The Development of Plant Virology and Serology in the Early 20th Century

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In the early twentieth century, *Tobacco mosaic virus* (TMV) was both an economic problem for tobacco, pepper and tomato growers, and an experimental model system for plant pathologists and virologists. It became the right tool for the job [1] in the first big push to understand the nature of a virus, in particular its constituent chemical components: RNA and coat protein that formed the virus particle. This work, as elaborated by Creager [2], reveals the intimate links between basic and applied biology and how TMV became a model system for virology, biochemistry, and structural biology between 1935-1960. Today, TMV continues as the right tool and has accumulated several 'firsts'. These include the use of its coat protein *trans*-gene to mediate protection from subsequent infection by related TMV strains, the biology and mechanism of how TMV spreads from cell to cell using the 30-kilodalton (kDa) movement protein, and the cloning of the TMV *N* gene from TMV-resistant tobacco followed by the identification of the novel features of the first cloned resistance genes [3-6]. Each of these current avenues of research has a direct lineage to the findings of Francis O. Holmes who was a plant

virologist at the Rockefeller Institute for Medical Research (RIMR) at its sites in Princeton (1932-1950) and New York (1950-1965).

F. O. Holmes and his work on TMV provide a template to view the development of the tools of plant virology and the ongoing efforts to understand the mechanisms that control host-pathogen interactions. As outlined by Corner in his survey of the first half-century of the Rockefeller Institute for Medical Research [7], Holmes made ten significant advances to the area of plant virology and pathology **(Table 1)**. Holmes' initial findings were made at the Boyce Thompson Institute for Plant Research (BTI, Yonkers, NY), under the direction of Louis O. Kunkel. During 1923-1932 Holmes' described the use of tobacco host plants for local lesion assays and how to use such plants (e. g., *Nicotiana glutinosa*) as a tool to measure the relative amount of virus in a preparation and to study virus movement in plants. I have discussed some of these techniques and advances previously [4, 5]. For the purposes of this research report, my summary will focus on Holmes' finding that there was a gene in certain tobacco plants that could be used to develop resistance to TMV in the field. The Francis O. Holmes Papers, 1924-1979 (RG450 H735), excluding his notebooks, at the Rockefeller Archive Center, have provided an interesting view of Holmes' enthusiasm for this work and his surprise at the outcome.

TABLE 1. Francis O. Holmes' Ten Advances in Plant Virology [7].

1. Local lesions caused by TMV
2. Spread of TMV in plants (virus movement)
3. Quantitative assay of virus infectivity (local lesion assay)
4. Genetic aspects of plant diseases (local lesion, systemic, yellow mutants)
5. Inherited differences of invasiveness of different strains of TMV. Reflect unit differences in structure of virus = single gene difference (1936)
6. Genetics of the plant itself (*N*-gene). Dominant Mendelian factor (1934)
7. Practical aim of developing virus resistant strains
8. Evolution and distribution of TMV and host plants (tobacco, pepper, tomato) suggest that TMV is a New World virus.
9. Classification and nomenclatures. Attempt to Latinize (binomial) nomenclature.
10. Practical measures. Holmes' ribgrass stain in plantain was infecting tobacco in field. Control of weedy hosts and location of tobacco field can be used to control disease.

Since this work occurred only 30 years after the rediscovery of Mendel's laws and concurrent with the discussions (and research) related to the identity of a gene,

particularly among agricultural scientists [8], Holmes may provide a new means to investigate the state-of-the-art and how he determined that a discrete factor (a gene) controlled resistance to a disease. As I become more conversant with the contemporaneous literature of the time and the historiography and insight provided by Keller [9], Kay [10,11], Creager [2], and Kohler [12] my thesis is that Holmes, working independently, made several significant discoveries that may have been underappreciated outside of the area of plant pathology/virology.

