PHYTOPATHOLOGICAL CLASSICS

VIRUSES IN VECTORS:
TRANSOVARIAL PASSAGE AND RETENTION

Selected Works
of

Hirotaro Ando • Teikichi Fukushi • Harold Haydon Storey

Edited by
C. Lee Campbell and G. W. Bruehl
With an introduction by
Karl Maramorosch

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Preface

The preparation of a Phytopathological Classic is a somewhat different enterprise than that of preparing many other types of publications. It is first and foremost a labor of love—a love for the history and origins of our science. It comes about as a commitment to the preservation and dissemination of research papers that are truly of classic stature but may not be readily available to the general phytopathological community. The result is a presentation of something we already knew but may have taken for granted and is a recognition of the foundation provided by those who have come before us.

The initial steps in the preparation of this Classic were undertaken at the 1979 meeting of the American Phytopathological Society in association with the IX International Congress of Plant Protection in Washington, DC. Bill Merrill (The Pennsylvania State University) was instrumental in the selection of the subject matter for the Classic and helped us to put the work (particularly the work of Fukushi and Storey) into perspective. From that point on, our work was primarily that of collectors, correspondents, and part-time biographical detectives.

The preparation of this work has been an enjoyable and rewarding task. It has been so because of the many kind people who replied to our letters and requests and provided helpful suggestions along the way. We would like to express our sincere appreciation and thanks to:

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INTRODUCTION

Leafhoppers and planthoppers are, next to aphids, among the most important vectors of plant viruses. Cicadellid vectors have been among the most difficult to discover because of the lapse of time between virus acquisition by the insects and transmission of the viruses to susceptible plants. Yet, leafhoppers provided the first demonstration of insect transmission of plant viruses, and this demonstration, in Japan, coincided in time with the earliest work on human virus transmission.

In 1889 and 1890, Theobald Smith and Fred L. Kilborne established that cattle ticks were the intermediate carriers of the pathogen that caused cattle tick fever ("Texas fever"). This pioneer work started a new field in medical science that led directly to the solving of the vector transmission of yellow fever, malaria, Rocky Mountain spotted fever, typhus, and others. Yellow fever provided the first instance in which the dependence on an insect vector for transmission of a human virus was proven. Walter Reed, together with J. Carroll and A. Agramonte (1901) are usually credited with this breakthrough. Actually, Carlos Finlay in Cuba preceded Reed by providing the needed background for conclusive proof that a mosquito vector was essential for the natural cycle of yellow fever disease.

In the 1880s and 1890s, plant pathologists and entomologists in Japan, unaware of the medical work done in the Western hemisphere, discovered the first vectors of plant viruses. They linked *Inazuma dorsalis* and *Nephotettix cincticeps* leafhoppers with rice dwarf disease and eventually proved that these insects were the vectors of the rice dwarf virus. At first the idea that leafhoppers merely carried plant viruses prevailed, but eventually they were proven to be alternate, reproductive hosts and reservoirs of these pathogens.

The connection of rice dwarf disease with leafhoppers had been noticed in Japan as early as 1883. Hashimoto, a rice grower in Shiga Prefecture, noticed the disease in that year and subsequently suspected a relationship between the disease and the abundant leafhoppers. Ten years later, in 1893, Hashimoto caged leafhoppers on rice plants in a glass container enclosed with cheesecloth. The following year he discovered the causal relationship between leafhoppers feeding on rice plants and the induction of rice dwarf disease. In 1895, Hashimoto proved experimentally the relationship between the leafhoppers and the rice disease. As far as could be established later, the leafhopper vector used by Hashimoto was *I. dorsalis*, which thus became the first insect proven *to induce* a virus disease of plants. By 1900, workers at the Shiga Agricultural Experiment Station attributed transmission solely to *N. cincticeps*, which thus became the first insect proven *to transmit* a plant virus.

A few years later, entomologists at Tokyo's Imperial Agricultural Experiment Station started experiments with rice dwarf. In 1910, Ando reared *N. cincticeps*, collected near Tokyo, on diseased plants obtained from Shiga Prefecture. Ando found that the second and third generation progeny of these leafhoppers produced infections in healthy plants. Approximately 15 days after feeding were required before noninfective leafhoppers became infective, and their ability to transmit decreased gradually. The fact that leafhoppers from the vicinity of

Tokyo, where the disease did not occur naturally, had to be placed on diseased plants from Shiga Prefecture before they became infective, led Ando to conclude that rice dwarf was not caused by mere leafhopper feeding, but that an unknown pathogen, acquired and later transmitted by the insects, was the actual cause of the disease. As early as 1902, the entomologist N. Onuki, at the Imperial Agricultural Station in Tokyo, demonstrated (apparently at Ando's suggestion) that leafhoppers only became infective after exposure to diseased plants. In 1908, this finding was confirmed by another entomologist, T. Nishizawa, at the Shiga Agricultural Experiment Station.

In 1915, Murata briefly mentioned that "the capability of producing dwarf disease" was transmitted from infective leafhoppers to the progeny through three or four generations, but he gave no experimental data and he did not realize that rice dwarf was caused by a virus transmitted through the egg of the vector. When Fukushi started his classic experiments in the 1930s, he paid little attention to Murata's statement because no reliable data had been presented. At that time, there was no record showing congenital transmission of a plant virus in an insect vector, and the concept that a plant-pathogenic virus could multiply in an invertebrate vector was almost heretic.

Fukushi, in his 1933, 1939, and 1940 papers, described the interaction between the rice dwarf virus and its vector *N. cincticeps* in great detail. He demonstrated transovarial transmission and multiplication of the virus in vectors. He found that not all individuals were able to acquire and transmit the virus. The minimum acquisition feeding period was three days, but most insects acquired virus after 10–50 days of feeding. Yet, even after 50 days, certain insects failed to acquire virus. The incubation period varied from 10 to 73 days, and most, but not all, vectors retained infectivity throughout their lives. Only in rare instances did the transmitting ability diminish with the age of the vector.

Fukushi demonstrated that viruses can multiply not only in plants but also in their vectors—invertebrate animals. His brilliant experiments provided overwhelming evidence for multiplication of rice dwarf virus in its vector, *N. cincticeps*. It is now probable that most viruses with well-defined incubation periods in their vectors also multiply in their vectors, but this cannot be accepted as an unqualified assumption. The findings that rice dwarf virus is pathogenic to *I. dorsalis*, and the evidence of multiplication and transovarial passage of several other plant-pathogenic viruses, indicate that these vectors are not fortuitous carriers of viruses but that they play a primary role as hosts in the evolution of plant and animal viruses.

In 1958, at the Annual Meeting of the American Phytopathological Society in Bloomington, Indiana, I was privileged to meet Fukushi for the first time. It was a thrilling and unforgettable experience. In 1965, we met again at a U.S.-Japan seminar on vectors held in Tokyo. In 1980, during my visiting professorship at Hokkaido University, I again admired his keen interest and thorough knowledge of current progress in virology. He not only retained his interest and critical grasp of current developments in the vastly expanding field of virology, but, despite advanced age, continued to inspire and advise many of his younger colleagues, as well as present and former graduate students. Currently, he is putting the finishing touches to the second edition of his *Plant Virology*, written jointly with his former student, Eishiro Shikata.

In 1958, at the VII International Congress of Microbiology, I had the first and only opportunity to meet H. H. Storey and to ask him several questions that came to my mind when I first read his accounts of needle inoculation of

leafhoppers with maize streak virus. At approximately the same time that Fukushi began his experiments in Japan, Storey (in Africa) attempted to determine what happened to the virus of maize streak in its vector, *Cicadulina mbila*. He found that in nature the leafhopper occurs in two morphologically indistinguishable strains that can interbreed freely. He called one the "active race" and the other the "inactive race." The ability to transmit the virus was governed by a single Mendelian, male sex-linked factor. In the active race, virus was found in the gut as well as in the hemolymph; in inactive individuals, virus was found only in the gut. The detection of the virus was made by the ingenious method of microinjection of extracts from leafhoppers into other leafhoppers; thus, the latter were rendered infective by needle inoculation. Inactive insects were unable to transmit virus acquired by feeding on diseased plants, except when their gut was punctured before or after feeding on an infected plant. In such previously "inactive" insects, virus could be detected in the hemolymph. Inactive insects could be rendered infective by needle inoculation using either plant sap or hemolymph of vectors as inoculum. Storey concluded that the difference between the active and inactive individuals was the permeability of the gut wall. In inactive insects, the passage of virus through the gut wall was prevented; they could not transmit the acquired virus because it did not reach their salivary glands. Storey found that virus was absent from naturally voided feces of vectors.

Why are leafhoppers highly specific as virus vectors? If it is because of gut permeability, it should be possible to render nonvector species into vectors by puncturing the gut wall. Storey tried this but found that *Peregrinus maidis* could not be rendered infective for maize streak by gut wall puncturing. Even when the virus was injected directly into the hemolymph of *P. maidis*, no transmission resulted. Storey also established that the length of the acquisition feeding period had no influence on the duration of the latent period.

Transmission of viruses from plant to plant by arthropod vectors is important not only because it is an indispensable step in the maintenance and perpetuation of plant-pathogenic viruses, but also because knowledge of virus-vector interactions can provide the means to curtail economic losses caused by infected food and fiber plants. Plant viruses are among the most important, complex, and extensively distributed plant disease agents in the world. Because of the intriguing mechanisms by which these viruses propagate and survive in plant and vector reservoirs, and the economically important diseases they cause, it is no wonder they have attracted numerous workers. Accomplishments in this field have transcended the boundaries of the agricultural sciences and have entered the advanced frontiers of medicine and biology, stimulating further studies in the vast and expanding array of plant and animal virology.

The understanding of the mechanisms of virus-vector interactions and the persistence or lack of persistence of viruses in vector reservoirs, fostered by the epochal discoveries in Japan and Africa, is of enormous importance for control strategies. In many human, animal, and plant diseases, control strategies have been based on attacking the epidemic phase, which is usually the least susceptible to control. Since control strategies aimed at interepidemic phases have greater potential efficiency, the contributions of Ando, Fukushi, and Storey provide information of immense basic and applied value.

Karl Maramorosch
New Brunswick, New Jersey

Hirotaro Ando (1871–1958)

Hirotaro Ando

1871–1958

Hirotaro Ando was born August 1, 1871, in Kaibara Town, Hyogo district. His early education was obtained in a local public school, where he was known as an infant prodigy. Following Daisan Koto Chugakuko (secondary school) in Kyoto, he enrolled in the Department of Agronomy, College of Agriculture, Imperial University (now Tokyo University), where he earned his B.S. degree in 1895. He was appointed to the newly founded National Agricultural Experiment Station in Nishigahara, Tokyo, and served there for 46 years. With this appointment he began a career in research and administration.

The Japanese government sent Ando abroad to Europe, North and South America, southern Asia, and China to study rice and its cultivation. He visited various parts of Japan as an expert on rice. He wrote *Inasaku Yoko (Principles of Rice Cultivation)* in 1903. The book encompassed breeding, cultivation, and control of weeds and pests of rice plants, and it contributed to the evolution of fundamental and practical research in the pioneering days of Japanese agriculture.

Among his contributions was the study on determination of transmission of rice dwarf virus, which emphasized that rice dwarf is not caused by leafhoppers but by certain unknown infective principles carried by the insect.

Ando's extensive interest in research was gradually concentrated in two major areas: the breeding of crops, and the response of crops to unusual climates (especially low temperature and frost, which frequently injured rice in the northern parts of Japan). He developed pure-line selection and cross-breeding methods of plant breeding in Japan, based on the Mendelian law, and his doctoral thesis, "Studies on the Cold Injury of Plants and Their Cold Resistance in Japan," was done at Tokyo Imperial University.

In 1920, he was appointed president of the National Agricultural Experiment Station, a post he held until his retirement in 1941. He organized the network of crop breeding systems in Japan and established facilities in experiment stations that contribute to the research on low temperature injury of crops. He held a joint appointment in the National Horticultural Experiment Station and the National Tea Experiment Station (1925–1941), where he organized and directed research. He also served concurrently as Professor of Kyushu Imperial University and Tokyo Imperial University.

Ando promoted the foundation of the Japan Society of Agriculture, the Japanese Society of Breeding, and the Genetic Society of Japan. He was a member and president of the Crop Society of Japan and a member of the Japan Academy. He also contributed to the development of agricultural policy as an adviser of the cabinet.

After his retirement, he studied the history of Japanese agronomy and published *Nippon Kodai Inasaku Kenkyu (Notes on the Ancient History of Rice*

Cultivation) in 1951, which detailed the introduction of rice into Japan and the establishment of its cultivation in ancient times.

In recognition of his contributions, Ando was honored with the title of "the person who had done distinguished service in the field of culture" in 1953 and was awarded the Order of Cultural Merit in 1956. Ando died in 1958.

On Rice Dwarf Disease[1]

by Hirotaro Ando

The results of the experiments carried out on rice dwarf disease are briefly reported in this paper.

Although Shiga Agricultural Experiment Station had reported that the disease was caused by a leafhopper, *Nephotettix cincticeps* Uhler, (Tsumaguro yokobai), the evidences that rice dwarf disease occurred in Shiga prefecture and in the vicinity of Kyoto, but not in the Tokyo district where the same leafhoppers were known to be present, aroused my doubt as to whether the leafhopper was the actual cause of rice dwarf disease, or if there was any other causative agent.

I started my experiments in 1905 using the leafhoppers *N. cincticeps*. The insects captured in the vicinity of Tokyo were reared on the diseased rice plants, which had been sent from the Shiga Agricultural Experiment Station, and their progeny were transferred to healthy rice plants. Almost all the rice plants tested were infected with the disease. On the other hand, the rice plants showed no symptoms of the disease when individuals of the Tokyo colony reared on healthy plants were used.

From the results obtained, it was suspected that the insects themselves were not the actual cause of rice dwarf disease, but that they might carry some causative agent of the disease. Therefore, further experiments were carried out to ascertain whether members of the Tokyo colony fed on the diseased rice plants were able to induce the disease in healthy rice plants. Tests were also performed to establish whether elimination of the causative agent within the insects would occur when the insects from the Shiga Agricultural Experiment Station, verified to have transferred the disease to rice plants previously, were successively transferred to healthy rice plants.

The experiments performed over a 3-year period since 1906 showed almost the same results.

In these experiments, the Tokyo colony insects did not cause rice dwarf disease. However, when they were confined on the diseased rice plants for about 15 days and then fed on the healthy rice plants, most of the test plants showed the symptoms of the disease. Similar experiments were carried out at the Shiga Agricultural Experiment Station using the Tokyo colony, and the leafhoppers fed on the diseased plants for 5–7 days became viruliferous.

Experiments were carried out to eliminate the causative agent carried in the infective leafhoppers by feeding them on healthy plants.

