, Noel made "simple and rapid decisions based on first order pros and cons," recalls UCR colleague Allan Dodds. Importantly, Noel was one of the social directors and role models who set the personality of the department. Furthermore, according to William Dawson, "Noel had a way of diffusing potential friction between members of the department. He was just so open about everything."

Noel taught several courses, most notably Physiological Plant Pathology. The course taught students the importance of both concept and detail in science and included historical background, current literature, and even unpublished data. Noel was a proponent of associating names with research and expected students to accurately connect researchers with their accomplishments. His method of course grading was ahead of its time and included journal article presentations and grant proposal writing because he believed these exercises had enduring value for graduate students. Noel had a terrifying reputation in Ph.D. qualifying exams for asking students very basic questions designed to measure their ability to think under pressure, as well as to determine if they had read the latest issues of *Science* and *Nature*. As Allan Dodds noted, "His favorite first question to the victim was 'What is a transistor?' This set the tone nicely." But all who knew Noel, throughout his life, always emphasize that his challenges to students and colleagues were intellectual, not personal. In fact, as William Dawson notes, "Noel was without any degree of arrogance. Beginning students, undergraduates, anybody interested in science was his colleague and equal. I think that is how he touched so many students that came through the department. No matter in what area they worked, there is hardly a student that will not tell you of the positive impact Noel had on their career."

Throughout his career, Noel believed in playing hard as well as working hard. He was athletic, an avid tennis player, and an organizer of numerous departmental volleyball games. His fierce competitive streak would show in these games, and if his team was losing he was like "a dog with a bone" and would not allow the game to end, even one year as a thunderstorm struck. Noel was a participant and usually an organizer or host in a variety of social events, including Fridays at the "Barn" and "Bull and Mouth", Chicken Liquor parties, St. Patrick's Day Wakes, hikes up the "C" mountain, cribbage, softball, volleyball, tennis, and badminton games, as well as the Departmental Coffee Hour. He had a passion to entertain and often threw parties at his home that included offerings of home-made "Mule" chili, cooked and raw oysters (despite a departmental experience of food poisoning at one of these gatherings). These parties were often preceded by a prep night, which was in effect, a preparty that on one occasion ended in a food fight with Noel as a participant in his own kitchen. Other entertainment favorites that he hosted included Halloween costume parties with ridiculous games in which all were required to participate. As a man of routine, Noel was the primary catalyst for Fridays at the Barn, and often with some prodding more than 20 department folks would attend. It was at the Barn that Noel introduced his colleagues to the Fox. The Fox was Noel's soul mate Diane, who became his beloved wife.

Diane asked Noel, "If I am a fox, what animal are you?" Noel replied that he was a mule. He explained that he was a mule because it is an honest, hardworking animal. In the presence of The Mule and The Fox, good times at the Barn are remembered by many.

In later years, Noel's strong curiosities and technical abilities supported passions outside of the lab for fine wines, fast sports cars, and high-end audio equipment. He and Diane hosted wine tasting events at their home, and he owned several sports cars, which were kept in mint condition. His discerning ear and knowledge of audio equipment put him in the league of connoisseurs who become reviewers of speakers and various peripherals for *Audio Asylum* and similar online publications. The last paragraph of a detailed 2001 review of Ensemble Megaflex speaker cables reveal the familiar Noel in this unfamiliar world. "Summing up, my system and my ears clearly preferred the Ensemble Megaflux FSF cables. However, I offer the caveat that I have worked hard to improve things in the crucial areas of vibration control and power conditioning . . . . But if things are right, or as close to right as your pocketbook will allow, the Ensemble cables faithfully reveal what's on the software and what the other components are doing. As with everything in this racket, when you take it to the limit, it is easy to fall over the edge. We of course all try to perch right on that edge!"