Francis Holmes used straightforward tools, direct inoculation of tobacco plants with TMV, and this may have been overshadowed by the new sciences of biochemistry and molecular biology. Although Holmes and Stanley were in the same laboratory, Stanley's physicochemical isolation of TMV crystals was a transformative event, welcomed with great enthusiasm by the public and scientists. Stanley's striking results also may be the temporal marker of the division between basic and applied plant pathology. This argument would mimic Fuerst's discussion that reductionist biology resulted in 'schools' of genetics and biology in the early- to mid-20th century [13]. In addition to 'schools' that generally marginalized the plant virologists, the plant pathologist can be further divided in 'clusters' of applied and basic science. This reductionist style, to include Tatum and Beadle's one-gene, one-enzyme and Stanley's virus crystals contrast with the holistic (or generalist) style of Holmes. This contrast, I argue, reveals how unusual Holmes was as a scientist (perhaps even to be regarded as a 'scientist's scientist').

Holmes embraced TMV as both a tool and an economically important agricultural problem. During his career he identified and worked on questions central to plant virology, including virus movement and genetic resistance. Yet he was able to work in both basic and applied research, and depending on his results the definition of 'basic' or 'applied' coalesced or changed. This argument is based on my reading of his observations and interactions with several of his peers. (Taking some liberties with Kohler's recent scholarship [14], Holmes may be viewed as someone equally comfortable in the landscape and labscape for his TMV research.) For the purposes of this report, I will focus on Holmes' *N*-gene work within the context of his career at RIMR.

In the early twentieth century, a primary question, following the discovery of bacteria and fungi, was to determine the nature of a virus. The study of the properties of viruses was of basic scientific interest and of practical value. As told by Creager [2] the major push towards solving the physicochemical properties of TMV occurred after Kunkel formed a plant virology group at RIMR-Princeton. By 1935, W. M. Stanley had 'solved' the problem in announcing that TMV was a crystal composed of protein. However, the actual solution was provided by F. C. Bawden and N. W. Pirie, in England, who showed that the virus contained phosphorus, and that the 'nature of the virus' was incomplete without its RNA [15]. The story of TMV from mid-1930s has been elaborated by historians of science [2, 3, 10, 11] and the plant virology community has provided accounts of TMV [4-6, 16].

My particular interest as a practitioner-historian is the TMV work performed by American scientists in the early 20th century, prior to World War II. During this period, before the introduction of 'big science', advances in agriculture occurred under the auspices of the Bureau of Plant Industry within the United States Department of Agriculture (USDA) [17], Land Grant Universities and Experiment Stations [17-19], and two private foundations, the Boyce Thompson Institute for Plant Research [5] and the Rockefeller Institute for Medical Research (RIMR). An extraordinary story is emerging on the research, creativity, and deductive insight of Francis O. Holmes, based on correspondence from the Rockefeller Archive Center (RAC), USDA publications, and manuscripts published in peer-reviewed journals.

The TMV research published and discussed by Holmes intersects with the early twentieth century interests in hybridization for breeding, following the rediscovery of Mendel's laws in 1900, and the plant improvement goals of scientists in land grant colleges, the USDA, and Agricultural Experiment Stations. It was a time that was rich in discussion and experimentation to try and decipher the structure of genes and the genetics of inheritance. The leaders in genetics used maize, mice, and *Drosophila* (fruit flies) as model organisms [9, 12, 20]. Phenotypes, such as maize kernel color, allowed for direct observation of genotypic effects. In turn, this allowed for improved agronomic features and crop improvement, as outlined by Paul and Kimmelman [8]. The link between

phenotype and genotype was pioneered by Thomas H. Morgan and Barbara McClintock, as elaborated on by Kohler [12] and Keller [9]. TMV, as I have previously argued, became a model system because it was of economic importance [4]. Tobacco was a high value (and valued) crop and the mosaic disease (TMV) resulted in significant losses. But more important, as described by Holmes, were the losses on tomato and pepper plants [21, 22]. Developing new strategies to control virus diseases was a priority for American agriculture [17].

As discussed by Keller, the central problem of the early 1940s was to determine the nature of heredity of bacteria and viruses, in particular to define the physicochemical properties of "the genetic apparatus, and especially their capacity to mutate" [9] (p. 162). This was the beginning of the use of bacteriophage as a model for genetics [23], yet a decade earlier it had been made evident by F. O. Holmes, H. H. McKinney, and Helen Purdy Beale (**Table 2**) that TMV had properties that could be distinguished by phenotype, serology, and that it accumulated heritable mutations [4,5,24-26].