The insects of the Shiga colony were fed on healthy rice plants. After the first set of plants inoculated showed disease symptoms, the insects were transferred to healthy plants. Before the second set of plants inoculated developed symptoms, the insects were transferred again to another set of healthy plants. Such transfers

[1] Ando, H. 1910. On rice dwarf disease. J. Jpn. Agric. Soc. 347:1-3. (English translation)

of the insects to healthy plants were carried out successively at intervals of 5–7 days. During these transfers, a portion of the insects were sometimes tested for their ability to cause the disease. The results showed that the insects tested were capable of causing the disease, but their first and second progeny did not do so. The same results were obtained in the experiments carried out at the Shiga Agricultural Experiment Station.

From the results mentioned above, it was concluded that the cause of the rice dwarf disease was not the leafhopper *N. cincticeps*, but that the disease may be caused by a certain unknown causative agent transmitted by the leafhoppers, like malaria parasites of human beings that were transmitted by mosquitoes.

At present, I could not establish what the causative agent of the disease is, but it should be noted that the leafhoppers are just a carrier of the disease agent. Leafhoppers other than *N. cincticeps* had no relation to the occurrence of the disease, such as *Macrosteles quadrimaculatus* (Yotsuten yokobai) and *Laodelphax striatellus* Fall. (Himetobi unka).

As for control measures, although the causative agent of the disease is still unknown, it is necessary to eliminate the diseased plants in the fields by roguing and burning them, in addition to destroying the leafhoppers.

Teikichi Fukushi (b. 1894)

Teikichi Fukushi

b. 1894

Teikichi Fukushi was born in Hokodate, Hokkaido, Japan, on September 5, 1894. He completed studies at the Hirosaki High School, Hirosaki, Tokoku, in 1913 and entered a three-year program at the Preparatory School of Hokkaido Imperial University. He transferred to studies in the Department of Agronomy at Hokkaido Imperial University and graduated in 1919. In 1920, Fukushi became a lecturer at the Agricultural College, Hokkaido Imperial University and, in 1921, was a professor at the Tottori Agricultural College.

In 1929, Fukushi was named Assistant Professor of Botany, Faculty of Agriculture, at Hokkaido University. He was awarded the doctorate degree in 1934 after presenting his thesis, "Studies on the Dwarf Disease of the Rice Plant." He was named Professor, Faculty of Agriculture, Hokkaido University, in 1934, and in 1947 became Director of the Botanical Garden at Hokkaido University. In 1958, the year he retired and became Emeritus Professor, Fukushi was an invited participant in the 50th anniversary meeting of the American Phytopathological Society. Still active in science, he accepted the position of Professor at the private College of Dairying, Rakuno Gakuen, in 1960.

Fukushi began his studies on plant diseases caused by fungi under the guidance of Kingo Miyabe at Hokkaido University. As early as 1921, he worked on the willow canker disease caused by *Physalospora miyabeana* Fukushi. By 1925, he had found varieties of apple resistant to *Gymnosporangium yamadae* Miyobe, which caused extensive damage in the Aomori prefecture and had completed anatomical investigations on gall tissue of this particular apple rust.

From 1925 to 1927, Fukushi studied in the United States and Europe. He studied with L. R. Jones at the University of Wisconsin on the asters yellow disease and with B. M. Duggar on tobacco mosaic virus at Washington University in St. Louis. He learned about the leafhopper transmission of the aster yellows agent from L. O. Kunkel during a visit at the Boyce Thompson Institute (then located at Yonkers, New York). During that period he also visited scientists in England, Germany, and Holland.

After his return to Japan, he had an intense curiosity about virus diseases of plants and began investigations on these diseases. He also promoted scientific research in plant virology in Japan. In 1932, he demonstrated that cigarettes were a source of tobacco mosaic virus infection in tobacco fields, and he succeeded (in 1933) in purifying TMV through kaolin.

Fukushi's most significant contribution to plant virology is his work on the rice dwarf virus disease, which was initiated in 1927. In 1931, he found inclusion bodies in rice leaves infected with rice dwarf virus (RDV) and confirmed that rice dwarf was a virus-induced disease. He performed precise studies on the relationship between RDV and its insect vectors and found that RDV could be transmitted through the eggs of the leafhopper vector (*Nephotettix cincticeps*) to progeny. Subsequently, in the late 1930s, Fukushi demonstrated successive

transmission of the virus through eggs for seven generations and concluded that RDV multiplies in the insect vector. By 1960, together with his former student, E. Shikata, he had found RDV particles in both the plant host and the insect vector by electron microscopy and had confirmed multiplication of the virus in the leafhopper.

Fukushi has also had a distinguished teaching career. Beginning in 1932, he offered the first lecture series in Japan on plant virus diseases. In 1952, he published *Plant Viruses* (in Japanese), the first book in Japan on plant virology.

Fukushi is an emeritus member of the Phytopathological Society of Japan, the Mycological Society of Japan, and the American Phytopathological Society and is an Honorary Fellow of the Indian Phytopathological Society. He served as president of the Phytopathological Society of Japan from 1956 to 1957 and as president of the Society of Japanese Virologists in 1957.

Throughout his career, Fukushi has been the recipient of numerous awards and honors. In 1941, he received the Japan Agronomy Prize for his studies of the rice dwarf disease. For his research on the insect transmission of plant viruses, he received the Japan Academy Prize in 1958. He was recognized for distinguished service to Aomori-Ken (Aomori Prefecture) in 1961, and was decorated in 1965 with the Second Order of the Sacred Treasure by the Japanese government in recognition of his brilliant contributions to education and science. In 1964, he was elected a member of the Japan Academy.

Transmission of the Virus Through the Eggs of an Insect Vector[1]

by Teikichi Fukushi
Botanical Institute, Faculty of Agriculture, Hokkaido Imperial University, Sapporo
(Comm. by S. Ikeno, M.I.A., October 12, 1933)

In the course of study on the special relationship which exists between the virus of dwarf disease of rice plant and its insect carrier, *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl., the writer obtained evidence to indicate that the virus may be transmitted through the eggs of the leafhopper, by breeding successive generations from infective parents or from crosses between infective females and non-infective males and vice versa. A brief description of the disease was given in the writer's previous paper (1931) and repetition in this short article would be superfluous. A more detailed account will be given in another publication presenting the results of transmission experiments and discussions on the etiology of the disease.

The experimental procedure adopted is as follows. A pair of male and female leafhoppers, either one or both infective, were confined to a young healthy rice plant in a glass tube* and allowed to remain there for one day, transferring to a new healthy plant every day. Precaution was taken to confine single nymphs to rice plants in separate glass tubes and to allow them to remain there until they grew into adults in order to obtain female leafhoppers which had never copulated. The eggs are laid in the leafsheath at a few spots near the ground and thrust transversely into the tissue lying one under the other. A few days after oviposition the spot where the eggs are deposited becomes easily discernible by its slightly discolored and more or less raised surface. The eggs hatch in about ten days. The tiny nymphs just emerged from eggs were transferred to healthy rice plants taking the necessary precautions so that they had no opportunity to feed upon plants on which the eggs had been deposited and which might have been affected by the disease in consequence of the infestation of the infective parents. Daily observations were continued as the hatching of eggs approached and the nymphs which had just emerged from eggs were transferred singly by means of a small camel's hair brush to a young healthy rice plant and glass tube was put on it. The leafhoppers were allowed to remain there and were kept under observation for two months or longer. The results of experiments will be shown in the following table.

[1] Fukushi, T. 1933. Transmission of the virus through the eggs of an insect vector. Proc. Imp. Acad. (Tokyo) 9:457-460.

*The glass tube was 30–40 cm long by 3 cm in diameter closed at the upper end by a thin cotton cloth and put on a rice plant.

TABLE I. Virulence of the progeny of infective leafhoppers.

parents		No. of progeny tested	No. of viruliferous progeny
female	male		
infective female × infective male			
e279-4-3 (♀)	e279-4-4 (♂)	7	5
e'34 (♀)	e'29 (♂)	14	13
e'39 (♀)	e'36 (♂)	8	8
e'49 (♀)	e'68 (♂)	4	4
e'53 (♀)	e'58 (♂)	1	1
infective female × uninfective male			
e279 (♀)	a (♂)	13	13
e151-4 (♀)	b (♂)	16	15
e151-1 (♀)	c (♂)	26	17
e151-4-4 (♀)	d (♂)	6	0
e279-4 (♀)	e (♂)	35	33
e279-1 (♀)	f (♂)	27	0
e151-1-16 (♀)	g (♂)	30	0
e'51 (♀)	h (♂)	8	8
e279-4-5-3-3 (♀)	i (♂)	5	5
e'62 (♀)	j (♂)	2	2
e'69 (♀)	k (♂)	2	0
e'65 (♀)	l (♂)	1	1
e'61 (♀)	m (♂)	12	6
uninfective female × infective male			
a (♀)	e151-1-22 (♂)	9	0
b (♀)	e151-1-13 (♂)	19	0
c (♀)	e'151-1-13 (♂)	5	0
d (♀)	e'47 (♂)	9	0
e (♀)	e279-4-32 (♂)	14	0
f (♀)	e'64 (♂)	4	0
g (♀)	e'72 (♂)	3	0

(♀ and ♂ denote infective male and female respectively while ♀ and ♂ stand for uninfective male and female.)

As shown above the majority of the offspring from the infective parents proved to be viruliferous and the progeny from the crosses between non-infective females and infective males were entirely free from virus whereas those from the crosses between infective females and non-infective males were either viruliferous or free from virus. It appears that the eggs are not affected by the virus after they have been deposited in the leafsheaths but probably at an early stage of their development in the ovary of the maternal insect body, because in no case has it been observed that the infective progeny emerged out of the eggs from non-infective females which had been laid in the leafsheaths of diseased plants. It is worthy of note that some individuals of the progeny from the infective females were viruliferous while others from the same parents were apparently free from virus. This appears to indicate that all the ova produced in an ovary are not always affected by the virus.

Although a number of virus diseases of plants have been demonstrated to be transmissible by the agency of certain insects, in no case has it been shown that

the virus is transmitted through the eggs of an insect carrier. McClintock and Smith (1918) reported that the virus of spinach blight (or spinach mosaic) is transmitted from infective aphids to their viviparously produced progeny up to the fourth generation. This was the only evidence yet recorded that indicates the possible transmission of virus from infective insect vectors to their offspring. Hoggan (1933), however, has been unable to confirm their conclusion.

Dwarf disease of rice plant, therefore, occupies a unique position among the virus diseases of plants for the reason of its transmission through the eggs of the insect vector.

According to Murata (1915, 1931), Onuki and Murata, formerly entomologists in the Imperial Agricultural Experiment Station were probably the first to notice that the offspring from infective leafhoppers are capable of producing dwarf disease in healthy rice plants. In 1902? they found that certain leafhoppers emerged from the eggs which had been taken from the Prefecture of Shiga where this trouble was prevalent at that time produced infections in healthy rice plants. However, they gave no detailed account of it. The idea generally prevailing at that time was that the disease was due to the infestation of leafhoppers and these entomologists considered the capability of producing dwarf disease to be a characteristic specific to the leafhoppers native to the Prefecture of Shiga, because all the leafhoppers of the same species taken in the vicinity of Tokyo failed to produce infections in healthy rice plants. Subsequently they found that even non-infective leafhoppers from Tokyo became infective after feeding upon dwarf diseased rice plants. Murata briefly stated that the capability of producing dwarf disease was transmitted from infective parents to their progeny through 3 or 4 generations but he presented no experimental procedures nor results which are considered to be indispensible to induce such very insignificant conclusions.

At any rate these entomologists were evidently in ignorance of the fact that they were dealing with a virus disease in dwarf disease of rice plant and that the virus may be transmitted through the eggs of the leafhopper, *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl.

MULTIPLICATION OF VIRUS IN ITS INSECT VECTOR[1]

by Teikichi Fukushi
Botanical Institute, Faculty of Agriculture,
Hokkaido Imperial University, Sapporo
(Comm. by S. Ikeno, M.I.A., July 12, 1935)

The assumption that a virus multiplies within the body of its insect vector is supported principally by the facts that certain viruses seem to require the so-called incubation period within the insect carriers and that some insects remain viruliferous for long periods without again having access to a source of the virus. A striking instance of the prolonged retention of virus within the insect bodies through three generations has been obtained in the course of a study on the relationship between the virus of dwarf disease of rice plant and its insect vector, *Nephotettix apicalis* var. *cincticeps*.

As previously reported[2] the virus may be transmitted through the eggs of the viruliferous leafhoppers to the progeny of the second generation. Certain nymphs emerging from the viruliferous eggs are capable of producing infection in healthy plants and retain their infectivity through their entire life.

It was attempted, therefore, to determine whether the virus is transmitted through the eggs of the leafhopper to the progeny of the third generation. A viruliferous female leafhopper designated as f 27, after mating with a non-viruliferous male, laid eggs hatching out after about 10 days. Single nymphs which had just emerged from these eggs were each transferred to a young healthy rice plant in a glass tube in the same way as previously described. They were transferred daily to new healthy plants. Some difficulties were experienced to manipulate young nymphs which were very active and some of them were eventually lost. Six insects were successfully transferred daily to new healthy plants through their entire life and 5 of them proved to be viruliferous, indicating that the virus had been transmitted through the eggs of the insect, f 27, to the progeny of the second generation. Attaining maturity, 2 of these viruliferous insects became females, one of which was designated as f 27-2. A non-viruliferous male was introduced into the glass tube enclosing this infective female which had become adult 7 days before. After pairing the insect, f 27-2 was again transferred to new healthy plants daily and 8 days later it began to lay eggs. This leafhopper emerged from the egg on July 19, 1934, became adult on August 13, and died on

[1] Fukushi, T. 1935. Multiplication of virus in its insect vector. Proc. Imp. Acad. (Tokyo) 11:301-303.
[2] Fukushi, T. 1933. Transmission of the virus through the eggs of an insect vector. Proc. Imp. Acad. (Tokyo) 9:457-460.
Fukushi, T. 1934. Studies on the dwarf disease of rice plant. J. Fac. Agric., Hokkaido Imp. Univ. 37(2):41-164.

September 7 after having laid 35 eggs and also having produced infections in 38 plants as shown below.

July	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26
	−	−	−	−	−	−	−	+	+	+	+
July	26–27	27–28	28–29	29–30	30–31	31–Aug. 1	1–2	2–3	3–4	4–5	5–6	6–7
	+	+	+	+	+	+	+	+	+	+	+	+
Aug.	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18
	+	+	−	+	+	+	+	+	+	+	+
Aug.	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29
	+	+	−	+	+	+	+	+	−	+	+
Aug.	29–30	30–31	31–Sept. 1	1–2	2–3	3–4	4–5	5–6	6–7		
	+	+	×	×	−	−	−	+	−		

[The sign (+) indicates positive infection, (−) no infection, (×) plant died, (|) moulting and (‖) the final moulting.]

From the 35 eggs above mentioned, 26 nymphs were successfully transferred to healthy plants as soon as they had emerged from the eggs. Six of these nymphs were transferred separately to new healthy plants every day, 9 of them were allowed to remain each in a glass tube enclosing a healthy rice plant and the others were lost on the occasion of transference. All the 15 insects which survived long enough proved to be viruliferous, showing that the virus had been transmitted through eggs to the progeny of the third generation. Six leafhoppers which were transferred daily to new healthy plants produced infections in 13, 55, 35, 11, 50 and 28 plants, respectively, as shown in the following table.

