FURTHER STUDIES ON THE DWARF DISEASE OF RICE PLANT

By

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CONTENTS

In a continuation of the studies on the dwarf disease of rice plant (FUKUSHI 1934) an attempt was made to gain an insight into the special relation of certain leafhoppers to the virus of this disorder. The results of experiments performed during a period from 1934 to 1939 will be presented in this paper, especially laying stress upon the transmission of the virus through some eggs of the insect vector during several generations.

The writer wishes to express here his sincere gratitude to Dr. K. MIYABE, Professor emeritus and Dr. S. ITO, Professor of plant pathology of the Hokkaido Imperial University for their valuable suggestions and kind encouragement during the course of the work. Also to Mr. T. WATANABE, Professor of plant pathology of the Utsunomiya College of Agriculture and Forestry to whom the writer is indebted for the material which he kindly supplied.

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The relation of *Nephotettix apicalis cincticeps* to the virus of dwarf disease of rice plant

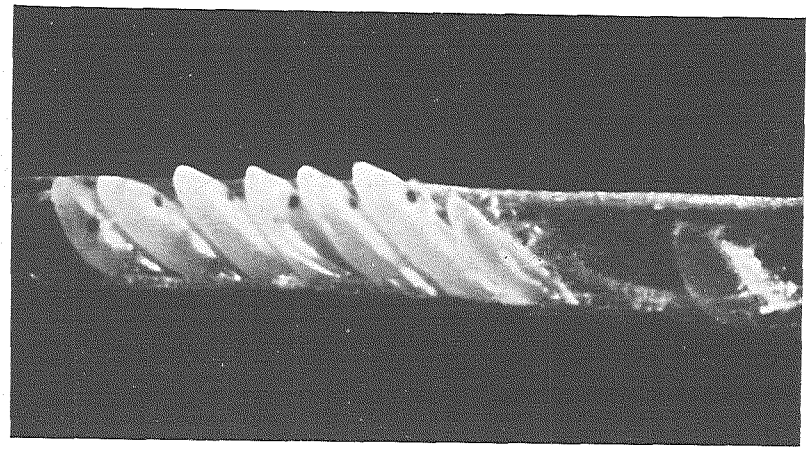
1) Transmission of the virus through the eggs of leafhoppers

As previously reported the virus under consideration may be transmitted through the eggs from a viruliferous female leafhopper to its progeny of the 2nd generation. It has been also stated that certain leafhoppers emerged from these viruliferous eggs may be capable of producing infections in healthy plants and retain the infectivity through their entire life without having renewed access to a source of virus. Accordingly an attempt was made to determine how and to what extent the virus may be passed on to the offspring of leafhoppers.

The experimental procedure, briefly stated, 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 2nd generation individuals are paired with nonviruliferous males and their offspring are also transferred to healthy plants as soon as they have hatched. Thereafter they are likewise transferred daily to 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 among the progeny of the leafhoppers.

Since it appears appropriate to describe in detail the procedure followed an account of it will be given. Each series of experiments was started with a viruliferous female leafhopper which had been obtained by confining single nymphs of the 3rd or 4th instars reared on affected plants upon healthy rice plants in separate glass tubes until they grew into adults and proved to be infective. The glass tube used was, as previously stated, 30–40 cm. long by 3 cm. in diameter closed at the upper end with a thin cotton cloth and put on a young rice plant of the variety, Bôzu No. 5, 10–30 days after germination. A male leafhopper, viruliferous or nonviruliferous, was introduced into the tube enclosing the viruliferous female, 7 to 10 days after the last moulting of the latter, because some female leafhoppers show a tendency not readily to mate with males, as they approach old age. In order to obtain a male leafhopper which had never coupled, single nymphs were confined on rice plants in separate glass tubes and allowed to remain there until they became adults. When such a male was introduced into the glass tube enclosing a female, mating of them took place before long. After pairing with the male, the female leafhopper was

transferred to successive new healthy plants at one day intervals. One to several days after mating the female leafhopper began to lay eggs and usually continued to do so for a period of 1 to 2 weeks or longer under

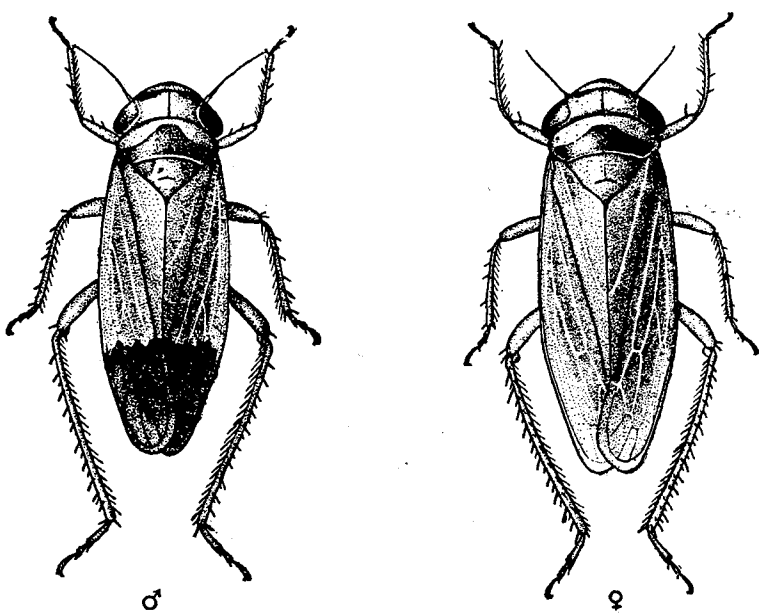


Eggs of the leafhopper, *Nephotettix apicalis cincticeps* Uhl.
(= *N. bipunctatus cincticeps*) (× ca. 30)

the writer's experimental conditions. The eggs were deposited in the tissues of leafsheaths of rice plant, generally 1 to 10 or sometimes more in a place and thrust transversely into the tissues lying one under the other. The eggs are about 1 mm. long, creamy white, elongate, and slightly curved tapering at the end. At first the eggs were scarcely visible since they had been pushed in so deeply that they were entirely imbedded in the tissues. However a few days after oviposition the spot where the eggs are deposited becomes easily discernible by its more or less raised surface and their shapes as well as the location of eyes are distinctly seen by means of transmitted light. (Pl. IV. figs. 1 & 2)