TABLE 2. On the nature of *Tobacco mosaic virus* in the early 20th century.

Methods	Year	Actors	Education	Institutions
Virus infectivity	1914	Allard	B.S. UNC-Chapel Hill	USDA (1906-1946)
Virus mutations	1926	McKinney	M.S. Wisconsin	USDA (1919-1959)
Centrifugation	1927			
Cross protection	1929			
Serology	1929	Beale	Ph.D. Columbia	BTI (1924-1952)
Local lesions	1929	Holmes	Ph.D. Hopkins	BTI (1924-1932)
N gene	1934			RIMR (1932-1960)

By the mid-1930s Holmes had clearly worked out the genetics of host-pathogen interaction and *trans*-species transfer of a resistance gene for TMV. McKinney (USDA) by 1929 had identified heritable TMV mutations by distinguishing phenotypic effects on plant hosts. It is also important to emphasize that McKinney used the term genetic mutation in his 1929 paper [24, 27] and by 1935 [28] he had isolated and characterized

mutants by phenotype, suggesting that viruses could experience both loss and gain of function [29]. At BTI, Beale was working closely with Stanley (at RIMR) in identifying mutants as strains based on her serological precipitin assays. Based on my findings at RAC, I am currently exploring how much of the "quest for the physical basis of the gene" had been limned by the workers at RIMR-Princeton, USDA, and BTI, prior to the bacteriophage courses at Cold Spring Harbor and the *Neurospora* work at Cal Tech and Stanford, as well as McClintock's research on maize genetics, and the Jackson Labs mice [2, 11, 12, 20, 23]. **Table 2** provides a general guide to the work and workers that I am interested in following prior to the time when W. M. Stanley was lauded for his work on the crystallization of TMV.

Holmes' work differed from that performed by *Drosophila*, *Neurospora*, and maize geneticists in that he 1) had strains (mutants) of TMV, 2) a variety of host plant species that were either susceptible or resistant to TMV, and 3) both the phenotype of the TMV mutants and the genetic effects of a host gene could be scored by observing the symptoms on plants and the effects of environmental conditions. Holmes' work was predicated in part on two contemporary scientists: Helen Purdy Beale of the BTI and H. H. McKinney of the Bureau of Plant Industry (USDA, Arlington, VA). From my preliminary findings, the published data and correspondence from Beale, McKinney, and Holmes suggest that these virology workers had conceptual ideas of the definition of the gene. By the mid-1930s, both in terms of inheritance and mechanistic processes, they had shown that TMV was an entity that could be studied, manipulated, and understood within the context of the host virus interaction. Each of these scientists contributed to findings that were reiterated by McClintock "that the genetic apparatus was . . . labile and flexible" [9] (p. 173). Although Stanley was able to insinuate himself in the medical and political community, the results of Beale, Holmes, and McKinney can be seen as more remote, perhaps due to their "style" of research [9] (p. xiii). These contributing styles may include their personalities, their identity as plant virologists (or plant pathologists), their agricultural institutional affiliations, attendance at applied research meetings, a lack of students or postdoctoral fellows, and choice of publication venues; one or more of these choices may have isolated them as the new biology developed.

Holmes' particular discoveries were i) that a resistance gene *N* could be transferred between plant species, ii) that plants with the *N* gene could be used to determine virus titer and movement, and iii) strains/mutants of TMV could be used with these plants to investigate symptom phenotypes and the genetic similarity or differences of TMV strains. These observations were both tools for basic biology to understand the genetics of host-plant resistance, the genetics of TMV, and practical applications including those used by Stanley to monitor the success of his purification/infectivity schemes to isolate TMV. Plant breeders benefited because they could now introduce a resistance gene into economically important crops such as tomato and tobacco.