No. of insect	Sept. 12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22
f 27-2-1	*−	−	−	−	−	−	−	−	+	+
f 27-2-9						*−	−	−	−	−
f 27-2-10						*−	−	−	−	−
f 27-2-11						*−	−	−	−	−
f 27-2-14							*−	−	−	−
f 27-2-15							*−	−	−	−

	Sept. 22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–Oct. 1
f 27-2-1	+	+	×	+	+	+	−	+	+
f 27-2-9	−	−	−	+	+	+	+	+	+
f 27-2-10	−	−	−	−	−	−	+	+	−
f 27-2-11	−	−	−	−	−	−	−	+	+
f 27-2-14	−	−	−	−	−	−	−	−	−
f 27-2-15	−	−	−	−	−	−	−	+	−

	Oct. 1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13
f 27-2-1	+	+	+	+								
f 27-2-9	+	+	+	+	+	+	+	+	+	+	+	+
f 27-2-10	−	−	−	−	+	+	+	+	+	+	+	+
f 27-2-11	−	+	+	+	+	+	+	+	+	+		
f 27-2-14	−	−	−	−	−	+	+	+	+	+	+	+
f 27-2-15	−	+	+	−	+	+	+	+	+	+	+	+

[*continued on next page*]

No. of insect	Oct. 13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23
f 27-2-1										
f 27-2-9	+	+	×	+	+	+	+	+	+	×
f 27-2-10	+	+	+	+	+	×	+	+	+	+
f 27-2-11										
f 27-2-14	+	+	+	+	+	+	+	+	+	–
f 27-2-15	+	+	+	+	+	+	×	+	+	+

No. of insect	Oct. 23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–31	31–Nov. 1
f 27-2-1									
f 27-2-9	+	+	+	+	+	+	+	+	+
f 27-2-10	+	+	+	+	+	+	×	+	×
f 27-2-11									
f 27-2-14	+	+	+	+	+	+	×	+	×
f 27-2-15	+	+	+	+	+	+	+	+	×

No. of insect	Nov. 1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13
f 27-2-1												
f 27-2-9	+	×	+	×	×	+	+	+	×	+	+	+
f 27-2-10	×	×	×	×	×	×	×	×	+	+	+	+
f 27-2-11												
f 27-2-14	+	+	×	+	×	+	+	+	+	+	+	+
f 27-2-15	×	–	×	–	×	–	–	–	×	–	–	

No. of insect	Nov. 13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23
f 27-2-1										
f 27-2-9	+	+	+	+	+	+	+	+	+	+
f 27-2-10	+	+	+	+	+	×				
f 27-2-11										
f 27-2-14	+	+	+	–	+	+	+	+	+	×
f 27-2-15										

No. of insect	Nov. 23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–Dec. 1	1–2
f 27-2-1									
f 27-2-9	×	+	+	×	×				
f 27-2-10									
f 27-2-11									
f 27-2-14	+	+	+	+	+	×	×	×	×
f 27-2-15									

No. of insect	Dec. 2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10
f 27-2-1								
f 27-2-9								
f 27-2-10								
f 27-2-11								
f 27-2-14	+	+	×	×	+	×	×	+
f 27-2-15								

* Date of emergence.

The average weight of a nymph just emerged from the egg being 0.06 milligram, the amount of virus originally contained in the body of the insect, f 27-2 must have been extremely small. Without assuming that the virus multiplies within an insect body, it can be hardly explained how such an insect was capable of producing infections in 38 healthy plants on which it was confined

for 24 hours on consecutive days and moreover distributing the virus into at least 15 eggs developing progeny which could produce infections in 201 healthy plants without again having access to a source of virus.

Retention of Virus by Its Insect Vectors Through Several Generations*[1]

by Teikichi Fukushi
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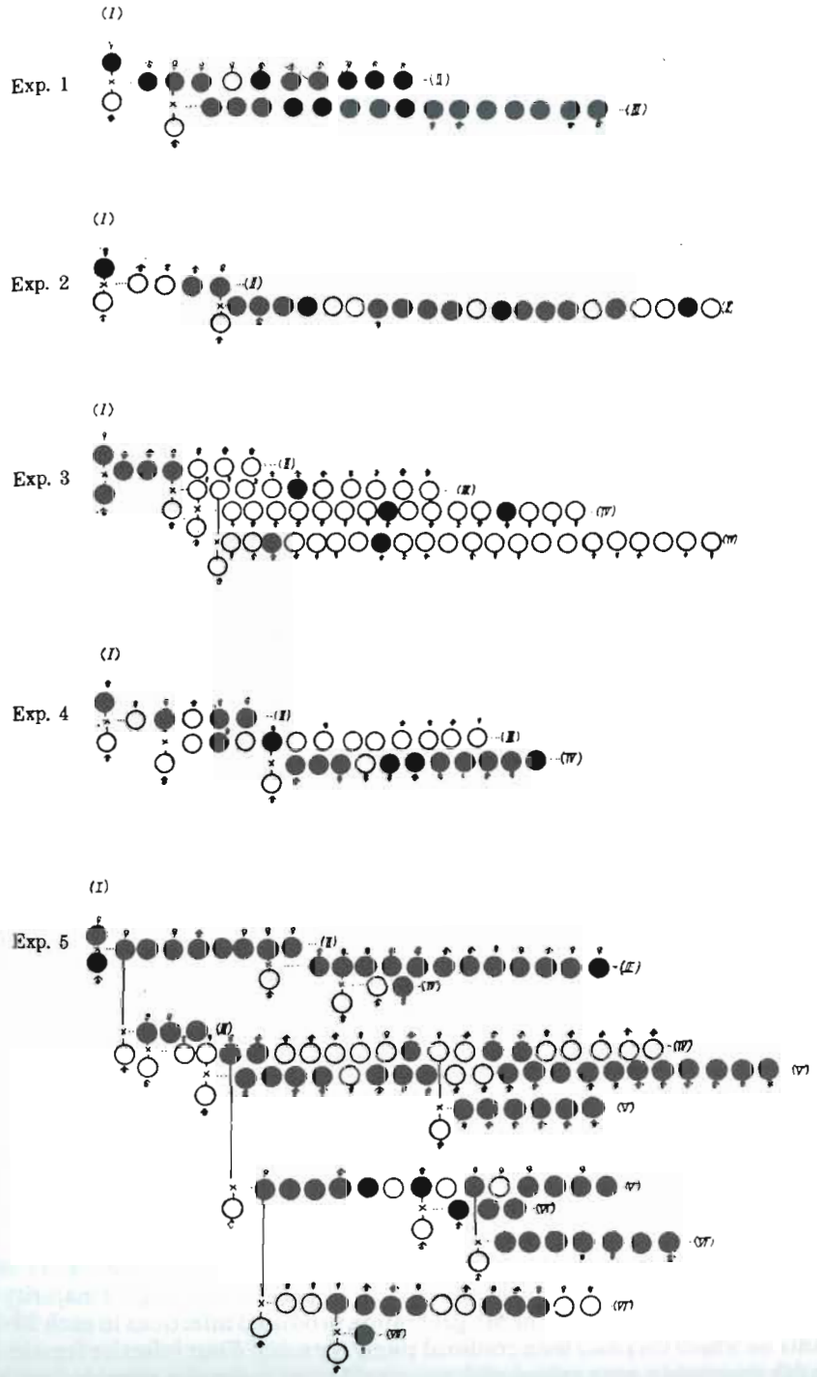
(Comm. by K. Miyabe, M.I.A., May 12, 1939)

In a previous paper[2] the writer has reported on a case of successful transmission of the virus of rice dwarf disease through the eggs of its insect vector passing on to the progeny of the 3rd generation. In view of the fact that rice dwarf disease occupies a unique position among plant virus diseases on account of its transmissibility through the eggs of a certain insect vector, an attempt was made to study this phase of the problem more extensively as a part of the investigations conducted on the special relationship between the virus of this disorder and its insect carrier, *Nephotettix apicalis cincticeps*, a leafhopper. The experimental procedure was similar to what has previously been described. It consists of picking up the nymphs bred from infective female leafhoppers immediately upon hatching to cage them singly on healthy rice seedling plants and subsequently transferring them daily to successive new healthy plants. The viruliferous females found among these second generation individuals are paired with non-viruliferous males and their offspring are also transferred to healthy plants as soon as they have hatched. Subsequently they are likewise transferred daily to successive new healthy plants in order to keep them from renewed access to a source of infection. In a similar way the experiment was continued as long as a viruliferous female was available in the progeny of the leafhoppers. As a consequence, during the past 5 years, two cases each were secured of transmission of the virus through eggs to the progeny of the 3rd and 4th generations as well as one case of its perpetuation to the 7th generation. In each case, the experiment was unavoidably ended because of the circumstances that either viruliferous females could not be obtained or that they died before laying eggs. The results of these experiments are presented in the following diagrams and table.

*The writer wishes to acknowledge gratefully his indebtedness to Profs. K. Miyabe and S. Ito for their kind advice and encouragement throughout the course of the investigation.
This work was made possible, in part, through a grant from the Japan Society for the Promotion of Scientific Research.
[1] Fukushi, T. 1939. Retention of virus by its insect vectors through several generations. Proc. Imp. Acad. (Tokyo) 15:142-145.
[2] Fukushi, T. 1935. Multiplication of virus in its insect vector. Proc. Imp. Acad. (Tokyo) 11:301-303.



● ○ indicate infective and non-infective leafhoppers, respectively.

Generation / Experiment No.	I	II		III		IV		V		VI		VII	
		Infective leafhopper	Non-inf. leafhopper	Infective leafhopper	Non-inf. leafhopper	Infective leafhopper	Non-inf. leafhopper	Infective leafhopper	Non-inf. leafhopper	Infective leafhopper	Non-inf. leafhopper	Infective leafhopper	Non-inf. leafhopper
1	♀ × ♂	9	1	15	0								
2	♀ × ♂	2	2	14	7								
3	♀ × ♂	3	3	1	9	4	34						
4	♀ × ♂	3	2	2	10	10	1						
5	♀ × ♂	8	0	15	0	6	15	36	6	17	6	1	0

♀ ♂ ♂ indicate infective female, infective male and non-infective male, respectively.

It is worthy of note that certain female leafhoppers which proved to be non-infective through their entire life produced inactive progeny as shown in experiments 3 and 5. On this point some explanations seem to be necessary. When a low temperature prevailed in the greenhouse, a much prolonged incubation period was usually required for the virus in the rice plant. On the other hand, some female leafhoppers which had grown too old would show a tendency not to mate readily with males. Accordingly, in experiment 3, certain females of the third generation which had been reared during the winter were allowed to mate with nonviruliferous males without waiting for their betraying infectivity. They eventually proved to be non-infective but some of their offspring produced infections in test plants. This phenomenon which was more definitely established in experiment 5 admits, in the writer's opinion, the following interpretation. Under low temperature conditions, which retard the manifestation of the disease in the rice plant, the multiplication of virus may be remarkably inhibited not only in the rice plant but also within the bodies of the insect carrier. In such a case the virus is perhaps more or less localized in its distribution and movement through the insect body. It is possible that in some leafhoppers the virus may migrate to the salivary glands and finally may be injected into the plant tissues through the proboscis to cause infections whereas in other individuals it may localize not in the salivary glands but in ovarian tubules to give rise to viruliferous eggs. Admittedly the latter individuals will prove to be non-infective but may produce infective progeny.

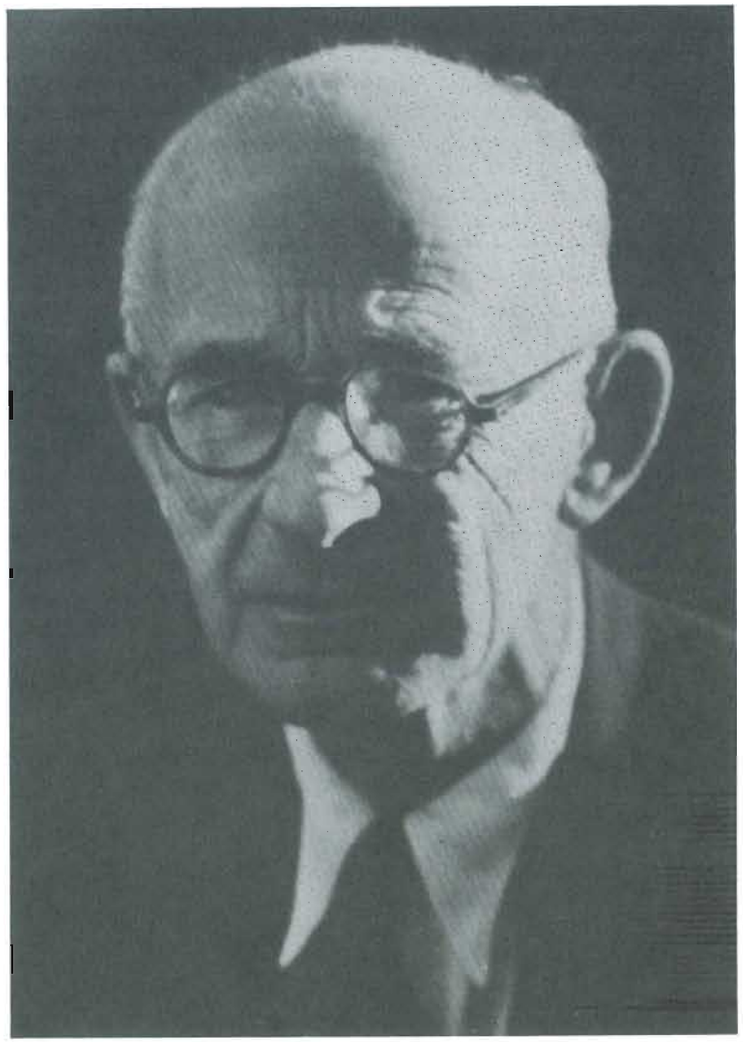
As shown in the diagrams, a most striking instance of prolonged retention of the virus by leafhoppers was obtained in experiment 5. In this case, the virus has been transmitted through the eggs to the progeny in the 7th generation; that is to say, it has been retained within insect bodies for a whole year during the period from April 7, 1938, to April 24, 1939.

It appears that the virus has practically not been reduced in virulence as a result of its retention by insect carriers through several generations, since a majority of the infective leafhoppers of the 6th generation produced infections in each 50–80 plants on which they had been confined singly for a day. Four infective females of the 6th generation were paired with nonviruliferous males at a suitable time but three of them deposited no eggs while the remaining one laid only a few normal

and several abortive eggs. Only one nymph of the 7th generation could be picked up at the proper time and confined on a test plant without daily transfers, which subsequently developed the signs of infection.