The eggs hatch in 8 to 13 days under favorable conditions but require a still longer time at lower temperatures. It is extremely important to pick up the nymphs in the act of emerging from the eggs so that they have no opportunity to feed upon the plants on which the eggs have been deposited and which may have contracted the disease in consequence of infestation by the infective parents. For this purpose it was very convenient to control the time of hatching adequately as follows. The plants harbouring the full grown eggs which would hatch in a day or two were placed in a cool place and covered with wooden boxes in the evening. In the next morning, when these plants were transferred to a sunny

warm place in the greenhouse, some of these eggs might soon begin to hatch out while the others were anticipated likewise to hatch in the following days. In the process of hatching the nymph forces its way, anterior end first, from the egg shell (chorion) and makes its appearance through the suture between the culm and the leafsheath. This is accomplished by an undulating movement of the body, which reaches a position more or less perpendicular to the plant surface before the unfolding of appendages. (Pl. IV. fig. 3 & 4). As the appendages unfold the contortions of the body become more vigorous until the tiny nymph gains a foothold on the plant



Adults of the leafhopper, *Nephotettix apicalis cincticeps* UHL. (×10)

surface and gets entirely out of the membrane. (amnion?) (Pl. IV. fig. 5 & Pl. V. fig. 6). The process is completed in 10 to 15 minutes. During the latter part of the hatching process, when the appendages have been unfolded the opportunity is afforded to lift the nymph off and transfer it to a healthy plant. The transfer can be effected by means of a sharpened pencil the tip of which is slightly moistened with spittle. The nymph was confined singly to a healthy rice plant in a glass tube and transferred daily to successive new healthy plants. Some difficulties were experienced

to manipulate the tiny nymphs of 2 to 5 days of age, since they were very sensitive and leap with surprising agility. Thus a number of nymphs were lost or accidentally killed during their transfer. As has been previously reported most of the nymphs are not infective immediately after the emergence from eggs and a period from 1 to 18 days, usually more than 5 days must elapse before they become capable of producing infections in healthy plants. Hence it is probable that most nymphs will not pick up the virus through feeding upon the rice plants on which they are allowed to remain for 5 days immediately after the emergence from eggs. If some of them should produce infections in the plants on which they feed during this period, they should be discarded. In the later experiments, therefore, this procedure was followed and proved to be very helpful. Thereafter each nymph was transferred daily to successive new healthy plants. As the nymphs confined in glass tubes grow old they can be more readily transferred. By means of patting the glass tube the nymph was induced to hop from the rice plant to the wall of the tube which was promptly lifted placing the hand over the opening in the bottom to prevent the escape of the insect. The tube was inclined at about 45 degrees and gently set upright placing over a new healthy plant lest the insect should drop out of it. The glass tube was then patted again on the side to cause the nymph inside to fall on the leaf of rice plant or on the soil enclosed. In most cases the nymphs dropped on the soil would migrate to the plants crawling up the stems but sometimes they climb up the walls of the tubes and should be dropped repeatedly until they succeed in reaching the rice plants since most younger nymphs are unable to migrate by themselves from the walls of glass tubes to the enclosed plants. Occasionally some nymphs could be transferred to the rice plant which had been brought in contact with the wall of glass tube in neighbourhood of the point where the insect was located. After the nymph has migrated to the plant care should be taken lest the leaf of rice plant comes in contact with the wall of glass tube bridging over the space between them because the insect may crawl along the leaf to reach the glass tube and eventually go astray. The insects almost in the 4th instar as well as in the subsequent stages can readily migrate by themselves to the enclosed plants no matter where on the walls of glass tubes they may locate. As stated before, insect transfers were made before the window in a small room. Since the leafhopper has a tendency to leap or fly towards a light source, any individual that escaped went to the window and could be easily recaptured by means of the pipette method originally used by KUNKEL (1926).

The viruliferous females found among the 2nd generation individuals were paired with nonviruliferous males. For this purpose a supply of nonviruliferous leafhoppers was maintained on healthy rice plants in insect-proof cages in the green house. The offspring of the 3rd generation were also transferred to healthy plants as soon as they had hatched and subsequently, or after 5 days, transferred daily to successive new healthy plants. In a similar manner the experiment was continued as long as a viruliferous female was secured among the progeny of the leafhoppers. As a long period was required to complete one experiment series some difficulties were encountered during the course of the work. In spite of scrupulous care some younger larvae were lost or accidentally killed in transferring them and certain others died prematurely for some obscure reason. Sometimes no viruliferous females were produced or they, if any, would not pair with males or died without laying eggs, contrary to expectation. In such cases the experiment was unavoidably ended. After all, during the past 5 years, 2 cases each were secured of transmission of the virus through eggs to the progeny of the 3rd and 4th generations as well as 1 case of transmission to the 7th generation. The results of the experiments will be presented in detail.

EXPERIMENT 1.

May 4, 1934, about 80 leafhoppers were furnished to the writer through the courtesy of Prof. T. WATANABE of the Utsunomiya College of Agriculture and Forestry who had collected them feeding on *Astragalus sinicus* L. grown on rice fields in the vicinity of Utsunomiya. All but one of them proved free from virus as mentioned in the previous paper. May 18, the offspring of the nonviruliferous leafhoppers were introduced into an insect-proof cage enclosing dwarf diseased rice plants to feed on them for about a month. When these insects became adults, in order to test their infectivity, they were confined individually in glass tubes each enclosing a healthy rice plant. Of 46 insects including 26 males and 20 females tested, 1 male and 2 females proved to be viruliferous. One of the latter designated as f27, after mating with a nonviruliferous male, laid eggs which hatched out after about 2 weeks. Single nymphs which had just emerged from these eggs were transferred to a young healthy rice plant in a glass tube. Subsequently they were transferred daily to successive new healthy plants. Much difficulty was experienced to manipulate these young nymphs 2–5 days of age which were very active and some of them were eventually lost. All but one of 10 leafhoppers which survived for a sufficiently long

period proved to be infective, indicating that the virus had been transmitted through the eggs to the progeny of the 2nd generation.