THE U.S.-JAPAN SEMINARS AND OTHER INTERNATIONAL ACTIVITIES

Noel was a prominent speaker at international conferences throughout his career. Representative of his activities are the many keynote talks he gave in the 1990s: the second European Federation of Plant Pathology Conference, Strasbourg, 1992; the fifth Conference of the International Society for Molecular Plant-Microbe Interactions, Seattle, 1992; the 1992 Annual Meeting of the South African Society for Plant Pathology; the 1994 Annual Meeting of the Israel Phytopathology Society; and the Garret Memorial Lecture at the 1995 Annual Meeting of the British Society for Plant Pathology. Noel was also honored with a Gatsby Traveling Fellow Lectureship by the John Innes Institute, Norwich, England, in 1992, and was a Distinguished Lecturer for the Japan Society for the Promotion of Science in 1998.

The latter honor was particularly meaningful because Noel had had a long relationship with many Japanese scientists and was a major participant in the U.S.-Japan Scientific Seminar Series. These seminars focus on host-parasite interactions and are supported by the National Science Foundation and the Japan Society for the Promotion of Science. The meetings typically alternate between the United States and Japan and have been held at 4- or 5-year intervals over the past 40 years (47). Noel first participated in the U.S.-Japan Seminars as a U.S. observer in the third seminar, in Lincoln, NE, in 1977, which focused on "Recognition and Specificity in Plant Host-Parasite Interactions". The fourth seminar in 1981 in Brainerd, MN, addressed "Plant Infection—The Physiological and Biochemical Basis." Seiji Ouchi of Kinki University recalls that a highlight of that meeting was Noel leading a "hot discussion on molecular models of gene-for-gene interactions during an unofficial evening beer party"—a characteristic activity of Noel and a harbinger of breakthroughs to come. The eighth seminar was entitled "Delivery and Perception of Pathogen Signals in Plants" and was co-organized by Noel and Shigeyuki Mayama of Kobe University in Marina del Rey, California, in 1999.

Noel invited collaborations with many Japanese scientists, including younger scientists as well as established scientists like Mayama, Masaaki Yoshikawa of Kyoto Prefectural University, and Shinji Tsuyumu of Shizuoka University. He visited Japan several times and gave lectures in many institutes and universities with each visit. Collaborators such as Ouchi, Mayama, and Tsuyumu have warm memories of being guests of Noel and his wife Diane in their home in Riverside. These memories include driving through the California sunshine in Noel's favorite sports car and looking down on a night view of Riverside from the perspective of the hot tub in Noel's yard.

Noel seemed to be a natural at most things, but according to Tsuyumu, the seventh seminar in Tsu-City, Mie, Japan, in 1991, revealed a weakness, namely in karaoke singing. However, Tsuyumu was amazed that when Noel next visited Japan, he sang "almost professionally." Tsuyumu concluded, "Nothing was impossible for Noel."

SCIENTIFIC CITIZENSHIP AND THE AMERICAN PHYTOPATHOLOGICAL SOCIETY PRESIDENCY

Noel served on the editorial boards of most of the major journals publishing plant pathology and molecular plant pathology research, including *Phytopathology*, *Molecular Plant-Microbe Interactions*, *Journal of Bacteriology*, *Applied and Environmental Microbiology*, *Plant Physiology*, *Journal of Phytopathology*, and *Annual Review of Phytopathology*. He also coedited with Gary Stacey several volumes of the influential *Plant-Microbe Interactions* series, which is published by APS Press.

Noel co-organized multiple scientific conferences, including the eleventh Annual Symposium in Plant Physiology at Riverside in 1988, the eighth U.S.-Japan Scientific Seminar (discussed above), and a National Academy of Sciences colloquium on "Virulence and Defense in Host-Pathogen Interactions: Common Features Between Plants and Animals" in Irvine, California, 1999. He served on several scientific advisory boards, including the External Advisory Board for the NSF Center for Engineering Plants for Resistance against Pathogens at the University of California, Davis; the External Advisory Board for the Noble Foundation Division of Plant Biology, Ardmore, OK; the National Research Council, Associate Program, Life Sciences Review Panel; and the National Research Council Committee on the U.S. Department of Agriculture National Research Initiative grants program.