The complete experimental data for the 5 cases mentioned above will be presented in detail in another publication. But it will be interesting to note here that in experiment 5, infections were produced in more than one thousand rice plants by 26 leafhoppers of 5 generations derived from one viruliferous egg, when they were confined singly on a rice plant for a day and transferred daily to new healthy plants.

To explain these experimental results it seems necessary to assume that the virus of rice dwarf disease multiplies within the bodies of its insect vectors, since the amount of virus originally contained in one egg must be extremely small.



Harold Haydon Storey (1894–1969)

Harold Haydon Storey

1894–1969

Harold Haydon Storey was born June 10, 1894, in Manchester, England. He attended school on the Isle of Man and received his Ph.D. from Cambridge University in 1928. He served in World War I (1914–1918) in the Royal Air Force as an engineer and as a fighter pilot. From 1922 to 1928, he served in the Department of Agriculture, Union of South Africa, in Natal. In 1928, he joined the staff of the East African Agricultural Research Institute at Amani, Tanganyika Territory. It was there, working in relative isolation, that he conducted his experiments elucidating mechanisms of transmission of the maize streak virus by the leafhopper *Cicadulina mbila*. In World War II (1939–1945), he served as Secretary and then Chairman of the East African Industrial Research Board, which planned and directed research on the production and supply in East Africa of paints, chemicals, edible oils, pottery, firebricks, building boards, and pyrethrum extract.

In 1946, he was appointed Secretary for Colonial Agricultural Research in the Colonial Office in London. He returned to East Africa in 1948 as Deputy Director and Acting Director (1956–1957) of the East African Agriculture and Forestry Research Organization, Nairobi, Kenya. From 1954 to 1956, Storey was a member of the Scientific Council of Africa. He continued active research in East Africa until his death in 1969.

In 1931, Storey proved that a white fly transmitted the tobacco leaf curl virus and also devised control measures for this disease, which was threatening the tobacco industry of Central Africa. He subsequently saved the Nyassaland (Malawi) tea industry from near extinction by proving that "tea-yellows" was a sulphur deficiency. He also initiated a cassava selection and breeding program to control the cassava mosaic and brown streak diseases. Genetics always held a special interest for him, and he spent the years preceding retirement studying the genetics of resistance of maize to *Puccinia polysora* and to maize streak virus.

For his exceptional contributions to research, Storey was elected a Fellow of the Royal Society in 1946 and, in 1948, was awarded the title of Companion of the Order of St. Michael and St. George (C.M.G.) for outstanding services to East African industry during the war years and for subsequent administrative contributions at the Colonial Office.

The Inheritance by an Insect Vector of the Ability to Transmit a Plant Virus[1]

by H. H. Storey
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(Communicated by F. T. Brooks, F.R.S.
Received July 27, 1932)

The leafhopper, *Cicadulina mbila* Naude, is normally an efficient vector of the virus of streak disease of maize (Storey, 1925). I have, however, encountered exceptional individuals of this insect-species which were unable to transmit the virus (Storey, 1928). An attempt to discover the reason for their anomalous behaviour led me to undertake studies of the breeding of this species. The results so obtained, which show that the ability to transmit is an hereditary character, have been briefly reported (Storey, 1931), and I now present a full account of this work.

Since these studies introduce a new conception into pathology, I find it necessary to give my definition of certain current terms and to utilise two new ones. I employ *transmission* in the restricted sense of the act of transfer of a virus by a vector from a diseased to a healthy plant *in the natural process of that vector's feeding*. I introduce the term *activity* to denote an insect's inherent* potentiality to transmit a virus; *inactivity* to denote the absence of that inherent potentiality. An insect is *viruliferous* or *non-viruliferous* according as it is, or is not, actually carrying a virus in such a way that it inoculates that virus into a plant on which it feeds, and in consequence causes the plant to develop the symptoms of the disease.

For clearness in the understanding of this account I may refer to the evidence which has been given elsewhere (Storey, 1928) that the leafhopper, *Cicadulina mbila*, is invariably non-viruliferous upon hatching from the egg; that thereafter the normal (i.e. active) leafhopper of either sex and in any stage of its development may take up the virus by feeding on a diseased plant; and that the leafhopper, following its first feed upon a diseased plant, will normally become viruliferous after a latent period not exceeding 3 days at the temperatures at which my experiments have been performed, and will usually thereafter remain viruliferous without further feeding upon a diseased plant.

In the execution of this work Mr. R. F. W. Nichols has taken an important part, which I gratefully acknowledge. The early pure line breeding was carried

[1] Storey, H. H. 1932. The inheritance by an insect vector of the ability to transmit a plant virus. Proc. R. Soc. London, Ser. B 112:46-60.

*The qualification "inherent" may appear tautological. A line of work now in hand indicates that the qualification is, on the contrary, essential.

out by him alone during my absence from Amani. In the latter work he has undertaken the greater part of the manipulation of the experiments.

Materials

In the first series of breeding experiments I used hoppers from a mixed culture of *Cicadulina mbila* which had been maintained for 2 years from parents originally collected near Tanga, Tanganyika Territory. From this mixed culture I isolated pure lines designated *Act.* I and II and *Inact.* I and II. Later, further collections of hoppers were made in the same locality, and from these were bred the pure lines of *Act.* IV and V and *Inact.* III and IV.

Preliminary evidence from the breeding of this species of leafhoppers indicated a falling-off of fertility in inbreeding. Consequently, in the course of the earlier breeding work not described, by crossing parallel lines whenever opportunity allowed, I tried to avoid inbreeding. Later experience showed, however, that my fears of inbreeding were exaggerated; while crosses are probably more prolific than inbred lines, I have found it possible to rear lines successfully by inbreeding.

Two main considerations have determined the experimental procedure adopted in this work:

(1) The test for the activity of a hopper rests upon whether it becomes viruliferous after feeding upon a diseased plant. A viruliferous leafhopper can be recognized as such only by studying the effect of its feeding upon a healthy plant.

(2) The leafhopper lays its eggs in the tissues of a living leaf and these eggs will hatch only if the leaf remains alive through the period of their development.

The method used in the testing of families for activity was as follows. While still in an early nymphal instar, the hoppers were caged together upon a fully streaked leaf of a diseased maize plant. They fed upon the diseased leaf as long as was needed for them to reach a late nymphal instar, but never for less than 4 days. Thereafter each nymph was placed separately in a small single-leaf cage of a design which I have described elsewhere (Storey, 1928), and allowed to feed upon the first leaf of a very young maize seedling until the disease symptoms appeared on that seedling usually in 5 or 6 days—or, failing successful transmission, for a period of not less than 14 days.

A test carried out in this manner, as I have already shown (Storey, 1928), demonstrates with certainty whether a hopper of the species, *Cicadulina mbila*, is viruliferous or non-viruliferous. I have also shown that it is usually safe to accept as inactive those hoppers which are non-viruliferous in such a test. Rarely, however, an active hopper, for some reason unknown, may fail to become viruliferous following the first period of feeding upon a diseased plant, and after a second feed on a diseased plant may become viruliferous. Consequently in many instances those hoppers which were non-viruliferous in the first test were returned to a diseased plant and then tested a second time. Usually I resorted to a second test only when the number of non-viruliferous hoppers was small in proportion to the number of viruliferous hoppers.

I have accepted the evidence of the second test as conclusive, since throughout this and earlier work I have encountered no instance where a hopper, later

proved to be active, has failed to become viruliferous at least during the second feed on a diseased plant.

The test may fail if the hopper dies during the test. Generally a proportion of the hoppers in each family died. These deaths may be ascribed in part at least to injuries received in transfer to the cages, to drowning in water exuded into the cages from the hydathodes of the plant, and to difficulties encountered in the final change of skin in the confined space of the leaf-cages. Many hoppers which died during the test nevertheless successfully inoculated the virus into the test plants. It would be unsafe to assume that hoppers which died during the test and failed to transmit were necessarily inactive. An uncertainty exists as to their status for the following reasons: (1) they may have been viruliferous, but may have died without feeding on the test plants, or (2) they may have been active but failed to become viruliferous after their first feed on a diseased plant, a situation which would have been revealed in a second test, had this been possible. To meet the former difficulty, in later work I adopted the procedure of discarding all individual tests in which the hoppers died during the first 2 days of the test, and excluding these tests from the totals. I assumed that no hopper would survive 2 days, under the conditions of my experiment, without feeding on the test plant. It is clearly impossible to confirm or disprove the possibility considered under the second of the preceding alternatives. The negative evidence which they provide must, however, at least be regarded as doubtful, and where it is in conflict with the mass of the evidence provided by the experiments in which it occurs, I have felt justified in not regarding it as significant.

The preceding paragraphs suggest that I have encountered considerable difficulties in the testing of families for activity. Actually, the exceptional behaviour of hoppers there considered has had a very small influence upon my results. The following figures are extracted from the records of the breeding of active races, which appear in Table I; of 531 hoppers in families which I have regarded as active, 519 were viruliferous in the first test, 7 were non-viruliferous in the first test and viruliferous in the second test, while 5 died during the tests without causing infection of the test plants.

The tests were carried out in a gauze-protected greenhouse in which the maize plants were raised from seed. Two rows of 12 seeds each were sown in long narrow wooden boxes. One row of plants was usually retained as a control for the test plants. During the whole course of this work no control plant became diseased.

At the end of the test, the leafhoppers which had been caged singly while still nymphs, had become adults and were ready for pairing. Each pair, selected as parents on the results of the tests, was confined in a glass tube, 20 cm. long by 25 mm. bore, closed at one end with muslin and at the other by a plug of cotton-wool. Through this plug was passed the tip of a leaf of a maize plant upon which the parent hoppers fed and laid eggs, and the newly hatched nymphs fed. The mean air temperature of the insectary varied from 19° to 23° C according to the season, with absolute maxima and minima of 30° and 11° C. The breeding tubes were not exposed to direct sunlight except for a short period in the early morning. The hoppers bred at all seasons but more rapidly at the higher temperatures. At first difficulty was encountered through the high humidity and deposition of water within these tubes, which apart from the direct ill-effects on the young nymphs, caused the premature death of the leaf-tip and consequently of many eggs laid in its tissues. This difficulty was overcome by a system of ventilation. An

Table I.—Breeding of Active Races.*

All parents selected as active.

Generation.	Family number.	Family number of parents.		Tests.	
		Female.	Male.	Number V.	Number N-V.
F1	A	From mixed culture		10	0
	B	,,		22	2
	C	,,		11	1
	E	,,		55	0
	F	,,		3	0
	G	,,		21	1†
	H	,,		25	8‡
	L	,,		7	0
	N	,,		19	6‡
F2	I	A	C	23	0
	II	A	C	10	0
	III	A	Mixed culture	17	0
	V	A	C	33	9‡
	IX	F	E	19	0
	XI	E	E	4	0
	XIII	E	E	5	2†
	XIV	E	E	8	0
	XVII	G	V (F2)	21	2
	XVIII	E	H	18	0
	XXI	L	20 (F3)	35	0
	XXII	L	20 (F3)	18	0
	XXIII	L	24 (F3)	10	2‡
	XXIV	N	N	11	6
	XXVII	N	N	31	0
	XXVIII	N	N	19	0
F3	1	II	I	24	0
	2	II	I	5	0
	6	I	II	7	1†
	7	I	I	19	0
	10	V	V	6	3‡
	14	V	H (F1)	20	3‡
	16	IX	2 (F3)	12	1†
	20	XVIII	XVIII	8	0
	24	XVIII	XVIII	14	0
	29	XXIII	XXIII	11	5
	31	XXIII	XXII	16	4
	32	XXIII	XXII	29	7
	33	XXIII	XXI	11	0
F4	*a*	1	2	7	0
	i	14	14	24	2‡
	q	7	7	9	0
	y	20	16	47	0
	b′	24	*i* (F4)	37	0
F5	BB	*i*	*i*	22	1
	DD	*i*	*i*	3	2
	EE	*i*	*i*	4	1‡
	JJ	*b′*	*b′*	9	0
	MM	*b′*	*b′*	22	0

* In the Tables, viruliferous and non-viruliferous are designated by V and N-V.

† Hoppers died without second test.

‡ One or more of the non-viruliferous hoppers in each group received second test and proved active.

electric fan forced air into a main duct from which it was led into the glass breeding-tubes by small rubber tubes passing through the wool plugs. A further development was the preliminary pairing of hoppers in small leaf-cages, from which they were transferred to the breeding tubes only after egg-laying had begun. By these means the useful life of the leaf-tip in the breeding tube was prolonged.

One further operation remains to be described. For the purpose of establishing bulked cultures of active races, I was required to breed a non-viruliferous progeny from parents, which having demonstrated their ability to transmit in the tests, were necessarily viruliferous and would infect any plant to which they had access for the laying of their eggs. For this purpose the usual breeding procedure was followed, a leaf of an originally healthy plant being exposed within the breeding tube. The feeding of the parents produced no visible effect upon this leaf, although the plant as a whole became streak-diseased. I have shown that a hopper rarely picks up the virus from the green part of a diseased plant (Storey, 1928). Consequently, by testing the young nymphs which hatched in the tube, as early as possible upon maize seedlings, I was able to separate for bulking those—generally a large proportion, which had failed to become viruliferous.

The Breeding of Pure Lines

Active Races.—The results of the first series of experiments in breeding from parents selected as active are presented in Table I. (This and subsequent Tables omit only those relevant pairings which produced no offspring.) In certain instances both parents were selected from one family; but as far as possible the parents were selected from different lines, owing to my fear of a falling-off in fertility resulting from inbreeding. This fact makes the Table difficult to follow, but the manner of presentation allows the parentage of any family to be traced back through all its generations.

Of the nine F1 families raised, four showed all members active, though on three of these the numbers were small. Of the subsequent families raised from parents selected from these F1 families, almost all came pure for activity. The exceptions are probably sufficiently explained by the death of hoppers during the tests, except in the instance of the F1 family No. L, the subsequent history of which in the F2 and F3 generations (see XXIII and 29, 31, 32) shows that it was not pure for activity, although the seven hoppers of which it consisted were all active.

Five of the F1 families contained both active and inactive hoppers. Parents selected as active from these mixed families gave in some instances pure progenies and in some instances mixed. Thus in the F2 generation, crossings of parents from C (mixed) and A (pure) gave two pure progenies and one mixed progeny. Inbreeding within the family N (mixed) gave two pure progenies and one mixed.

The breeding was carried on to the fifth generation, although it is clear that for the mere purpose of obtaining pure lines the work might have been stopped earlier. In starting bulked cultures of pure lines I assumed that I might accept a line as pure if parents, taken from apparently pure families, produced a progeny similarly pure. The following bulked cultures were thus established:—

Act. I—Parents from IX, XI, (F2) and 1 (F3).
Act. II—Parents from *y* (F4).

Further bulked cultures, Nos. *Act.* IV and V, were established by breeding from female hoppers, caught as adults in the field. A small proportion of these females gave progenies which proved to be apparently pure for activity. The F2 inbred generation from these was tested and bulked. The details of the families appear in Table II.