Table 1. Infectivity of the progeny of f27

Insect No.	Date of emergence from egg	Sex	Virulence
f27-1	July 14	male	infective
f27-2	15	female	"
f27-3	"	"	"
f27-4	19	"	noninfective
f27-5	"	male	infective
f27-6	"	"	"
f27-7	24	"	"
f27-8	"	female	"
f27-9	"	male	"
f27-10	25	"	"

Six of these insects, from f27-1 to f27-5 and f27-10 were successfully transferred daily to new healthy plants through their entire life and 2 of them, attaining maturity, became infective females, one of which was designated as f27-2. August 20, a nonviruliferous male was introduced into the glass tube enclosing this infective female which had emerged into the adult stage 7 days before. After pairing, the insect, f27-2 also was transferred daily to successive new healthy plants and 8 days later it began to lay eggs. This leafhopper emerged from the egg on July 15, became adult on Aug. 13 and died on Sept. 7 after having laid 35 eggs and produced infections in 38 plants as will be shown in the following table.

Table 2. Results of daily transfers of f27-2 to test plants

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

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

From the 35 eggs deposited by the leafhopper, f27-2, 26 nymphs were successfully transferred to healthy plants as soon as they had hatched out. Six of these larvae were transferred separately to successive new healthy plants every day, 9 of them were allowed to remain singly in separate glass tubes enclosing healthy rice plants and the others were lost on the occasion of transfer. All the 15 insects which survived long enough proved to be viruliferous indicating that the virus had been transmitted through eggs to the progeny of the 3rd generation.

Table 3. Infectivity of the progeny of f27-2

Insect No.	Date of oviposition	Date of emergence from egg	Sex	Virulence
f27-2-1	Aug. 29	Sept. 12	n	infective
f27-2-2	31	14	"	"
f27-2-3	"	"	"	"
f27-2-4	"	"	"	"
f27-2-5	"	"	"	"
f27-2-6	"	"	"	"
f27-2-7	Sept. 1	16	"	"
f27-2-8	"	"	"	"
f27-2-9	2	17	female	"
f27-2-10	"	"	male	"
f27-2-11	"	"	n	"
f27-2-12	3	"	"	"
f27-2-13	"	18	"	"
f27-2-14	"	"	male	"
f27-2-15	"	"	"	"

n stands for a nymph which died prematurely

Six leafhoppers which were transferred daily to new healthy plants caused infections in 13, 55, 35, 11, 50 and 28 plants, respectively, as shown in the following table.

Table 4. Results of daily transfers of infective progeny of f27-2 to test plants

Insect No.	Sept. 12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	20-21	21-22	22-23
f27-2-1	−	−	−	−	−	−	−	−	+	+	+
f27-2-9					−	−	−	−	−	−	−
f27-2-10					−	−	−	−	−	−	−
f27-2-11					−	−	−	−	−	−	−
f27-2-14						−	−	−	−	−	−
f27-2-15						−	−	−	−	−	−

Insect No.	Sept. 23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–Oct. 1	1–2	2–3
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. 3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14
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. 14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25
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. 25–26	26–27	27–28	28–29	29–30	30–31	31–Nov. 1	1–2	2–3	3–4
f 27–2–1										
f 27–2–9	+	+	+	+	+	+	+	+	×	+
f 27–2–10	+	+	+	+	×	+	×	×	×	×
f 27–2–11										
f 27–2–14	+	+	+	+	×	+	×	+	+	×
f 27–2–15	+	+	+	+	+	+	×	×	−	×

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

Insect No.	Nov. 15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26
f27-2-1											
f27-2-9	+	+	+	+	+	+	+	+	×	+	+
f27-2-10	+	+	+	×							
f27-2-11											
f27-2-14	+	−	+	+	+	+	+	×	+	+	+
f27-2-15											

	Nov. 26–27	27–28	28–29	29–30	30–Dec. 1	1–2	2–3	3–4	4–5	5–6
f27-2-1										
f27-2-9	×	×								
f27-2-10										
f27-2-11										
f27-2-14	+	+	×	×	×	×	+	+	×	×
f27-2-15										

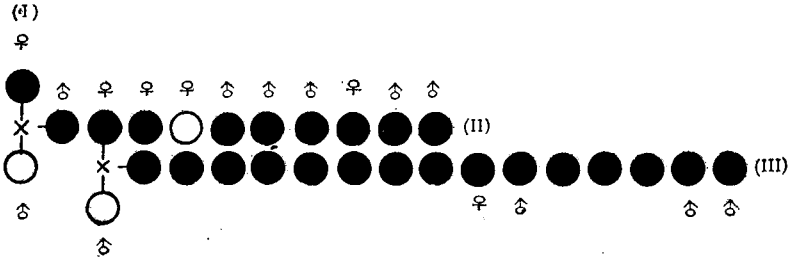
	Dec. 6–7	7–8	8–9	9–10
f27-2-1				
f27-2-9				
f27-2-10				
f27-2-11				
f27-2-14	+	×	×	+
f27-2-15				

The leafhopper of the 3rd generation designated f27–2–9 only, was a female and after mating with a nonviruliferous male, laid 4 eggs which were left unnoticed until the larvae had hatched out of them. Hence the experiment could not be extended further.

As shown above, the female leafhopper, f27 emerging from a viruliferous egg was capable of producing infections in 38 plants on which it was confined for 24 hours on consecutive days and moreover of transmitting the virus to at least 15 eggs developing progeny which could produce infections in 201 healthy plants without again having renewed access to an affected plant.

The results of Experiment 1 are presented in the following diagrams.