H. A. Allard, an assistant physiologist for the Bureau of Plant Industry (USDA), described in 1914 that *Nicotiana glutinosa* "invariably failed to produce any indications of disease" [30] (p. 12) when inoculated with TMV. This was in contrast to mosaic disease on *N. tabacum* and "many distinct species of *Nicotiana*" and "a great variety of solanaceous plants" [30] (p. 10). With TMV Allard [30] showed this "both in the greenhouse and in the field," performing assays with tomato, pepper and petunia (p. 10). Importantly, he observed that many plants did not become infected with "visible symptoms of the disease" (p. 11) and he used various *Nicotiana* species to investigate the nature of the exceptions to TMV infection. In 1914, Allard determined that when *N. tabacum* was crossed with *N. glutinosa* pollen, the first generation seed (F1) produced plants that were resistant to TMV infection, but were infertile so he could not proceed with further analysis of the type of immunity displayed by the *N. glutinosa* genetic background.

However, this work was not pursued at the time. As Holmes noted, the "dominant gene *N* of *Nicotiana glutinosa* . . . played an important role in the study of tobacco-mosaic virus, in part even before its identity was recognized" [21]. Allard's finding "was not the first to be exploited;" instead, the *N* gene paved the way for Holmes in "facilitating quantitative measurement" of TMV "by inducing prompt formation of conspicuous primary lesions" to determine virus titer [21]. Holmes also discovered that inoculation of TMV onto common bean (*Phaseolus vulgaris* L.) resulted in similar necrotic lesions, presumably due to an ortholog of the *N* gene. As he told Geoffrey

Samuel, a plant pathologist in Australia, Holmes used beans because the results were obtained much faster than using tobacco—bean plants were inoculated 10 days after planting compared to several weeks for tobacco, and the lesions could be scored and plants discarded five days later [31]. The second "contribution of this dominant [*N*] gene was in allowing accurate separation of strains" of TMV. The third contribution, "though envisaged first and long delayed in accomplishment," was the *N* gene itself in "conferring a desirable type of disease resistance . . . for decisive control" of TMV in the field. It is curious that Holmes did not include the use of the *N* gene to study virus movement, something that was championed by Samuel. I am investigating these details, with the intent to determine what resulted in Holmes' proposition, and subsequent proof, that the tiny lesions were a genetic factor and, in fact, represented a resistance response that was specifically induced by TMV infections on *N*-gene containing plants. Plant breeders are still studying Holmes' findings, with a particular interest in the remarkable stability of *N*-gene resistance and its retention in Burley tobacco on the H chromosome [32].

Although Holmes is best known by plant pathologists and virologists for his *N* gene research with tobacco, there is prior work [33] that deserves renewed attention—genetic crosses between TMV-susceptible pepper plants (mottling type; *ll*) and resistant lines (necrotic type; *LL*). Using *Capsisum frutescens,* Holmes discovered "a dominant Mendelian genetic factor that causes localization of tobacco-mosaic virus in inoculated leaves" [33]. This, he believed, was "the first description of such a genetic factor affecting a virus disease in its plant hosts" [33], and he described that the gene was not linked to seven readily observable traits in pepper, such as flower color and pungency, and the hybrids were fully fertile.

In the same paper he described the difficulty of working with the *N. tabacum* X *N. glutinosa* crosses, and reported only on the effects of TMV on F1 plants. He later acquired fertile amphidiploid plants (*N. digluta*) from R. E. Clausen [34] at the University of California-Berkeley and successfully introgressed the *N* gene (from the H chromosome of *N. glutinosa*) into commercially important tobacco lines. By October 1937 Holmes clearly described the dominant genetic effects of the *N* gene in tobacco. In a letter to Johnson he mentions that "breeding with the *N. glutinosa* derivatives is very easy" and "resistance is completely dominant" to the extent that "it should even be feasible to grow

F1 hybrids commercially at first, if desired, as in corn" [35]. The genetics of the *N* gene was also clearly associated with the chromosome, based on Holmes' letter to James Johnson [35], of the University of Wisconsin, in response to the availability of seed of *N. digluta* or *N. tabacum* with the gene *N*. He further noted, "the earlier derivatives [of tobacco] bore the whole H chromosome of *N. glutinosa* or so large a part of it as to prevent pairing of this type of chromosome in backcrosses." It is not clear how he had identified that the H chromosome was the location of the *N* gene, but "the improved types now in use are more closely like ordinary tobacco than were early derivatives, though even these were surprisingly tobacco like" [35].