The bulked cultures have been maintained for a considerable period—*Act.* I and II since October 23, 1930, and March 2, 1931, respectively. Table III shows the results of periodical sampling tests of certain cultures. These tests indicated a satisfactory maintenance of purity, with the exception of the one test of culture *Act.* I and April 11, 1931. The 10 non-viruliferous hoppers in this test all died before a second test could be made. It will be noticed that in a test of the same culture at a later date all hoppers were active.

In the breeding-up of large numbers of active hoppers, I have found it advantageous to make uncontrolled crossings between several bulked pure lines. These crossings have resulted in cultures pure for activity, as the sample tests in Table III demonstrate.

Inactive Races.—The results of breeding for inactivity are summarised below:

Parents—Selected as inactive, from original mixed culture.
F1—Six families raised, averaging 36 hoppers per family—All inactive.
F2—Ten families raised, averaging 9 hoppers per family—All inactive.
F3—Eleven families raised, averaging 13 hoppers per family—All inactive, except 2 hoppers, one in each of two families.
F4—Six families raised, averaging 11 hoppers per family—All inactive, except 1 hopper.
F5—Ten families raised, averaging 11 hoppers per family—All inactive.
F6—Five families raised, averaging 19 hoppers per family—All inactive.

Table II. Breeding of Active Races.

Races inbred from wild active parents.

Race number.	Generation.	Family number.	Tests.	
			Number V.	Number N-V.
Act. IV	F1	IV	47	0
	F2	IV.1	21	0
		IV.2	5	0
		IV.4	12	0
		IV.6	14	0
		IV.7	5	0
		IV.8	10	0
		IV.9	9	1*
		IV.10	19	0
Act. V	F1	V	26	1*
	F2	V.1	16	1*
		V.3	6	0
		V.6	13	0

* Hoppers died without second test.

Table III.—Sample Tests of Bulked Active Cultures.

Sample of hoppers taken from culture, fed for few days on streak-diseased maize and tested singly.

Culture number.	Date of test.	Tests.	
		Number V.	Number N-V.
Single Lines—			
Act. I	December 31, 1930	29	1*
	April 11, 1931	66	10*
	October 23, 1931	18	1*
	April 30, 1932	35	0
Act. II	May 15, 1931	15	0
Crossed Lines—			
Act. I × *Act.* IV	March 26, 1932	25	0
Act. II × *Act.* IV	March 26, 1932	20	0

* Hoppers died without second test.

The breeding from inactive parents thus resulted in progenies, which were entirely inactive, except that two families in the F3 generation and one in the F4 generation each contained one active hopper. In the two former instances the activity of the hoppers was confirmed in a second test. It seems impossible at present to explain these anomalous individuals except on the grounds of experimental error, for which in the necessarily complicated technique there is some opportunity. Whatever be the true explanation, however, these few exceptions—3 in 700—do not invalidate the general conclusion that lines may be bred true for the factor of inactivity.

In the course of the preceding pure line breeding, I established two bulked inactive cultures, designated *Inact.* I and II. Two more bulked cultures, *Inact.* III and IV, were started by inbreeding from wild females collected in the field, as shown in Table IV. It will be seen from this Table that the original female parents

Table IV.—Breeding of Inactive Races.

Races bred from wild parents.

Race number.	Generation.	Family number.	Parents.		Tests.	
			Female.	Male.	Number V.	Number N-V.
Inact. III	F1	III	Active	?	7	13
	F2	III.1	Inactive	Inactive	0	6
		III.3	Inactive	Inactive	0	12
	F3	III.1.1	Inactive	Inactive	0	23
		III.3.1	Inactive	Inactive	0	37
Inact. IV	F1	IV	Active	?	26	43*
	F2	IV.4	Active	Inactive	11	10
	F3	IV.4.1	Inactive	Inactive	0	22
	F4	IV.4.1.1	Inactive	Inactive	0	24
		IV.4.1.2	Inactive	Inactive	0	24

* Surviving non-viruliferous hoppers, all males.

were active, but gave mixed F1 progenies. In the instance of line III, I was able to select from the F1 family inactive males and females, which when paired gave progenies pure for inactivity. The line IV gave an F1 family in which all the surviving inactive members were males, so that it was necessary to pair these with active females of the same family. The resulting F2 family was consequently mixed, but parents selected as inactive from this family gave pure inactive progenies in the F3 and F4 generations.

Sample tests of these bulked cultures and of certain crossed bulked cultures invariably revealed a maintenance of purity for inactivity as shown in Table V.

The Crossing of Pure Lines

The results of the two reciprocal crosses between the pure active and inactive races are shown in Tables VI and VII. It will be seen that the cross active female × inactive male gave an entirely active progeny in the F1 generation. The F2 generation of this cross consisted of active females and both active and inactive males. The reciprocal cross, inactive female × active male, resulted in the F1 generation in only active females and inactive males. Both active and inactive males and females appeared in the F2 generation.

I offer the hypothesis that in the species *Cicadulina mbila* the male is heterozygous for sex, that the factor for activity is dominant to that for inactivity, and that this factor is linked with sex (in the manner exemplified in the well-known instance of *Drosophila*); that is (employing the conventional notation) that the inactive male has the constitution (aX)(Y), the active male (AX)(Y), the inactive female (aX)(aX), and the active female either (Ax)(AX) or (AX)(aX). This hypothesis requires in the several generation of crosses the ratios shown opposite "Expectation" in Tables VI and VII. An examination of the figures in these tables shows a close agreement of the observed totals with the expectation. In particular, it will be noted that the hypothesis requires that in the F1 and F2 generations of Table VI and the F1 of Table VII certain categories shall not appear in the families. In every instance this requirement has been realised. Thus, for example, the hypothesis requires that the F1 generation in Table VII shall contain no inactive females or active males; these categories were absent from the five independent families reared.

Table V.—Sample Tests of Bulked Inactive Cultures.

Sample of hoppers taken from culture, fed for a few days on streak-diseased maize and tested singly.

Culture number.	Date of test.	Tests.	
		Number V.	Number N-V.
Inact. I	October 22, 1931	0	27
	November 19, 1931	0	27
	April 23, 1932	0	33
	May 15, 1932	0	33
Inact. II	March 18, 1931	0	65
Inact. I × *Inact.* III	April 11, 1932	0	13
Inact. I × *Inact.* IV	April 11, 1932	0	20

The closeness of the agreement of the observed totals with the expectations is obvious by simple inspection in all instances except in the F2 generation of the cross inactive female × active male (Table VII). The χ^2 test applied to the totals of this generation gives a value of $P = 0.065$, which falls just above the value ($P = 0.05$) generally accepted as implying a significant fit to the expected ratios. If the individual families be examined, all show a significant fit to the expected ratios (family A8 barely so), except family A5, which shows a wide deviation from the expectation. I am unable to explain this anamalous result. If it may be regarded as due to experimental error, the totals of the remaining 10 families show a reasonably close fit to the expected ratios ($P = 0.3$ approximately).

Comparison of Active and Inactive Races

There can be little doubt that both active and inactive races judged by the usual criteria of the systematist fall into the one species *Cicadulina mbila*. I have failed to find any point of difference in the external morphology of members of the two races. Mr. T. W. Kirkpatrick, Entomologist of the East African Agricultural Research Station, has confirmed my conclusion after an independent examination. In particular we find no difference in male genitalia of the races, which agree with the figures of China (1928).

Crosses between the two races, as has already been noted, are fertile.

Both races feed freely on a maize leaf and I have observed no difference in their length of life. Sections of maize leaves, upon which the two kinds of hoppers have fed, show microscopically no difference in the character of the feeding channel. The feeding stylets of the two races are not detectably different in average length. Nevertheless, although the active race of hoppers usually became viruliferous after a short period of feeding upon a diseased maize leaf under my experimental

Table VI.—Crosses—Active Female × Inactive Male.

Generation.	Family number.	Parents.		Females.		Males.	
		Female.	Male.	Active.	Inactive.	Active.	Inactive.
F1	A	Active	Inactive	15	0	14	0
	B	Active	Inactive	13	0	5	0
	C	Active	Inactive	18	0	21	0
			Totals	46	0	40	0
			Expectation	43	0	43	0
F2	A.1	Active	Active	5	0	1	4
	A.2	Active	Active	8	0	4	2
	B.1	Active	Active	5	0	4	1
	B.2	Active	Active	6	0	2	3
			Totals	24	0	12	10
			Expectation	23	0	11·5	11·5

condition, the inactive race, as the evidence already given shows, invariably failed to become viruliferous. The same is true at temperatures outside the range encountered in that work. In an experiment, groups of about 20 inactive hoppers were each fed on diseased maize for 2 days at temperatures of 10° C, about 24° C (room temperature), 30° C, and 40° C. Only five hoppers survived the highest temperature. No hopper in any series become viruliferous. Similar failures were encountered in every instance where the period of feeding on diseased maize was reduced to 1 hour and extended to several days.

Discussion

The possibility of a differing ability to transmit a virus in different races of one vector-species has probably been considered by many workers who have studied insect-transmission. K. M. Smith (1931a), for example, has recently drawn attention to this possibility. Hoggan (1929–1931), in her work upon the vector-species, *Myzus persicae* Sulz., took special precautions to avoid errors through the use of a single strain of the aphids. I believe, however, that the work described provides for the first time a definite demonstration that strains of unequal ability to transmit a virus may exist within a single species.

Table VII.—Crosses—Inactive Female × Active Male.

Generation.	Family number.	Parents.		Tests.			
				Females.		Males.	
		Female.	Male.	Active.	Inactive.	Active.	Inactive.
F1	A	Inactive	Active	27	0	0	14
	B	Inactive	Active	3	0	0	7
	C	Inactive	Active	8	0	0	14
	D	Inactive	Active	17	0	0	23
	E	Inactive	Active	27	0	0	19
		Totals		82	0	0	77
		Expectation		79·5	0	0	79·5
F2	A.1	Active	Inactive	8	11	8	7
	A.2	Active	Inactive	1	1	1	1
	A.3	Active	Inactive	0	1	1	0
	A.4	Active	Inactive	0	0	3	3
	A.5	Active	Inactive	7	9	12	1
	A.6	Active	Inactive	2	4	3	0
	A.7	Active	Inactive	4	2	1	0
	A.8	Active	Inactive	1	5	0	1
	A.9	Active	Inactive	0	3	4	4
	B.1	Active	Inactive	4	5	2	3
	B.2	Active	Inactive	2	2	3	3
		Totals		29	43	38	23
		Expectation		33·25	33·25	33·25	33·25
Omitting family A.5		Totals		22	34	26	22
		Expectation		26	26	26	26

This phenomenon is possibly of a more general occurrence than has yet been shown. It is true that Kunkel (1926) decided that, in the species *Cicadula sexnotata* Fall., "most, if not all, individuals are capable of taking up the aster yellows virus." This conclusion, however, is based upon the study of only 30 hoppers, whose origin, although not clearly stated by Kunkel, may have been an inbred line. On the other hand, unexplained failures are a common feature of the majority of published descriptions of experiments in the insect transmission of plant viruses. The factors concerned in these failures are doubtless highly complex; of these the physiological condition and genetical constitution of the experimental plants are not the least important. Of the factors which concern the vector comparatively little is known, although definite advances have been made in the demonstration of a latent period of the virus in the insect (Carsner and Stahl, 1924; Kunkel, 1926; Linford, 1932; Severin, 1921; Smith, 1931; Storey, 1928), of the necessity for certain vectors to take up the virus in a particular stage of their development (Linford, 1932; Samuel and Bald, 1931), and of the loss of the ability to inoculate the virus in the ageing of certain vectors (Kunkel, 1926; Storey, 1928). A further insight into the part played by the vector is now afforded by the proof that hereditary factors may determine the ability of an insect to transmit a virus.

The conclusion that I have bred races of *Cicadulina mbila* which, in the natural process of feeding, are unable to transmit the virus of streak disease, can hardly be doubted. Under exactly parallel conditions the active races have transmitted, while the inactive races have failed. Under abnormal conditions as to length of the period of feeding on a diseased plant and the temperature at which this feeding takes place, the inactive races have failed equally. It is true that my method of testing might lead to erroneous conclusions, if the difference between the active and inactive races lay in a prolonged latent period of the virus in the latter. I consider, however, that my evidence excludes this possibility. In many instances the period of observation of the test plants has extended over considerably more than 5 weeks from the first exposure of the hopper to the virus. Conclusive evidence, however, is provided by the bulked cultures, in which supposed inactive hoppers, after a period of feeding on a diseased plant and a second period of testing, were caged for the rest of their lives on maize plants. In no instance did the plants develop the disease. It is improbable that the difference between the active and inactive races lies in a rapid loss of the ability to inoculate the virus, after the manner of that reported by Kunkel (1926) for *Cicadula sexnotata*, since in certain instances the period of feeding on a diseased plant, preliminary to the test, has been reduced to as short a period as 1 hour, without causing any inactive hoppers to give evidence of being viruliferous.

Studies are now in progress in an attempt to discover the basis of the difference in behaviour of the two races. At this stage I may say that by certain treatments I have been able to obtain viruliferous hoppers from an inactive race. I have, however, no evidence to cause me to doubt the truth of the statement that, *in the natural process of feeding*, the inactive race hoppers will invariably fail to transfer the virus of streak disease.

Summary

(1) Races have been bred of the species, *Cicadulina mbila* Naude, which are on the one hand able, and on the other unable to transmit the virus of streak disease

in the natural process of feeding on maize plants.

(2) The crossing of the pure races has demonstrated that the ability to transmit is inherited as a simple dominant Mendelian factor, linked with sex.

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Investigations of the Mechanism of Transmission of Plant Viruses by Insect Vectors - I[1]

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The studies here described aim at the elucidation of the action of a plant virus within the insect that is its specific vector. It is widely held that insect transmission is not normally a mechanical process; but of the nature of the biological relation, into which virus and insect are supposed to enter, little is definitely known. By the use of the method of mechanical inoculation of the virus into the insect I have obtained certain direct evidence bearing upon this problem.

The virus studied is that which causes streak disease in the maize plant. A single strain has been used, maintained in the course of my experiments by repeated transfers to maize seedlings in the greenhouse. Conceivably this strain is a complex of viruses, but if so it has shown no sign of splitting into its components during the period of the work now described. The vectors of the streak virus are the leafhoppers, *Cicadulina mbila* Naude and *Cicadulina zeae* China. The process of transmission has been the subject of considerable past studies (Storey, 1925, 1928, 1932a; Storey and McClean, 1930), and recently I have shown that within the species *C. mbila* there occur races, to which I have applied the terms *active* and *inactive*, able and unable respectively to transmit the virus in the normal process of feeding* (Storey, 1932b). Similar races probably exist in the species *C. zeae*, but inactive races have alone been available for use in these investigations. The several races have been bred through many generations, and frequent sampling tests, performed during the course of this work, have shown that purity has been maintained, except possibly in the instance of one active race.