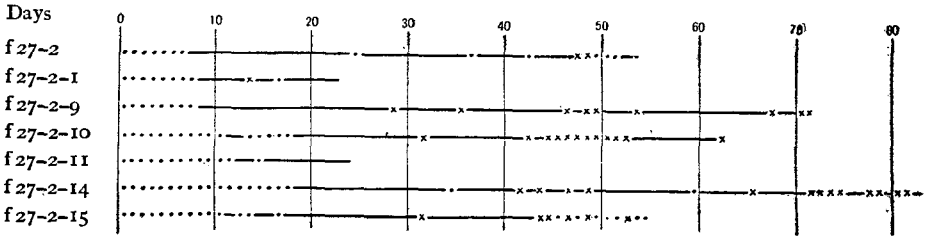
Diagram 1.



● ○ indicate infective and noninfective leafhoppers, respectively

Diagram 2.

Results of daily transfers of infective leafhoppers to test plants in Exp. 1



..... non-infective period (each dot represents one day)
——— infective period
× × × × unknown because the test plants were killed

EXPERIMENT 2.

A portion of the progeny of the leafhopper, *f27* mentioned in the foregoing experiment was maintained on dwarf diseased rice plants in an insect-proof cage in the green house. April 2, 1936, 12 larvae from this cage were transferred singly into glass tubes enclosing healthy rice plants in order to find infective females. Thus an infective female designated as f187 was secured. April 23 this insect having proved to be infective was mated with a nonviruliferous male. The nymphs of the 2nd generation were transferred singly to healthy rice plants enclosed in separate glass tubes immediately upon emergence from eggs. After that they were provided daily with successive new healthy plants. Of 4 insects which survived for a sufficiently long period 2 individuals proved to be infective.

Table 5. Infectivity of the progeny of f187

Insect No.	Date of emergence from egg	Sex	Virulence
f187-4	May 7	male	noninfective
f187-7	"	female	"
f187-9	10	male	infective
f187-11	"	female	"

The infective female, f187-11 hatched out of the egg on May 10 and emerged into the adult stage on June 9. It was weakly infective producing infections in only 11 of 85 plants on each of which it had been confined for one day. It remained noninfective throughout its nymphal stage and did not betray its infectivity until 5 days after the last moulting.

Table 6. Results of daily transfers of f187-11 to test plants

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

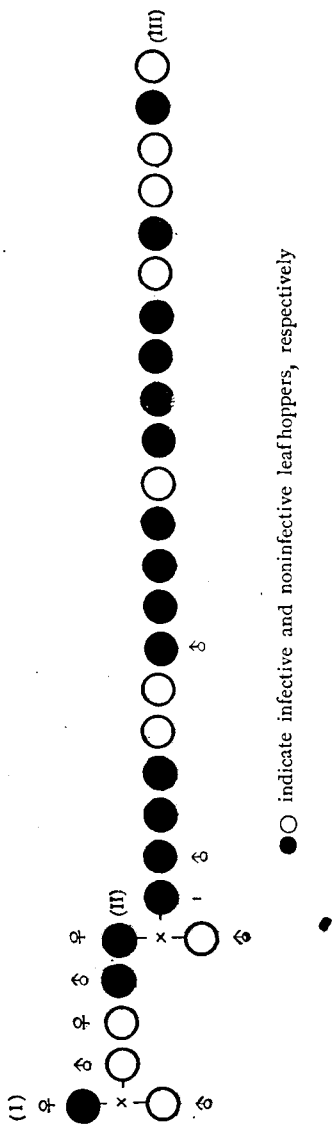
On June 15, six days after the emergence into adult stage this insect was paired with a nonviruliferous male and began to lay eggs on the next day. The nymphs of the 3rd generation were also transferred singly immediately on hatching, to healthy plants enclosed in separate glass tubes. Fourteen of 21 leafhoppers surviving for a sufficiently long period proved to be infective as shown in the following table.

Table 7. Infectivity of the progeny of f187-11

Insect No.	Date of oviposition	Date of emergence from egg	Virulence
f187-11-1	June 15	June 27	infective
f187-11-2	"	"	"
f187-11-3	"	"	"
f187-11-4	"	"	"
f187-11-5	16	"	noninfective
f187-11-6	"	"	"
f187-11-7	17	28	infective
f187-11-8	"	"	"
f187-11-9	18	29	"
f187-11-10	"	"	"
f187-11-11	"	"	noninfective
f187-11-12	"	"	infective
f187-11-13	"	"	"
f187-11-14	"	"	"
f187-11-15	20	July 1	"
f187-11-16	"	2	noninfective
f187-11-17	22	4	infective
f187-11-18	"	"	noninfective
f187-11-19	24	5	"
f187-11-20	"	"	infective
f187-11-21	"	"	noninfective

The results of this experiment also indicate that the virus has been transmitted through eggs of the leafhopper passing on to the 3rd generation. Since the virulence of the female of the 2nd generation, f187-11 appeared to be rather weak the experiment was not extended further. The summarized results of Experiment 2 are shown in the following diagram.

Diagram 3.



EXPERIMENT 3.

As stated above, a portion of the offspring of the leafhopper, f27 was maintained on dwarf diseased rice plants in an insect-proof cage in the green house. On June 30, 1936, six nymphs from this cage were confined singly in glass tubes enclosing healthy rice plants in order to obtain infective females. An infective female designated as f199 was thus secured and mated with a viruliferous male on July 17. On July 29 and 31, respectively, 8 and 3 nymphs were hatched out of eggs laid by this insect. These nymphs were transferred singly immediately on hatching, to individual healthy rice plants enclosed in separate glass tubes. Subsequently they were transferred daily to successive new test plants. Of 6 leafhoppers which survived for a sufficiently long period 3 individuals proved to be infective.

Table 8.
Infectivity of the progeny of f199

Insect No.	Date of emergence from egg	Sex	Virulence
f199-3	July 29	male	infective
f199-5	"	"	"
f199-6	"	female	"
f199-7	"	"	noninfective
f199-8	"	male	"
f199-9	31	"	"

The infective female, f199-6 emerged from the egg on July 29 and into

the adult stage on Aug. 21. It became infective 11 days after hatching from the egg, producing infections in 25 of 40 test plants on each of which it had been confined for one day.