However, this long list does not capture the essence of Noel as a scientific citizen, which could be seen in the Noel alone in an Amsterdam poster hall at a meeting of the International Society for Plant-Microbe Interactions, carefully studying posters on behalf of the poster award committee, or the Noel who *Molecular Plant-Microbe Interactions* editor Jan Leach reported was never too busy to review a manuscript; or the Noel who strongly promoted women scientists in the program and organizing committees of the U.S.-Japan Seminars and other meetings and who seemed to have a way of responding to any junior scientist who needed help.

Noel made important contributions to the American Phytopathological Society. Most notably, he served as Vice President, 1999–2000, President Elect, 2000–2001, and he was serving as President, 2001–2002, at the time of his death. Noel saw the declining support for agricultural research that occurred in the 1990s as an ominous trend (18), and his campaign statement for the APS presidency in the May 1999 issue of *Phytopathology News* stated, "I do see APS as a vehicle for improving the common good of plant pathologists and other agricultural scientists in the U.S., namely to facilitate increased competitive funding for agricultural research." In his "Message from the APS President" in the February 2001 *Phytopathology News*, Noel proposed to focus the plenary session at the 2002 APS meeting in Milwaukee on the funding issue to "bring together key congressional, USDA, industry, and policy people to frankly discuss the failure of the country in recent years to adequately support agricultural research, especially competitive funding." Noel also saw declining funding as an important factor underlying another ominous trend in plant pathology, the failure of American students to enter the agricultural sciences (18).

As Noel put it, "increased funding for plant pathology and the rest of the food, fiber, and natural resources enterprise would go a long way toward remedying this and other problems. Otherwise, plant pathology will wither, exacerbating disease control and food production in the century we are entering" (18). Noel used his stature in science to work tirelessly to increase agricultural research funding (22, 49). Unfortunately, plant pathology has lost one of its greatest champions in this battle.

RECOGNITION AND LEGACY

As Noel's achievements accumulated, he gained increasing recognition by various scientific organizations. In 1995, Noel and Brian Staskawicz shared the American Phytopathological Society Ruth Allen Award, which honors individuals whose innovative research has changed the direction of research in any field of plant pathology. In 1996 Noel also received the Secretary's Honor Award from the U.S. Department of Agriculture and the Award of Merit from The Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture. Noel became a Fellow of the American Phytopathological Society in 1991, a Fellow of the American Association for the Advancement of Science in 1996, a Fellow of the American Academy of Microbiology in 1997, and on April 29, 1997, he was inducted into the National Academy of Sciences. His inaugural article was entitled "Characterization of a 34-kDa soybean binding protein for the syringolide elicitors" (11). Noel's outstanding contributions to science were also recognized at UCR. In 1996 he was the Faculty Research Lecturer, the highest honor granted by the campus for research. In 1997 he became a Distinguished Professor and assumed the William and Sue Johnson Endowed Chair in Molecular Plant Pathology. Noel's prominence at UCR is also indicated by the inclusion of his image (with the pectate lyase parallel β-helix) in a mural at the entrance to the campus (**Figure 1**).

Figure 1

This larger-than-life painting depicts Noel and the pectate lyase C parallel β-helix as part of a campus history mural near the entrance to the University of California at Riverside.

Noel was diagnosed with leukemia following blood analyses that were done during his recovery from an automobile accident in 1999 when another driver ran a red light. He kept his condition private and continued unabated with his myriad scientific activities and responsibilities. These responsibilities included taking on the demanding burden of APS President because he felt, "it was time to give back to the society." One telling incident (of many recalled by colleagues) during this period of Noel's illness occurred when Shinji Tsuymu visited Riverside in 2002. Tsuyumu had arranged for a limousine to take him on the long drive back to the Los Angeles airport, but Noel insisted on driving him despite obvious and unusual exhaustion (which Noel explained as possibly due to "a cold"). Within a month, Noel could no longer hide his condition and wrote to Shinji that he had to cancel a planned trip to Japan because of leukemia.