For certain secondary studies I have used *Peregrinus maidis* Ashmead, the vector of a virus recently found in East Africa and believed to be identical with that causing the stripe disease of maize in Cuba (Stahl, 1927), and *Aphis maidis* Fitch, the vector of the common grass or sugar cane mosaic disease (Brandes, 1920).

[1] Storey, H. H. 1933. Investigations of the mechanism of transmission of plant viruses by insect vectors. I. Proc. R. Soc. London, Ser. B 113:463-485.

*The assumption that the transmission process occurs during the *feeding* of the insect has been generally made. Its truth can hardly be doubted, although I know of no evidence that goes further than proving that *contact* between the insect and the plant is necessary. In this paper, however, I offer evidence showing that a period of contact with a diseased plant results in the appearance of the virus in the intestine of the insect.

Methods

My method of inoculating the virus-bearing fluid into leafhoppers, a process which has apparently not hitherto been used in virus studies, is now described.

Two needles are required; one, the "holding needle" is a blunt-pointed steel needle with one flat side; the other, the "inoculating needle" is a stainless steel entomological pin (Kreye's "Insektennadeln aus rostfreiem Kruppstahl," No. 5) ground on an oilstone to a finely tapering point. For certain purposes the puncture in the insect has been made with a finely pointed steel sewing needle, which is more robust than the entomological pin but resistant to wetting and so less suitable for the transfer of inocula. The needles are sterilized before use by immersion in boiling water. Upon the stage of a binocular dissecting microscope is placed a movable stage consisting of a sheet of composition cork material, which is soft enough not to damage the delicate point of the inoculating needle. This stage is sterilized by burning its surface slightly in a flame.

For inoculation the insect is anaesthetized with chloroform and laid on its back on the stage. In the normal inoculation, which has been used throughout most of these studies, the insect is steadied by the holding needle and the inoculating needle, bearing the inoculum is thrust into the middle of the abdomen, the point passing usually just through to the opposite side, fig. 1.

It should be noted that the two species of *Cicadulina* are small insects of an overall body length of 2.5–3 mm, and in the early work no attempt was made to direct the inoculating needle into any particular internal organ or to decide at the time of the inoculation whether any organ had been penetrated. With the normal puncture the inoculum was certainly introduced into the blood and possibly into some organ of the abdomen. For certain purposes I have tried to avoid all organs by making a shallow puncture in the abdomen, the point only of the needle being introduced in a sloping direction just under the skin. This object has been more certainly attained by making the puncture in the femur of a hind leg. On the other hand in certain experiments I have aimed at the penetration of organs in the abdomen by making a deep puncture, the point of the needle passing right through the abdomen, fig. 2. I shall produce experimental evidence to support the view that in deep inoculation the needle at least occasionally may penetrate the intestine.

In certain instances, e.g., the inoculation of faeces or blood, it has been possible to transfer a minute drop of fluid on the point of the inoculating needle. Usually, however, the amount of inoculum introduced has been the film adhering to the needle after dipping in the fluid. In order to increase the dose of inoculum I used in some experiments a needle that was encircled close to its point by a minute spiral of fine brass wire, fig. 3. This "reservoir needle" was used in the following manner: the point of the needle having been introduced into the abdomen of the insect, the fluid was drawn down from the reservoir to the puncture with the point of the holding needle. It was possible to see that a quantity of the fluid passed into the puncture. The quantity, however, was not measurable; I refer to this inoculation as "heavy" as compared with the "light" inoculation by the normal method. A refinement of the technique is the substitution for the inoculating needle of a capillary glass pipette, fig. 4. This pipette, made by drawing out a fine glass tube in a micro-burner, is sealed (with De Khotinsky cement) to the end of a standard hypodermic needle. The use of a Burroughs Wellcome "Agla" micrometer syringe allows of the ejection of an

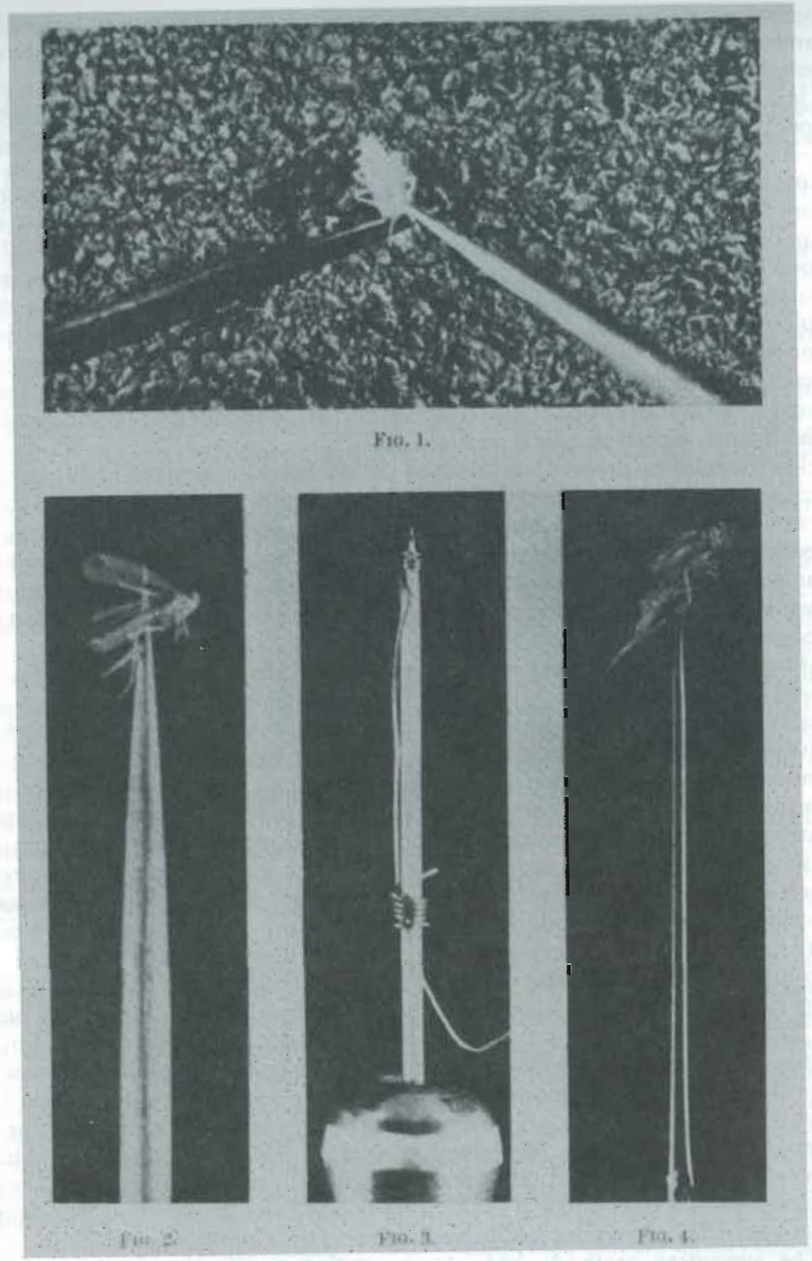

FIG. 1.—The method of inoculation of *Cicadulina mbila*. The insect is steadied by means of the "holding needle" on the left, and punctured in the abdomen with the "inoculating needle" on the right. ×7.

FIG. 2.—The method of "deep inoculation" of *Cicadulina mbila*. The point of the needle is thrust right through the anterior part of the insect's abdomen. ×7.

FIG. 3.—The "reservoir needle" used for "heavy inoculations." ×3.

FIG. 4.—The method of inoculation of *Cicadulina mbila* by means of a micro-pipette. ×7.

approximately known volume of the inoculating fluid. There are, however, a number of practical difficulties with this method and I have used it only to a limited extent.

It is surprising that the very severe wounding involved in these methods of inoculation—the severity of which may be judged by the photographs in figs. 1, 2, 4—has not usually proved fatal to the insects concerned. The wound in the exoskeleton is rapidly sealed by coagulation of the blood fluid. On recovering from the anaesthetic the insects usually feed at once. In a large number of experiments with *C. mbila* employing the normal puncture, a mean of 90% of the inoculated insects survived 2 days and 60% survived a week. In many instances inoculated insects survived for 5 weeks or more. Insects inoculated by a deep puncture and by pipette showed a somewhat lower survival rate, while those inoculated with a shallow puncture showed a higher one. Even nymphs successfully underwent the change of skin within a few days of inoculation. I have raised small families of *C. mbila* from parents that, punctured while still nymphs, subsequently paired as adults and laid eggs.

The inoculation process has involved the introduction into the body cavity of various alien fluids, seldom sterile and usually not specially adjusted to be isotonic with the insect's blood. Particularly is this true of inoculations by pipette, where a relatively large volume of fluid injected has often caused the whole insect to be extremely distended. Nevertheless, no wide deviation from the usual survival rate has been noted, except in one of several experiments in the inoculation of voided faeces by needle.

An essential part of every experiment has been the test of the insects for the power to cause infections. My method for testing *C. mbila*, confined singly in a small cage clipped on to a leaf of a maize seedling, has been described elsewhere (Storey, 1928). Long experience of this technique has caused me to place great confidence in it as a decisive test whether the insect is *infective* or *non-infective**. The insects have been retained on the test seedlings usually for 7 days. This period is amply long enough to ensure infection of the test plants by normally infective leafhoppers. Recent evidence shows, however, that on rare occasions a hopper, made infective by inoculation, may fail to infect a plant within this time. However, the errors introduced into my results from this cause can have been only slight. For tests of groups of insects I have used an enlarged form of the clip-on cage, in which the leaf of the test plant is pressed against the open end of a glass tube of 15 mm bore. It is convenient to seal on to this end of the tube a piece of "bolting-silk," which prevents the accidental escape of the insects during manipulation but does not hinder their feeding on a leaf pressed into contact with the silk. The first symptoms of streak disease have usually appeared within 3–6 days, but the test plants have been retained under observation generally for 20 days. All tests have been performed in a gauze-protected glasshouse, where control seedlings have been maintained alongside the test seedlings. In the course of this work only one control plant became diseased and this infection was clearly associated with an escaped leafhopper captured on a neighbouring plant.

The preceding methods have been applied to the inoculation of the appropriate viruses into *Peregrinus maidis* and *Aphis maidis*. A limited series of experiments, however, gave entirely negative results.

*I use these terms in preference to, but in the same sense as, the "viruliferous" and "non-viruliferous" of an earlier paper (Storey, 1932b). An insect is "infective" if it is actually carrying the virus in such a manner that it inoculates that virus into a plant upon which it feeds.

The Inoculation of Insect Vectors

In the course of later sections of this paper many experiments in the inoculation of active races of *C. mbila* with a variety of inocula will be described in detail. At this stage it will be sufficient to summarize results that demonstrate the possibility of this process.

In nine separate experiments, in which undiluted fresh juice of streak-diseased maize seedlings was inoculated by a normal puncture into the abdomens of 150 active individuals of *C. mbila*, a total of 106 became infective. In one experiment all of 15 insects inoculated became infective; otherwise a proportion only of those inoculated became infective. The mean percentage of successful inoculations in the nine experiments was 73 ± 6.6. The successful experiments included both sexes of adults and also nymphs.

In the preceding experiments the exact site of deposition of the inoculum was uncertain; further experiments showed, however, that the inoculation might be effective if the virus was introduced into the blood alone. A heavy inoculation into a shallow puncture of the abdomen made 13 out of 18 leafhoppers infective. A heavy inoculation into a puncture of the femur—certainly avoiding all internal organs—gave 18 infective hoppers out of 28.

The inoculum used was undiluted fresh juice pressed from crushed young diseased maize seedlings grown in the greenhouse. Experiments showed that this fluid, stored in the dark at 23° C, contained inoculable virus up to the fourth day after preparation, but not on the eighth day. Fresh juice was effective by heavy inoculation when diluted with distilled water by 10^{-2} and rarely by 10^{-3}. Clarification of the juice by passage through paper pulp often rendered it ineffective, even when inoculated in relatively large quantities by pipette. It may be noted, however, that the virus may usually be recovered from such clarified juice by feeding it to leafhoppers through a membrane (Storey, 1932a).

A study of the transmission by inoculated insects revealed a difference in their behaviour from that of insects made infective by feeding on a source of the virus. The ultimate effect of infection by either class of vector was the same, but following infection by an inoculated insect, the disease usually developed in the plant more slowly. The inoculated insect caused infections more irregularly in short periods of exposure. Ultimately the inoculated insect became non-infective, whereas the insect that had taken up the virus by mouth usually continued to cause infections up to the time of its death. Detailed studies of these features of the transmission process will be described in later communications.

One further method of the introduction of the streak virus into *C. mbila* may be mentioned here. If the abdomen of the insect be gently pressed, a drop of fluid appears at the anus. On releasing the pressure the drop is drawn back into the rectum. By bringing into contact with the anus a reservoir needle bearing the juice of streaked maize and then releasing the pressure, I have supposed that some of the virus-bearing fluid would be drawn into the rectum. This experiment has been done three times, but the 77 active hoppers so inoculated all remained non-infective.

The Virus in the Vector Insect

By the inoculation into non-infective active *C. mbila* of fluids obtained from other infective *C. mbila* I have tried to follow the behaviour of the streak virus in

this insect. The infective insects, unless otherwise stated, had obtained the virus by feeding for several days upon diseased maize. In order to obtain infective insects that had not recently taken up the virus in feeding, the insects were caged on single leaves of an immune species of plant (e.g., a suitable variety of sugar cane) or of a healthy maize seedling. Although the latter will be infected as a result of the insects' feeding, I have shown that the virus is only rarely picked up from the part of a plant through which it receives the virus (Storey, 1928). As an additional precaution the insects were transferred to a fresh leaf every second day.

The inoculation of faeces voided by infective hoppers during feeding on a diseased plant, slightly diluted with sterile distilled water, gave negative results when inoculated into a total of 44 insects in two separate experiments. Similar negative results were obtained by feeding* voided faeces, diluted with 20% sucrose solution to active hoppers. This result confirms that of Severin (1931) with *Eutettix tenellus* Baker and the virus of curly-top of sugar beet. If, however, the abdomen of an anaesthetized leafhopper be gently pressed with a needle a drop of clear fluid will usually appear on the anus. This drop may be picked up with a second needle and inoculated into a non-infective hopper. The results of such experiments (Table I, experiments 1 and 2) show that this fluid contains the virus, if the hopper from which it is taken has recently fed on diseased maize. The low proportion of successes obtained by this inoculation suggests that the virus content of the fluid is small. If the infective hopper has recently fed only on healthy plants the fluid exuded usually contains no virus (Table I, experiments Nos. 3, 4, 5). In one of these experiments the fluid from infective hoppers, fed during the preceding 6 days only on healthy maize, gave a single infection out of 33. Where the time of feeding on healthy plants was increased to 10 and 20 days, no infections resulted.

I believe that the drop of fluid exuded from the anus is actually the contents of the rectum, and not blood, such as might be expected to exude had the wall of the rectum been ruptured by the pressure exerted. If the pressure on the abdomen be heavy, however, it is possible to rupture the rectum; the sudden appearance of a large volume of blood—often bearing globules of fat—is characteristic and easily recognized. Furthermore, whereas the supposed contents of the rectum may in some circumstances, as I have shown above, contains no virus, the evidence given

*By the method described in an earlier paper (Storey, 1932, *a*).