Table 9. Results of daily transfers of f 199-6 to test plants

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

On Aug. 24, three days after its last moulting this insect was paired with a nonviruliferous male and from the next day on it intermittently laid eggs for 13 days. The nymphs derived from these eggs were transferred singly immediately on hatching to individual healthy rice plants enclosed in separate glass tubes and after that they were transferred daily to successive new healthy plants. Twenty larvae were lifted on hatching but only a half of them survived long enough, the others having been lost or having died during the course of experiment. Only one of the 10 insects of the 3rd generation proved to be infective. It was a male designated f 199-6-5.

Table 10. Infectivity of the progeny of f 199-6

Insect No.	Date of oviposition	Date of emergence from egg	Sex	Virulence
f 199-6-1	Aug. 25	Sept. 4	female	noninfective
f 199-6-2	"	"	"	"
f 199-6-3	"	"	male	"
f 199-6-4	"	"	"	"
f 199-6-5	"	"	"	infective
f 199-6-6	"	"	"	noninfective
f 199-6-8	30	9	female	"
f 199-6-10	"	"	male	"
f 199-6-15	31	10	"	"
f 199-6-18	"	"	"	"

The leafhopper f 199-6-5 became infective 25 days after hatching and produced infections in 33 of 67 rice plants on each of which it had been confined for one day as shown in the following table.

Table 11. Results of daily transfers of f 199-6-5 to test plants

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

The progeny of the 3rd generation were reared in a glass house where a low temperature prevailed. Under such condition a much prolonged incubation period was usually required for the virus in the plant and consequently it took a much longer time to decide whether or not a leafhopper was infective. On the other hand, as stated before, some female leafhoppers would show a tendency not to mate readily with males as they approached old age. Accordingly 2 females of the 3rd generation, f 199-6-1 and f 199-6-2 were allowed to mate with nonviruliferous males on Oct. 12 without waiting for their betraying infectivity. Those 2 females eventually proved to be noninfective but some of their offspring produced infections in test plants to which they had been transferred immediately upon emergence from eggs as shown in Tables 12 and 13.

Table 12. Infectivity of the progeny of f 199-6-1

Insect No.	Date of oviposition	Date of emergence from egg	Sex	Virulence
f 199-6-1-1	Oct. 14	Oct. 27	female	noninfective
f 199-6-1-2	"	"	male	"
f 199-6-1-3	"	"	"	"
f 199-6-1-4	"	30	"	"
f 199-6-1-5	"	"	"	"
f 199-6-1-6	"	"	"	"
f 199-6-1-7	"	"	female	"
f 199-6-1-9	17	Nov. 3	male	infective
f 199-6-1-10	"	"	?	noninfective
f 199-6-1-11	"	"	male	"
f 199-6-1-12	"	"	"	"
f 199-6-1-13	"	"	"	"
f 199-6-1-14	"	"	"	infective
f 199-6-1-16	26	5	female	noninfective
f 199-6-1-17	"	"	"	"
f 199-6-1-18	"	7	"	"

The leafhoppers, f 199-6-1-8 and f 199-6-1-15 killed the plants on which they had been confined and so it was not decided whether they were infective. Two of 16 individuals of the 4th generation proved to be infective.

Table 13. Infectivity of the progeny of f 199-6-2

Insect No.	Date of oviposition	Date of emergence from egg	Sex	Virulence
f 199-6-2-1	Oct. 13	Oct. 27	female	noninfective
f 199-6-2-2	"	"	male	"
f 199-6-2-3	15	30	"	infective
f 199-6-2-4	"	"	"	noninfective
f 199-6-2-5	"	"	female	"
f 199-6-2-6	"	"	"	"
f 199-6-2-7	"	"	n	"
f 199-6-2-9	"	"	male	infective
f 199-6-2-10	"	"	"	noninfective
f 199-6-2-11	"	"	"	"
f 199-6-2-12	26	Nov. 5	n	"
f 199-6-2-13	"	"	male	"
f 199-6-2-14	"	"	female	"
f 199-6-2-15	"	"	"	"
f 199-6-2-16	"	"	n	"
f 199-6-2-17	"	"	n	"
f 199-6-2-18	27	7	male	"
f 199-6-2-19	"	"	female	"
f 199-6-2-20	"	"	male	"
f 199-6-2-21	"	"	n	"
f 199-6-2-22	"	9	female	"
f 199-6-2-23	"	"	"	"

n stands for nymphs which died prematurely

The leafhopper, f199-6-2-8 killed the test plant and accordingly it was impossible to decide whether it was infective. In this case 2 of 22 individuals of the 4th generation proved to be infective. The summarized results of Experiment 3 are presented in the following diagrams.

Diagram 4.

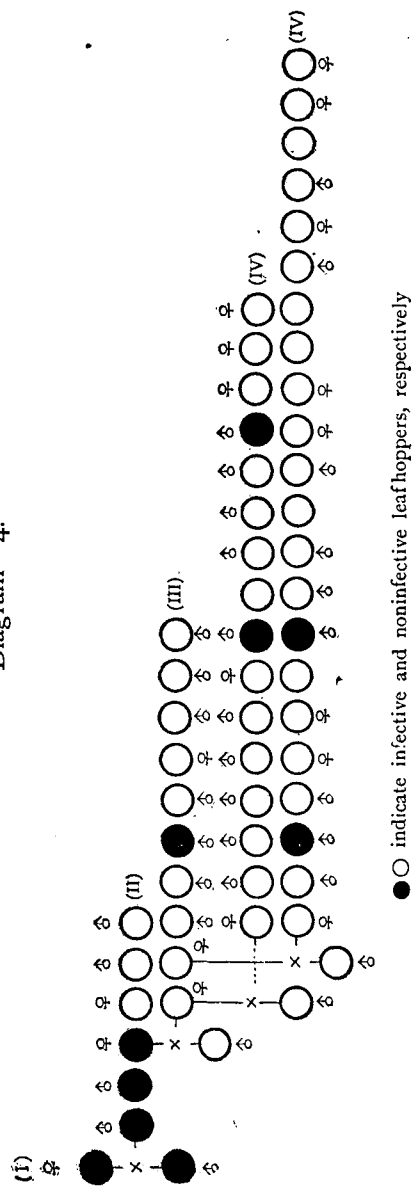
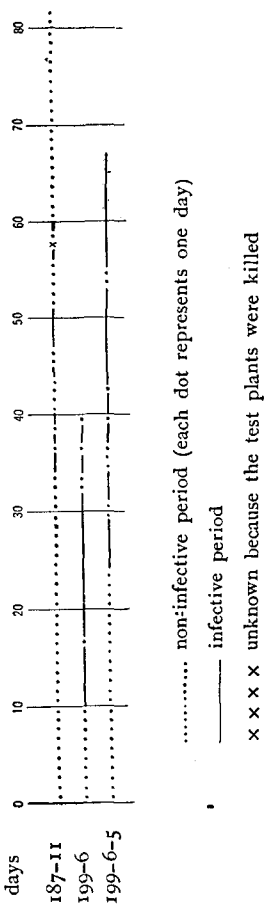