By September 1937 Holmes had F5 plants "in hand" and by year-end he hoped to have the sixth generation of "tobacco bearing the *N. glutinosa* gene for necrotic response to infection with tobacco mosaic virus" and was interested in testing his plants in tobacco growing regions of the US [35]. His delight in his research is revealed in correspondence with James Jensen [36], then at USDA Puerto Rico, in reporting that "the inheritance of resistance to tobacco-mosaic disease works so well as to be almost unbelievable" and had plans to study strains of virus in early 1937 [36]. Holmes had made "tentative arrangements" with W. D. Valleau, a professor and tobacco breeder at the University of Kentucky for the *N*-gene would be deployed in Burley (the predominant commercial tobacco variety) as a backcrossed line. Holmes wanted to pursue similar tests in Wisconsin if Johnson was willing to make the backcrosses to commercial varieties used in that region. Interestingly, Holmes knew that although the *N* gene from *N. glutinosa* was dominant it "does not give the complete protection that the Tabasco gene gives in peppers . . . for there is no leaf abscission to remove virus from the plant" [35]. This was a problem, in that Holmes' knew that "there is danger to the individual plant in stem necrosis, or even systemic necrosis under some cultural conditions." The population (or field) of plants was protected, because of the low amounts of circulating virus, but individual losses could accumulate" [35]. Kunkel was equally enthusiastic about the results: in a letter to Kenneth Starr Chester he commented, "Dr. Holmes has succeeded in getting the *Nicotiana glutinosa* factor for localization of tobacco mosaic virus into tobacco varieties. I think these resistant varieties may be of commercial value" [37].

From the genetics standpoint, it may be pertinent to note that within a decade, Holmes knew that the *N*-gene was located on the H chromosome of *N. glutinosa* [38]. A significant question is to determine if Holmes was using cytogenetics in addition to phenotypic assays (i.e., the necrotic local lesion induced by the TMV-host interaction when the *N* gene is expressed) to plan experiments and make his selections. Holmes continued to improve on this work [21] and in 1960 reported the introgression of two minor genes for resistance (*rm1rm1* and *rm2rm2*) from *N. tabacum* var. Ambalema along with the *N* gene. This resulted in new tobacco lines (*NNrm1rm1rm2rm2)* that responded to TMV infection with an agronomically useful trait of nonhaloed, pinpoint, and later-developing lesions [22]. This effect further reduced the amount of inoculum transferred plant-to-plant and, perhaps more importantly, greatly decreased the potential of TMV infecting tomato and pepper plants in neighboring fields.

At the request of Firman Bear [39], the science editor of *Country Home Magazine*, Holmes wrote a lengthy note on the relevance of the TMV work [40] where he emphasized that the importance of the *N* gene was to reduce the amount of TMV in the environment to protect economically important crops such as tomato and pepper. In January 1939 he reported that TMV was "one of the less important causes of loss" in tobacco, but in tomatoes "reductions in yield as high as fifty percent may occur" and losses of "up to one hundred percent" could occur in pepper "although each plant still stands" [40] (p. 6). The losses were economically significant in both yield loss and downgrading of quality due to size, misshapen fruit, and blemishes. In this letter, Holmes anticipated "good prospects" for resistance in pepper and tobacco, but predicted diminished success for tomato. However, the importance of the *N* gene-tobacco was "the key to the control of the disease in other crops, for without infected tobacco, and the consequent reservoir in dried products of tobacco [cigarettes], the disease as we know it cannot exist" [40]. Therefore, the key to disease prevention susceptible crops was to reduce the total incidence of TMV in tobacco and the subsequent carry over of TMV on smoker's hands as they planted or watered pepper and tomato plants, which was sufficient to cause an epidemic. H. H. McKinney echoed this point of view in a letter to Holmes three years later. McKinney had studied several Ambalema tobacco lines. The "better lines", in McKinney's hands, had "greatly reduced the hazards from the

mechanical transmission of virus to susceptible vegetable crops, and the importance of seasonal carry over of virus in the soil refuse" [41].