Table I.—The inoculation of the rectal contents of infective active *C. mbila* into active *C. mbila*.

Experiment number.	Inoculum. History of infective hoppers before inoculation.		Test insects. Number inoculated.	Test insects. Number infective.
		days		
1	On diseased maize	>2	25	7
2	,,	>2	26	2
3	On healthy maize	6	33	1
4	,,	20	26	Nil
5	On healthy sugar cane, Co 205	10	19	Nil

in the next paragraphs shows that in those same circumstances the blood will contain the virus.

Inoculations with the general body contents of infective hoppers gave positive results. To obtain the inoculum the anaesthetized insect was divided at the junction of the thorax and abdomen with a heated needle; the mixed contents of each half were then picked up by the needle. The experiment was successful whether the contents of the abdomen or of the head and thorax were used; it was successful whether the insects, being infective, had fed recently on diseased or healthy plants. The method of obtaining the inoculum allowed of no decision whether the virus had come from some internal organ (e.g., the intestine) or from the blood of the insect.

I have attempted to obtain the blood alone by making a shallow puncture into the abdomen, thorax or leg, and exerting gentle pressure.* The results given in Table II show that inoculation of the blood of infective hoppers gives positive results, whatever may be the site of the puncture or the recent history of the hoppers. In one experiment (No. 6) the blood was obtained by puncture of the tibia of a hind leg; the positive results obtained dispels all possibility that the virus in the inoculum used had come from some internal organ of abdomen or thorax accidently punctured in obtaining the blood. The blood, unlike the contents of the rectum, still contains virus after the insect has fed on a healthy plant, although there is an indication of a falling off of virus concentration if the period on a healthy plant be prolonged (Table II, experiments 9 and 11). On the other hand, the virus concentration is at least fully maintained if the insects continue to feed on diseased maize (Table II, experiment 2).

*A colourless fluid is obtained from such a puncture. The assumption that this fluid is the blood seems to be justified. Occasionally it bears globules of fat, derived probably from the "fat body."

Table II.—Inoculation of blood of infective active *C. mbila* into active *C. mbila*.

Experiment number.	Inoculum. Source.	Inoculum. History of infective insects.		Test insects. Number inoculated.	Test insects. Number infective.
			days		
1	Abdomen	On diseased maize	6	27	16
2	,,	,,	1	27	3
	,,	,,	2	21	1
	,,	,,	4	21	5
	,,	,,	21	27	12
3	,, *	,,	5	26	Nil
4	,, *	,,	14	21	7
5	Thorax	,,	23	21	16
6	Tibia of hind leg	,,	5	21	3
7	Abdomen	On healthy maize	15	25	8
8	,,	On healthy cane	10	27	10
9	,,	,,	29	26	4
10	Thorax	On healthy maize	8	23	6
11	,,	,,	30	19	Nil

* Nymphs.

In the foregoing experiments, it was impossible to obtain the inoculum aseptically. It might be argued that the insect was naturally contaminated with virus externally and that it was from that source that the virus in my inoculum came. I have carried out a control experiment, in which blood was transferred from a non-infective insect to the surface of the abdomen of an infective insect, and then inoculated back again into the original insect. Negative results were obtained with 14 such transfers. Further control is provided by the entirely negative experiments, recorded later, in the inoculation of the blood of inactive hoppers fed on diseased maize. If an insect feeding upon a diseased plant were liable to be externally contaminated with virus, inactive insects would be expected to be as subject to contamination as active ones.

The time relations of the appearance of virus in the blood and the development of the power to cause infections were studied in the following experiment. *C. mbila* undergoes a "latent" or non-infective period following its first feed on a source of virus (Storey, 1928), similar to that demonstrated for a number of vectors of virus diseases (Balo and Samuel, 1931; Carsner and Stahl, 1924; Kunkel, 1926; Linford, 1932; Severin, 1921; Smith, K. M., 1931). Groups of 10 active hoppers were fed for 3 hours on diseased maize and then tested, still in groups, for successive 3-hour periods on healthy maize. This procedure gives a value for the "minimum latent period"* at the temperature of the experiment (a mean of 23° C). It will be seen from Table III, experiment 1, that 2 out of 4 groups caused infections after 12–15 hours from the start of the experiment. The blood of 10 similarly treated insects was inoculated after each time interval into about 20 non-infective active hoppers. The results (Table III, experiment 2) demonstrate that the virus appeared in the blood in between 6 and 9 hours from the start of the first feed on diseased maize. It is thus seen that the appearance of

*The exact interpretation of this value is uncertain. In different individual insects the duration of the latent period varies widely (Storey, 1928). The latent period of a group, as here determined, may be regarded as the period required for the most "rapid" member of the group to reach the stage of inoculating a minimum infective dose of virus within the time allowed for each test, or as the period required for the doses inoculated by all members of the group to aggregate to a minimum infective dose.

Table III.—Comparison of minimum latent period in groups of 10 active *C. mbila*, fed on streak-diseased maize, with the appearance of the virus in the blood of similar groups.

Test Periods. (Times from first exposure to virus.)

Hours :→	0–3	3–6	6–9	9–12	12–15	15–18	18–21	21–24
Experiment 1.—*Tests for latent period*—								
No. of groups tested	—	4	4	4	4	4	4	4
No. of groups infective	—	Nil	Nil	Nil	2	2	3	1
Experiment 2.—*Tests for virus in blood*—								
No. of insects inoculated	20	20	19	20	—	—	—	15
No. infective	Nil	Nil	5	7	—	—	—	5

virus in the blood of the insects precedes the development of the power to inoculate in feeding a minimum infective dose.

The blood of an active leafhopper that has been inoculated with the virus may contain the virus some time after the original inoculation. In one experiment, the blood of six active hoppers, that had been inoculated 14 days previously with the blood of infective hoppers and had successfully infected plants during the preceding 4 days, was inoculated into 18 non-infective hoppers. Of these one became infective. In a similar experiment, in which the inoculum was obtained from hoppers that had become infective as a result of inoculation, 19 days previously, with juice of diseased maize, 1 out of 23 hoppers became infective. There are thus indications that the virus may persist in the blood after inoculation, although the proportion of successes was less than with blood obtained from hoppers that had taken up the virus in feeding.

The Virus in Inactive Races of Vector Species

Although uninjured inactive *C. mbila* will never transmit the streak virus in the course of feeding, yet they may be inoculated with the virus and may then become infective. By the inoculation of the juice of diseased maize into a normal puncture, 41 out of 105 inactive hoppers were made infective. The mean percentage of successes in the eight separate experiments from which these totals were obtained was 38 ± 7.0. This percentage is significantly less than that obtained by similar inoculation of active hoppers. Heavy inoculation into the femur of a hind leg resulted in 5 infective hoppers out of 25. Thus inactive hoppers may be made infective by the introduction of the virus into the blood alone; but here again there is an indication that the inoculation is less effective than with active races.

Inoculated inactive insects, like active ones, produce infections irregularly and tend to become non-infective in course of time.

The normal behaviour of the virus in inactive leafhoppers has been studied by transferring inocula obtained from them to active hoppers. The general body contents of inactive hoppers proved to be virus-bearing only when taken from the abdomen and only if the hoppers had recently fed on diseased maize (Table IV). The blood of uninjured inactive hoppers, after feeding on diseased maize, never contained the virus (Table V). The fluid from the rectum was found to contain the virus, if the insects had recently fed on diseased maize, but not if a period of feeding on healthy plants had followed the feed on a diseased plant (Table VI).

These results show that the inactive insect is able to take up the virus in feeding and to pass it through the intestine. But, in contrast to its behaviour in the active insect, the virus does not pass out from the intestine into the blood. The results obtained by the inoculation of the general body contents conform to this explanation. For within the thorax the intestine is a narrow tube, through which food material is believed to pass rapidly; in the abdomen the intestine enlarges to form the crop (fig. 5), whence, in the experiments of Table IV, the virus was doubtless obtained.

The rectal contents, which I have shown to contain the virus may be used as an inoculum for the inoculation of inactive hoppers. In the experiment of Table VII the rectal contents of inactive hoppers, immediately after a feed on a diseased plant, were inoculated by a shallow abdominal puncture into the identical

Table IV.—Inoculation of general body contents of inactive *C. mbila*, fed on streak-diseased maize, into active *C. mbila*.

Experiment number.	Inoculum.			Test insects.	
	Site.	History of insects.		Number inoculated.	Number infective.
			days		
1	Abdomen	On diseased maize	>2	16	4
2	Thorax	,, ...	>2	16	Nil
3	Abdomen	On diseased maize, then on healthy maize	5	15	Nil
4	Thorax	,, ,,	5	14	Nil
5	Abdomen	On diseased maize, then on healthy sugar cane, Co 205	6	26	Nil

Table V.—Inoculation of blood of inactive *C. mbila*, fed on streak-diseased maize, into active *C. mbila*.

Experiment number.	Inoculum.			Test insects.	
	Site.	History of hoppers.		Number inoculated.	Number infective.
			days		
1	Abdomen	On diseased maize	3	26	Nil
2	,, ...	,, ...	4	28	Nil
3	,, ...	,, ...	6	31	Nil
4	,, ...	,, ...	14	18	Nil
5	,, *.....	,, ...	4	20	Nil
6	,, *.....	,, ...	5	26	Nil

* Nymphs.

Table VI.—Inoculation of contents of rectum of inactive *C. mbila*, fed on streak-diseased maize, into active *C. mbila*.

Experiment number.	Inoculum.		Test insects.	
	History of insects.		Number inoculated.	Number infective.
		days		
1	On diseased maize	2	28	8
2	,, ...	3	19	1
3	On diseased maize, then on healthy Co 205	5	24	Nil

hoppers from which they were obtained. Two out of 21 hoppers were made infective in this way. A control series of hoppers was first gently pressed to cause an exudation at the anus and then a shallow abdominal puncture was made with a sterile needle. None of the control insects became infective. This experiment involved the *external* transfer of material from the rectum to the blood-fluid. The transfer may equally be done *internally*, by puncturing the rectum. Of 22 hoppers, recently fed on diseased maize and punctured by introducing a needle into the anus, 4 became infective (Table VIII, experiment 1). The hoppers may also become infective if the period of feeding on diseased maize is deferred until after the rectum has been punctured. In two experiments (Table VIII, experiments 2 and 3) this treatment was successful with 9 hoppers out of 35.

These experiments prepare the way for the understanding of an observation made early in the course of this work. If inactive leafhoppers be punctured in the abdomen with a sterile needle, and particularly if the puncture be deep, some of these inactive leafhoppers may now behave as if they were active. That is, if the puncture be made immediately before or immediately after the hopper has fed on a diseased plant, that hopper may become infective. This experiment has been repeated many times and has indeed become a routine laboratory procedure for obtaining infective inactive hoppers. I summarize in Table IX a number of experiments on these lines. The proportion of successful infections by this method is not high, but is sufficient to demonstrate the possibility of the process. An analysis of the figures of the several experiments showed no significant difference in the proportion of successes obtained by feeding the insects on diseased maize immediately before or after the puncture had been made. I would repeat that the inactive leafhoppers, if uninjured, will never become infective by simply feeding on a diseased plant. Simultaneously with the majority of the experiments listed in Table IX, and in those that follow, a random sample of 20 to 30 hoppers was removed from the supposed inactive culture, fed on diseased maize, and tested in a group on a maize seedling (see Controls of Table IX). In no instance did infection follow the feeding of any control sample. In some instances the actual leafhoppers, subsequently used in the experiments, were first subjected to a similar control test and proved to be non-infective.

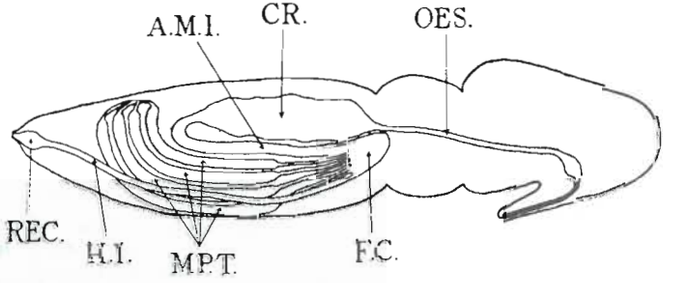

FIG. 5.—Diagrammatic vertical longitudinal section of *Cicadulina mbila*, showing digestive system. OES., œsophagus; CR., crop; A.M.I., ascending portion of mid-intestine; F.C., filter chamber; H.I., hind intestine; REC., rectum; M.P.T., four Malpighian tubules. (These tubules undergo considerable convolutions, which are not depicted in this diagram.)

As further controls to the foregoing experiments, two series of 45 hoppers in all were punctured but not fed on a diseased plant, while a third series of 24 hoppers was anaesthetized only and then fed on a diseased plant. None of the control hoppers became infective. On the other hand an abdominal puncture was without effect on the normal transmission by active hoppers. Twenty-eight active hoppers were punctured and then fed on diseased maize; all became infective.

Table VII.—Inoculation of inactive *C. mbila* with their own rectal contents, after feeding on streak-diseased maize.

Experiment number.	Inoculation method.	History of hoppers.		Tests.	
				Number inoculated.	Number infective.
1	Shallow puncture	On diseased maize	days 6	21	2
Control	Shallow puncture (no inoculum)	,, ...	6	23	Nil

Table VIII.—Inoculation of inactive *C. mbila*, fed on streak-diseased maize, by puncture of rectum through anus with sterile needle.

Experiment number.	History of hoppers.		Tests.	
			Number inoculated.	Number infective.
1	On diseased maize before puncture	days 2	22	4
2	On diseased maize after puncture.....	2	14	5
3	,, ,, ...	2	21	4

Table IX.—Inoculation of inactive *C. mbila* by abdominal puncture with sterile needle and feeding on streak-diseased maize.

History of insects.	Number of experiments.	Test insects.	
		Total inoculated.	Total infective.
Fed on diseased maize before puncture	9	211	31
Fed on diseased maize after puncture	14	271	57
Controls—Fed on diseased maize, not punctured	13*	353	Nil

* In many instances one control sample served for two or more experiments performed on the same day.

If the puncture was made into inactive leafhoppers, that had fed on a diseased plant and then for a period on a healthy plant immediately before puncturing, the hoppers did not become infective (Table X). If on the other hand the first feed on a diseased plant was postponed until 2 to 8 days after the puncture has been made, some of the hoppers became infective (Table XI). The mean percentage of success in these experiments was, on the figures available, not significantly different from that obtained by feeding the punctured hoppers on a diseased plant at once. I shall show later, however, that a puncture made 40 days previously is no longer effective in causing an inactive hopper to become infective following a feed on a diseased plant.