Diagram 5.

Results of daily transfers of infective leafhoppers to test plants



EXPERIMENT 4.

On April 14, 1937 about 60 leafhoppers were collected on *Astragalus sinicus* L. growing on the rice fields in the vicinity of Utsunomiya. A majority of them were at their nymphal stage. They were confined singly on individual young healthy rice plants enclosed in a glass tube to test their infectivity. Only one of them proved to be infective furnishing further evidence to indicate that the virus under consideration may overwinter in its insect carrier. In the middle of June, 12 and 2 nonviruliferous female and male leafhoppers, respectively were introduced into an insect proof cage enclosing dwarf diseased rice plants. On July 17 their offspring in the 3rd or 4th instar were confined singly each on a healthy rice plant enclosed in a glass tube to test their infectivity and an infective female designated h66 was thus secured. On July 31 this insect was found to be infective and was mated with a nonviruliferous male 2 days later. Thereafter this female leafhopper laid more than 30 eggs which hatched in 10 to 15 days. Sixteen nymphs were lifted immediately on emergence from eggs transferring singly each to a young healthy rice plant enclosed in a glass tube. After 7 days they were transferred daily to successive new healthy rice plants. Only 5 of them survived for a sufficiently long period producing 3 infective individuals.

Table 14. Infectivity of the progeny of h66

Insect No.	Date of emergence from egg	Sex	Virulence
h66–4	Aug. 26	female	noninfective
h66–5	"	"	infective
h66–8	"	male	noninfective
h66–14	27	female	infective
h66–15	"	"	"

The infective female of the 2nd generation designated as h66–5 became infective 11 days after emergence from the egg and produced infections in 30 out of 41 rice plants on which it had been confined for one day, as shown in Table 15.

Table 15. Results of daily transfers of h66-5 to test plants

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

On Oct. 4, eleven days after emergence into the adult stage, this insect was paired with a nonviruliferous male. It began to lay eggs on the next day. Twenty nymphs of the 3rd generation were lifted immediately on hatching and transferred singly each to a healthy rice plant in a glass tube where they were allowed to remain for 5 days. Thereafter they were transferred daily to successive new healthy rice plants. Two of 12 leafhoppers which had survived long enough proved to be infective.

Table 16. Infectivity of the progeny of h66-5

Insect No.	Date of oviposition	Date of emergence from egg	Sex	Virulence
h66-5-1	Oct. 5	Oct. 20	n	noninfective
h66-5-4	8	"	male	infective
h66-5-5	"	"	n	noninfective
h66-5-6	9	"	female	infective
h66-5-7	"	"	n	noninfective
h66-5-8	"	"	female	"
h66-5-9	10	"	n	"
h66-5-11	12	"	n	"
h66-5-12	"	"	male	"
h66-5-13	"	"	female	"
h66-5-17	13	21	male	"
h66-5-19	"	22	female	"

n stands for nymphs which died prematurely

The results of daily transfers of the infective leafhoppers, h66–5–4 and h66–5–6 to successive healthy rice plants are shown in the following table.

Table 17.

Results of daily transfers of the infective progeny of h66–5 to test plants.

Insect No.	Oct. 20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–31
h66–5–4	–	–	–	–	–	–	–	–	–	–	–
h66–5–6	–	–	–	–	–	–	–	–	–	–	–
	Oct. 31–Nov. 1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	
h66–5–4	–	–	–	–	–	–	+	+	+	+	
h66–5–6	–	–	+	+	+	+	+	+	+	+	
	Nov. 10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21
h66–5–4	+	+	+	+	+	+	+	+	+	+	+
h66–5–6	+	+	+	+	–	–	–	–	–	–	–
	Nov. 21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–Dec. 1	
h66–5–4	+	+	+	+	+	–	+	+	+	+	
h66–5–6	–	–	–	–	–	–	–	–	–	–	
	Dec. 1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12
h66–5–4	+	+	–	–	+	+	+	–	–	–	–
h66–5–6	–	–	–	–	–	–	–	–	–	–	
	Dec. 12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23
h66–5–4	–	–	–	–	–	–	–	–	–	–	–
h66–5–6											
	Dec. 23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–31	31–Jan. 1		1–2
h66–5–4	–	–	–	–	–	–	–	–	–		–
h66–5–6											
	Jan. 2–3	3–4	4–5	5–6	6–7	7–8	8–20				
h66–5–4	–	–	–	–	–	–	+				
h66–5–6											

As shown above the infective male, h66–5–4 became infective 18 days after emergence from the egg and continued to be so for about a month but was noninfective during the next month as if it had exhausted the

virus. However it produced infection in a plant on which it was allowed to feed for the last 12 days of its life. After all this insect produced infections in 30 of 70 and more test plants on which it had been confined for one day. The female, h66-5-6 became infective 14 days after hatching and produced infections in 12 of 46 rice plants on which it was allowed to feed for one day. On Nov. 23, nine days after emergence into the adult stage it was mated with a nonviruliferous male and began to lay eggs 2 days later. Thirteen larvae of the 4th generation were lifted immediately on emergence from eggs and transferred singly each to a healthy rice plant enclosed in a glass tube where they were allowed to remain for 7 days. Thereafter they were transferred daily to successive new healthy plants. All but one of 11 leafhoppers which survived for a sufficiently long period proved to be infective.