Yet McKinney had some reservations about the utility of the *N* gene: it was environmentally sensitive and in warm climates, in particular the southern tobacco growing states, the necrotic reaction was no longer a local lesion, and "not enough emphasis has been placed on the fact that the necrosis in plants carrying the *N* factor may be lethal to the entire plant" [41]. He also raised questions about the effects of strains of TMV, noting that "mutation occurs in plants giving the necrotic reaction" and that "one can conceive of a systemic mutant 'running away' from a margin of a necrotic zone and setting up a general infection" [41]. If this occurred there was a possibility that a "new and destructive virus" could be "established in a pure state and in considerable quantity, thus creating a hazard of maximal proportions" [41]. Yet, this does not appear to have occurred and the *N*-gene remains the gold standard for resistance to TMV in tobacco. The observations of both Holmes and McKinney of temperature sensitivity of the N-gene has been confirmed using the tools of molecular biology [4, 42-44].

Holmes also shared McKinney's interest in virus mutants, and was involved, along with Helen Purdy Beale, in characterizing the biological properties of TMV variants. McKinney, a career employee of the USDA [25], was the first to report identifiable (phenotypic) TMV mutants on tobacco host plants [27]. His practical application of this observation resulted in the development of cross protection, a method that uses a mild strain of virus to protect plants from a subsequent infection by a more severe, economically important, strain of the same virus [4, 45]. McKinney's insight continues to be deployed today for protection of greenhouse and citrus crops and was the basis for the first demonstration of the use of transgenic plants to protect from virus infections. McKinney also developed the use of centrifugation for purification of plant viruses and published the results of using prototype machine in July 1927 that was capable of spinning at 50,000 rpm [46]. The correspondence and publications of Holmes (RAC), McKinney (USDA, National Agriculture Library), Johnson (University of Wisconsin), Valleau (University of Kentucky), and Allard (University of North Carolina-Chapel Hill) may provide important insights into the ideas and background for conceiving these experiments and deciphering their meaning and usefulness. The

notebooks of Francis Holmes, a more detailed investigation of his correspondence, and the annual reports will be important sources to trace the development of Holmes' interest in the necrotic phonotype being 'captured' for use as a resistance gene.

Creager and Kay have elegantly demonstrated the importance of the plant virology group at RIMR to basic research on the physicochemical aspects of viruses [2, 10]. This was reflected by James Johnson in a 1947 letter to Holmes, writing that he was "extremely sorry to learn that the laboratory at Princeton is to be abandoned, since we had come to look to this as the chief plant virus center in the United States . . . I have come to regard it as more and more essential that the plant virus workers keep abreast with the new work in the animal virus field and it seems to me also to be equally important that the animal virus workers know what is going on in the plant virus field" [48]. My particular interest is to trace the work of the plant virologists in the context of plant pathology and genetics, before the mid-century transition to biochemistry and molecular biology. Holmes' methodology and experimentation is an important component of a historical analysis of early efforts to determine the 'nature of a virus'.

Furthermore, from the perspective of the practitioner-historian, Holmes' work remains at the forefront in deciphering the molecular genetic details of host-pathogen interactions, both in basic biology and agricultural applications. The identification and cloning of the tobacco *N* gene and the properties of its encoded protein was not completed until the mid-1990s [49, 50] and the report of its introduction into tomato in 1996 [44] provided the molecular proof of Holmes' observations and analyses six decades earlier [21, 33, 51]. From this brief summary, there is a wealth of material yet to be analyzed both in the interactions of Holmes with others during the process of his work on virus movement and resistance, his notebooks, and a detailed analyses of his published materials. The plant virology research of Francis O. Holmes resonates today: each semester his eponymous local lesion assay is used for undergraduate teaching demonstrations and his manuscripts are regularly cited as tools and background for current research on molecular plant-virus interactions, virus movement, and breeding for improved resistance to agriculturally important crop plants.

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