The position in which the puncture is made is important. A deep puncture into the anterior part of the abdomen is more effective than one near the tail (Table XII, experiment 1). A puncture made by a needle passed horizontally through the abdomen is probably less effective than a vertical puncture but more effective if it be near the dorsal surface than if it be near the ventral surface (experiment 2). A shallow puncture into the abdomen was successful with 1 hopper only out of 48 (experiments 3 and 4). A puncture into the thorax proved fatal within 2 days to 38

Table X.—Inoculation of inactive *C. mbila* by abdominal puncture with sterile needle after feeding first on diseased maize and then on healthy maize before puncture.

Experiment number.	History of insects before puncture.		Tests.	
			Number inoculated.	Number infective.
1	On diseased maize, then on healthy maize	days 7	30	Nil
2	,, ,, ...	9	19	Nil
3	,, ,, ...	14	21	Nil

Table XI.—Inoculation of inactive *C. mbila* by abdominal puncture with sterile needle, followed by feeding on healthy maize and then on diseased maize.

Experiment number.	History of insects, after puncture.		Tests.	
			Number inoculated.	Number infective.
1	On healthy maize	days 2	19	2
	On diseased maize	2		
2	On healthy maize	8	16	2
	On diseased maize	2		
3	On healthy maize	7	20	3
	On diseased maize	2		

out of 47 hoppers, but none of the surviving 9 hoppers infected a test plant (experiment 5). A severe compression of the abdomen, by a needle held transversely to the abdomen, without puncturing the exoskeleton, caused 1 out of 13 hoppers to become infective (experiment 6). This evidence of position is to be considered in relation to the anatomy of *C. mbila*. In fig. 5 is shown diagrammatically the digestive system of this species, which appears, in the features essential to the present argument, to be typical for this group of insects (*cp.* the description of *Cicadula sexnotata* by Dobroscky [1931]). The anterior dorsal portion of the abdomen, in which as I have shown the puncture is most effective, is occupied by the organ usually called the "crop," relatively large and consequently, it may be supposed, the most vulnerable portion of the intestine. It is suggested that penetration of the intestine by the needle is the necessary condition for an inactive hopper in these experiments to become infective.

Certain direct experimental evidence appears to support this suggestion. A series of 20 inactive hoppers, fed on diseased maize, was punctured deeply in the anterior part of the abdomen. Upon withdrawal from each inactive hopper the needle was immediately pricked into the abdomens of two non-infective active hoppers; that is, the fluid picked up by the needle in each inactive hopper was transferred to two active hoppers. Both series were then put on test. Of the first series, three hoppers became infective. Of the second series one pair of hoppers became infective, and these hoppers had been inoculated from one of the three inactive hoppers that became infective. I have shown that the blood of an uninjured inactive hopper, fed on diseased maize, does not contain the virus. This was confirmed in a control experiment parallel to that described above, in which the blood of 20 inactive hoppers (selected at random from the sample of hoppers used in that experiment) was inoculated into active hoppers, none of which became infective. In the one successful experiment, therefore, the needle, in puncturing the inactive hopper, picked up the virus from some source other than the blood. I suggest that this source was the intestine, which is known to have contained the virus. Since in any case the amount of virus picked up is likely to be small, my failure to transfer virus from the remaining two inactive hoppers, that became infective, is not unexpected.

Table XII.—Inoculation of inactive *C. mbila* by puncturing with sterile needle in different places and feeding on diseased maize.

Experiment No.	Puncture.		Fed on diseased maize.	Test insects.	
	Nature.	Site.		Number inoculated.	Number infective.
1	Deep	Abdomen, anterior end	After puncture	23	8
	,, ...	Abdomen, posterior end	,,	27	Nil
2	Transverse	Abdomen, near dorsal surface	,,	27	2
	,,	Abdomen, near ventral surface	,,	24	Nil
3	Shallow	Abdomen, middle	Before puncture	25	1
4	,, ...	,, ...	,,	23	Nil
5	,, ...	Thorax	After puncture	9	Nil
6	No puncture	Abdomen compressed	After treatment	13	1

The evidence thus causes me to picture the infective inactive leafhopper of these experiments as one in which a part of the virus-bearing food ingested by mouth has passed out through a hole in the intestinal wall into the blood of the body cavity. That this is a true picture is supported by the abnormally distended appearance of many of the treated insects. I have been able, furthermore, to show experimentally that soon after puncture the blood may contain the virus. A series of 20 inactive hoppers was punctured and then fed for one day on diseased maize. Thereupon a little blood from each hopper was inoculated into two non-infective active hoppers. Tests of all these hoppers showed that two of the inactives of the first series (designated Nos. 4, 7), and four of the pairs of actives of the second series (Nos. 4, 5, 7 and 18), became infective. Thus two inactive hoppers, that became infective as a result of treatment, were shown to have had virus in their blood. Two others (Nos. 5 and 18), that failed to infect their test plants, also had virus in their blood. It may be noted, however, that these two inactive hoppers died within the first two days of the test, and may not have lived long enough to infect plants even though potentially infective.

Inoculated inactive hoppers may continue to show virus in their blood, so long as they remain infective. Two inactive *C. zeae* (which behave in a similar manner to *C. mbila*—see below) were inoculated with the streak virus and proved to be infective in two successive tests. At the conclusion of the second test, 17 days after the original inoculation, the blood of each hopper was inoculated into five active *C. mbila*. One hopper in each series of five became infective. On the other hand, as I have already mentioned, the inactive hopper, made infective by one of the methods described above, will usually remain infective for only a short time. Hoppers that have ceased to be infective appear to contain no virus in their blood. An inactive hopper, pricked before feeding on diseased maize, infected one test plant, but later failed to infect a second. Ten active hoppers, inoculated with its blood, were non-infective. In a similar experiment four inactive hoppers, made infective by puncture before feeding on diseased maize, were given a second test of 2 days' duration. In this second test two only successfully infected their plants, but the incubation of the disease in the plants was unusually prolonged. This feature I have come to associate with infections produced by hoppers that are on the point of becoming non-infective. The blood from the 4 hoppers inoculated into 30 active hoppers failed to cause any to become infective.

Inactive races of *Cicadulina zeae* behave in a similar manner to *C. mbila*. Infective individuals of inactive *C. zeae* were obtained by inoculation of the virus, and by deep puncture before and after the insects had fed on diseased maize (Table XIII). Again control tests confirmed the inability to transmit of uninjured random samples of the races of hoppers used in these experiments.

The Reinoculation of Insects that Had Become Non-infective

I have already noted that an active insect, made infective by feeding upon a source of the virus, will normally remain infective up to the time of its death. Exceptionally, however, an insect will lose the power to cause infections. One such insect, after feeding on diseased maize, was infective for about three weeks and then failed in two successive weekly tests. After a second feed on a diseased plant, it infected plants in four weekly tests, but then failed in two tests. Thus

after becoming non-infective this insect was not resistant to reinoculation by mouth. It will be noticed, however, that the original loss of infective power was not merely accidental, for even after reinoculation the same tendency to loss was manifested.

The inoculated insect, as I have mentioned, usually becomes non-infective in course of time. The results shown in Table XIV prove that both active and inactive hoppers, successfully inoculated in various ways and later proved in two or more successive weekly tests to have become non-infective, may be successfully reinoculated by needle. Experiment No. 4 indicates, however, that a deep puncture, performed 40 days previously, is no longer effective in causing an inactive hopper to become infective by simple feeding on diseased maize.

The Behaviour of Non-Vector Species

The success obtained in the inoculation of inactive races of vector species led to experiments in the inoculation of other species, known to be unable normally to transmit the virus employed. For this purpose I inoculated species that are

Table XIII.—Inoculation of inactive *Cicadulina zeæ* by various methods.

Experiment number.	Nature of puncture.	Source of inoculum.	Test insects.	
			Number inoculated.	Number infective
1	Normal	Juice of diseased maize	25	12
2	,, ...	,, ...	28	3
3	Deep, abdominal	Fed on diseased maize *after*	33	5
4	,, ...	,, *before*	20	2

Table XIV.—Reinoculation of *C. mbila*, originally made infective by inoculation and later proved to have become non-infective in two or more tests.

Experiment No.	Race.	Original inoculation.	Reinoculation.	Tests after reinoculation.	
				Number inoculated.	Number infective.
1	Active	Juice of diseased maize	Juice of diseased maize	5	3
2	Inactive....	,,	,,	4	3
3	,, ...	Punctured. Fed on diseased maize	,,	1	1
4	,, ...	,, ,,	Fed on diseased maize only*	1	Nil

* 40 days after the original puncture.

recognized to be vectors of viruses other than that used in the inoculation. There is every probability that a species able to transmit one virus is structurally not unfitted to transmit another virus, and it was therefore possible that its inability to transmit the second virus might be overcome by the treatment successfully applied to inactive races of vector species.

The results of all experiments to this end have been uniformly negative. *Cicadulina mbila* has been inoculated with the viruses of mosaic and stripe of maize. *Peregrinus maidis* and *Aphis maidis* have been inoculated with the virus of streak. The methods employed have been the direct inoculation of the virus by needle, and a deep puncture following feeding upon diseased plants. The evidence provided by the inoculation of *Peregrinus maidis* with the streak virus is the most conclusive. The inoculation in different ways of 75 individuals of this species resulted in no infections of the test plants. Nevertheless, by inoculating into active *C. mbila* the rectal contents of *P. maidis*, recently fed on streak-diseased maize, I was able to prove that the virus might be ingested by this species and passed through the intestine. The blood, however, of *P. maidis*, fed on streaked maize, failed to make *C. mbila* infective. On the other hand, the inoculation of the rectal contents of *Aphis maidis*, fed on streaked maize, was unsuccessful.

Although the negative evidence provided by this series of experiments is not conclusive, there is a strong probability that the inoculation of non-vector species is impossible.

Discussion

I have thus shown that after *Cicadulina mbila* has been in contact with a streak-diseased maize plant, the virus is present in the contents of the rectum. A part therefore of the virus, which must be assumed to have been ingested by mouth, passes through the length of the intestine. When, however, the contents of the rectum are naturally voided, the virus, for some reason that I am unable to explain, rapidly disappears.

In the active insect a part of the virus passes out from the intestine into the blood, where I have been able to demonstrate its presence. In the inactive insect, on the other hand, the virus is confined to the intestine and fails to reach the blood. If, however, the virus be introduced by inoculation into the blood of an insect of either class, that insect may become infective. Thus I am led to regard the presence of the virus in the blood of an insect as an essential step towards its becoming infective. It will be seen that this step precedes in time the development of the power to cause infections.

Since the introduction of the virus into an active insect's rectum is ineffective, it appears that the site of passage of the virus through the intestinal wall is located elsewhere than in the rectum and possibly the remainder of the hind intestine.

The inactive insect differs from the active one primarily in a property of the wall of the intestine that resists the passage of the virus. I have shown that this resistance may be overcome by a puncture of the intestine. This puncture may be made by needle through the rectum or crop or probably occasionally by simple rupture of the intestine following pressure on the abdomen. The evidence that I offer leaves little doubt that I have thus correctly interpreted the nature of the operation performed on these insects. The survival of an insect in which a hole in

the intestine allows the food materials to flow out into the blood implies a surprising hardihood. Nevertheless, I believe that to be a true statement of the condition of such insects, and that they will survive, sometimes to the normal span of life, is certain.

While the primary mechanism of the resistance of inactive *C. mbila* thus appears to lie in some property of the intestinal wall, some imperfect secondary mechanism must operate also. It is necessary to postulate this in order to explain the significant difference in the proportion of successes obtained in the inoculation of active and inactive insects. I can, however, offer no suggestions as to the nature of this secondary resistance.

Insects that have lost the power to cause infections are not resistant to reinoculation. If therefore the original loss of the virus were due to a resistance developed by the insect, that resistance could be only very weak. On the whole the available evidence rather suggests that the loss is due to an exhaustion of the supply of virus in the insect.

In these investigations I have thus traced the behaviour of the virus up to its appearance in the blood of the vector insect. It is generally believed that the saliva is the vehicle by which the virus is transferred from the insect into the plant. If this assumption be correct, it may be supposed that the blood is the vehicle by which the virus is transported from the intestine to the salivary glands. Investigations of the virus in the insect's saliva will be described in a later communication.

My studies of non-vector species, though of limited extent, suggest that they cannot be caused to transmit by treatments successfully applied to inactive races of *C. mbila*. Although *Peregrinus maidis* may take up the streak virus and may pass it through its intestine, yet so far as my experiments show, no treatment of the insect will cause it to transmit this virus. Nor was I more successful in attempts to cause *C. mbila* to transmit the stripe and mosaic viruses. The inability of non-vector species to transmit a virus appears to rest upon a more complete mechanism of resistance than that of inactive races of *C. mbila*.

This work has been carried out at the East African Agricultural Research Station, Amani, East Africa. I acknowledge the help that I have received from my assistant, Mr. R. F. W. Nichols.

Summary

(1) This paper deals primarily with experiments in the mechanical inoculation of insects with plant viruses. The method employed was the introduction of the virus into a puncture of the abdomen or leg made with a finely pointed needle or glass micro-pipette. A large proportion of the insects survived this treatment.

(2) The inoculation of active races of *Cicadulina mbila*, the vector of streak disease of maize, was successful, when the inoculum introduced was the juice of diseased maize seedlings, fresh or kept for four but less than eight days, undiluted or diluted with distilled water by 10^{-2} (rarely, by 10^{-3}).

(3) Inoculation by causing the virus fluid to be drawn up into the rectum per anus failed.

(4) By the inoculation of the appropriate fluids, it was shown that the virus was present in infective active *C. mbila*, (a) in the contents of the rectum, if the insect has recently fed on a diseased plant, but not otherwise, (b) in the general contents of thorax or abdomen, and (c) in the blood, whether the insect had fed recently upon diseased or healthy plants. The virus was not found in the naturally voided faeces. The appearance of the virus in the blood preceded in time the development of the power to cause infections.

(5) Inactive races of *C. mbila*—normally unable to transmit the virus—were made infective by needle inoculation with the streak virus. The proportion of successes was, however, significantly less than with active races.

(6) After a feed on a diseased plant, the inactive insect was found to have the virus in its rectum, but never in its blood.

(7) A simple puncture of the abdomen with a sterile needle, either following or followed by a feed on a diseased plant, sometimes caused inactive *C. mbila* to become infective. By comparison of the efficacy of different positions for the puncture, it was concluded that the treatment was successful only if the needle had penetrated some part of the intestine.

(8) Inactive races of *Cicadulina zeae* proved to be susceptible to inoculation with the streak virus by the methods successful with *C. mbila*.

(9) *C. mbila* was not successfully inoculated with the viruses of maize stripe and mosaic diseases; nor *Peregrinus maidis* and *Aphis maidis* with the virus of streak.

(10) It is concluded from these observations that, in active *C. mbila*, the streak virus, entering the intestine by mouth, passes through the intestinal wall into the blood; and that, in the inactive insect, the cells of the intestinal wall resist the passage of the virus. It is recognized that there may be some secondary mechanism of resistance; nevertheless, in many inactive individuals, once the barrier of the intestinal wall has been passed, the virus behaves as in an active insect.

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