Table 18. Infectivity of the progeny of h66-5-6

Insect No.	Date of oviposition	Date of emergence from egg	Sex	Virulence
h66-5-6-1	Nov. 25	Dec. 4	male	infective
h66-5-6-2	"	"	n	"
h66-5-6-3	"	"	female	"
h66-5-6-4	26	6	"	noninfective
h66-5-6-5	"	"	"	infective
h66-5-6-6	27	9	male	"
h66-5-6-8	28	10	female	"
h66-5-6-9	"	11	male	"
h66-5-6-11	"	13	"	"
h66-5-6-12	Dec. 2	14	female	"
h66-5-6-13	4	16	n	"

n stands for nymphs which died prematurely

The leafhopper, h66-5-6-4 which survived for 71 days was transferred daily to new healthy plants during 50 days but allowed to feed on one rice plant for the last 20 days. In no case, however, did it produce infections in test plants. The results of daily transfers of some leafhoppers of the 4th generation are shown in the following table.

Table 19.

Results of daily transfers of the progeny of h66-5-6 to test plants

Insect No.	Dec.	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15
h66-5-6-1		–	–	–	–	–	–	–	–	–	–	+
h66-5-6-6							–	–	–	–	–	–
h66-5-6-9									–	–	–	–
h66-5-6-11											–	–
h66-5-6-12												–
h66-5-6-13												

	Dec.	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26
h66-5-6-1		+	+	×	+	×	+	×	+	+	+	+
h66-5-6-6		–	–	–	–	–	–	–	–	–	–	–
h66-5-6-9		–	–	–	–	–	–	–	–	–	–	–
h66-5-6-11		–	–	–	–	–	–	–	–	–	–	–
h66-5-6-12		–	–	–	–	–	–	–	–	–	–	–
h66-5-6-13			–	–	–	–	–	–	–	–	–	–

	Dec.	26–27	27–28	28–29	29–30	30–31	31–Jan. 1		1–2	2–3	3–4	4–5
h66-5-6-1		+	+	+	+	+	+		+	+	+	+
h66-5-6-6		–	–	–	–	–	+		–	–	–	–
h66-5-6-9		–	–	+	–	–	–		+	–	–	–
h66-5-6-11		–	–	–	–	+	–		–	–	–	–
h66-5-6-12		–	–	+	–	–	–		+	+	+	+
h66-5-6-13		–	–	–	–	–	–		–	–	–	–

	Jan.	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16
h66-5-6-1		+	+	+	+	+	+	+	×	+	+	+
h66-5-6-6		–	–	–	–	–	–	–	–	–	–	–
h66-5-6-9		–	–	–	–	+	–	–	–	+	×	–
h66-5-6-11		–	–	–	–	–	–	–	–	–	–	–
h66-5-6-12		+	+	+	+	+	+	+	+	+	+	+
h66-5-6-13		+	–	–	–	–	+	–	–	–	–	×

	Jan.	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27
h66-5-6-1		+	+	+	+	+	+	+	+	+	+	×
h66-5-6-6		–	–	–	–	–	–	–	–	–	–	–
h66-5-6-9		–	+	–	–	–	–	–	–	–	–	–
h66-5-6-11		–	–	–	–	–	–	–	–	–	–	–
h66-5-6-12		+	+	+	+	+	+	+	+	+	+	+
h66-5-6-13		+										

Insect No.	Jan. 27–28	28–29	29–30	30–31	31–Feb. 1	1–2	2–3	3–4	4–5	5–6
h66–5–6–1	+	+	+	−	+	+	+	+	+	+
h66–5–6–6										
h66–5–6–9	−	−	−	−	−	−	−	−	−	−
h66–5–6–11	−	−	−	−	−	−	+	−	−	−
h66–5–6–12	+	+	+	+	+	+	+	+	+	+
h66–5–6–13										

	Feb. 6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17
h66–5–6–1	+	+	+	+	−	−					
h66–5–6–6											
h66–5–6–9	−	−	−	−	−	−	−	−	−	−	−
h66–5–6–11	−	−	−	+	−	+	−	−	−	+	−
h66–5–6–12	+	+	+								
h66–5–6–13											

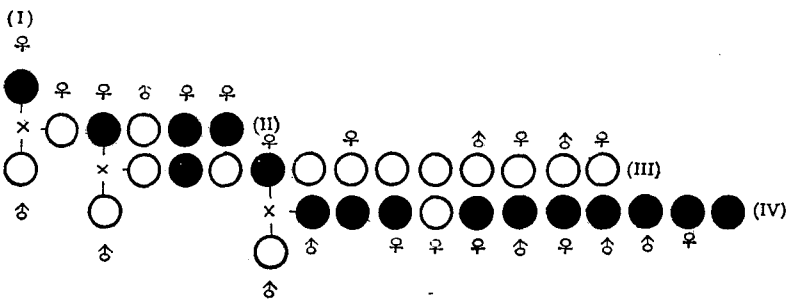
	Feb. 17–18	18–19	
h66–5–6–1			
h66–5–6–6			
h66–5–6–9	−	−	−(19–24)
h66–5–6–11	−	+	−(19–III 10)
h66–5–6–12			
h66–5–6–13			

As shown above the leafhopper, h66–5–6–1 became infective 11 days after emergence from the egg and remained viruliferous throughout its subsequent life producing infections in 52 plants. The insect h66–5–6–9 became infective 18 days after hatching but proved to be weakly virulent producing infections in 5 of 59 plants on each of which it had been confined for one day. The insect h66–5–6–11 also became infective 18 days after hatching and was infective to the same degree as the insect just mentioned. The female leafhopper, h66–5–6–12 was evidently infective 15 days after emergence from the egg and appeared to be very virulent but it was killed accidentally after it had produced infections in 40 plants. The insect h66–5–6–13 became infective 21 days after hatching and died prematurely producing infections in only 3 plants. In short, 2 individuals of the 4th generation were remarkably infective whereas the other 4 were weakly virulent. The other insects, h66–5–6–2, h66–5–6–3, h66–5–6–5 and h66–5–6–8 became infective before they were transferred daily to new healthy plants and so all were discarded. From the results of this experiment it is evident that the virus under consideration was

transmitted through the eggs of leafhopper to the progeny of the 4th generation.