Noel died at the height of his career at age 61. His sister, Judith J. Carr, and his wife, Diane Ill Keen, Head of Access Services at the UCR Science Library, survive him. Diane established two memorial funds that support two annual events that honor Noel's contributions to the field of molecular plant pathology and to molecular biology at UCR. The first award is the American Phytopathological Society's Noel T. Keen Award for Research Excellence in Molecular Plant Pathology. The second is the Noel T. Keen Symposium which is sponsored by the UCR Center for Plant Cell Biology (CEPCEB). In April 2003, on the first anniversary of Noel's death, UCR named its former Bio-Agricultural Library in Noel's honor. The renovated facility, which had become the Core Instrumentation Facility of the Genomics Institute and Center for Plant Cell Biology, was named "Noel T. Keen Hall."

Noel's scientific legacy touches all aspects of molecular plant pathology. He contributed more than 170 technical publications, many of which had enormous impact on the field. One indicator of this impact is that Noel is listed in *ISIHighlyCited.com* as being in an elite group of one half of one percent of all publishing researchers for the period of 1981–1999 regarding the numbers of citations to their work. Seminal reviews he wrote for Annual Reviews had a particularly large impact (4, 17, 18, 30). Noel also leaves a legacy of many students and postdocs who have gone on to become important contributors to science themselves. To pick just one example, Robert B. Horsch started in Noel's lab as a Riverside high school student and eventually went on to become a prominent plant scientist at Monsanto and a 1998 recipient of the President's National Medal of Technology for pioneering achievement in plant biology and agricultural biotechnology. "It was Keen, personally, more than any other person, who taught me what the scientific process is," Horsch said at the time of his award. Prominent among the many young scientists that Noel helped train were those who pursued their Ph.D. thesis research in his lab and then went on to successful careers in industry, the U.S. Patent Office, and in faculty positions in diverse institutions, including several Land Grant universities and an Ivy League medical school.

Noel also leaves a unique legacy as a larger-than-life model of the pure scientist. As noted by his former UCR colleague William Dawson, Noel seemed to lack the emotions of jealousy or envy. Although famously competitive in sports, in the otherwise competitive realm of research, Noel was always truly happy for the successes of his competitors. It seemed as if in science the only thing that mattered was the science itself—and the wide circles of younger scientists and collaborators around the world that he could help. In the end, for the many who knew and worked with Noel, there was the experience of meeting right on the advancing border between discovery and mystery with someone who was there purely for the science, who saw things so clearly that he sharpened the mind of everyone in his presence, and whose vision was so broad that it could advance an entire field. That is the Noel who will be missed.

DISCLOSURE

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Several perceptive summaries of Noel's life were published by Noel's colleagues, and they provided a rich source of material for this chapter. These are "Tributes to Noel T. Keen, Respected Plant Pathologist Dedicated to Science," by James J. Sims, in the July 2002 *Phytopathology News*; "Remembering Noel Keen," by Patricia S. McManus, in *The Pathogen: Department of Plant Pathology*, *University of Wisconsin*, *Newsletter*, August 2003; "In memoriam," by Donald Y. Kobayashi and Scott Gold in *Plant-Microbe Interactions*, *Vol. 6*, 2003, edited by G. Stacey and N.T. Keen; and "In Memoriam" for the University of California, Riverside, Faculty Senate by James J. Sims (Chair), Donald A. Cooksey, Dennis D. Focht, Bradley C. Hyman, Linda L. Walling, and Frances A. Jurnak (**http://www.universityofcalifornia.edu/senate/inmemoriam/NoelT.Keen.htm**). We thank several colleagues who shared their experiences with Noel: Carol Bender, William O. Dawson, Allan Dodds, Frances Jurnak, Jan E. Leach, Shigeyuki Mayama, John Mildenhall, Seiji Ouchi, James J. Sims, Michael Stanghellini, Brian J. Staskawicz, Shinji Tsuyumu, Paul Williams, and Ching-Hong Yang. And, above all we thank Diane Ill Keen for providing photographs and additional insights into many aspects of Noel's life.

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The *Pseudomonas syringae* pv. *glycinea* AvrD avirulence protein directs the synthesis of a small-molecule elicitor of the race-specific resistance.

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