The results of Experiment 4 are presented in the following diagrams.

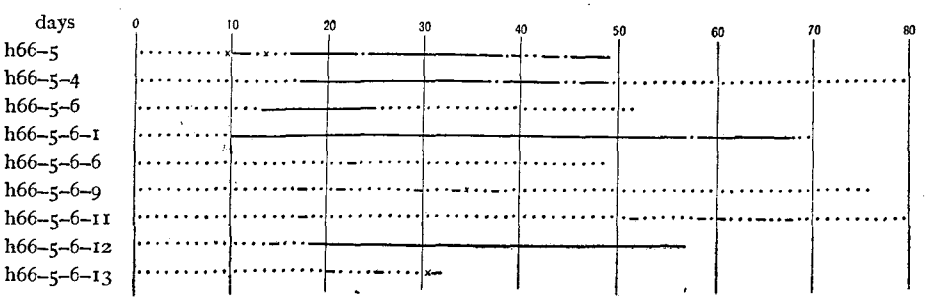
Diagram 6.



●○ indicate infective and noninfective leafhoppers, respectively

Diagram 7.

Results of daily transfers of infective progeny of h66 to test plants



..... noninfective period (each dot represents one day)

——— infective period

× × × × unknown because the test plants were killed

EXPERIMENT 5.

On Jan. 25, 1938 a nonviruliferous female leafhopper was mated with an infective male, h66–5–6–1 and their offspring were reared on dwarf diseased rice plants in an insect-proof cage in the green house. On Mar. 20, some nymphs in the 3rd or 4th instar were confined singly each on a young healthy rice plant enclosed in a glass tube to test their infectivity.

On April 7 an infective female designated as h99 was secured and paired with an infective male. Ten nymphs of the 2nd generation were lifted immediately on emergence from eggs transferring singly each to a young healthy rice plant enclosed in a glass tube. They were allowed to remain there for 5 days and thereafter transferred daily to successive new healthy plants. Two of them died prematurely and the remainder all proved to be infective.

Table 20. Infectivity of the progeny of h99

Insect No.	Date of emergence from egg	Sex	Virulence
h99–1	April 19	female	infective
h99–2	"	n	"
h99–3	"	female	"
h99–4	"	male	"
h99–5	"	n	"
h99–6	"	female	"
h99–8	21	"	"
h99–10	"	"	"

n stands for nymphs which died prematurely

The results of daily transfers of the infective females, h99–1 and h99–8 will be shown below.

Table 21.

Results of daily transfers of infective offspring of h99 to test plants

Insect No.	Apr. 19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30
h99–1	−	−	−	−	−	−	−	−	−	−	−
h99–8			−	−	−	−	−	−	−	−	−
	Apr. 30–May 1		1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10
h99–1	+		+	+	+	+	+	+	+	+	+
h99–8	−		−	−	+	+	+	+	+	+	+
	May 10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21
h99–1	+	+	+	−	−	−	−	−	−	+	−
h99–8	+	+	+	+	+	+	+	+	+	−	−

Insect No.	May	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–31	31–1
h99–1		–	–	–	–	+	–	–	–	–	–	+
h99–8		–	–	–	+	+	–	+	+	–	–	–
	June	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12
h99–1		–	+	+	+	+	–	+	+	+	+	+
h99–8		+	+	–	–	+	–	+	+	+	+	+
	June	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23
h99–1		+	–	+	–	+	–	–	–	–	–	–
h99–8		+	–	–	–	–	+	+				
	June	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–July 1		1–2	2–3
h99–1		–	–	–	–	–	–	–	–		–	+
h99–8												
	July	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14
h99–1		–	–	–	–	–	–	–	–	–	–	–
h99–8												
	July	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	
h99–1		–	–	–	–	–	–	–	–	–	–	
h99–8												

The leafhopper, h99-1 became infective 12 days after emergence from the egg and retained its infectivity for 13 days but the virulence decreased gradually until the virus appeared to be entirely exhausted. Ultimately this insect produced infections in 29 of 91 plants on each of which it had been confined for one day. Seven days after emergence into the adult stage this female leafhopper was paired with a nonviruliferous male. Six larvae of the 3rd generation were transferred singly immediately on hatching each to a young healthy rice plant enclosed in a glass tube. They were allowed to remain there for 5 days and subsequently transferred daily to successive new healthy plants. Three of them died prematurely while the remaining 3 proved to be infective.

The insect, h99-8 became infective 13 days after hatching and retained its infectivity for about 50 days producing infections in 31 healthy rice plants. Nine days after emergence into the adult stage this female was mated with a nonviruliferous male and thereafter laid a good many eggs. Eighteen nymphs derived from these eggs were transferred singly immediately on hatching each to a healthy rice plant enclosed in a glass tube. From 5 days after that they were transferred daily to successive new healthy plants. Twelve of them which survived for a sufficiently long period proved to be infective.

Table 22. Infectivity of the progeny of h-99-1 and h99-8

Insect No.	Date of oviposition	Date of emergence from egg	Sex	Virulence
h99-1-1	May 26	June 7	female	infective
h99-1-2	"	8	"	"
h99-1-5	June 5	19	n	"
h99-8-1	"	"	male	"
h99-8-2	6	"	female	"
h99-8-4	7	20	"	"
h99-8-6	9	23	"	"
h99-8-7	10	25	"	"
h99-8-9	"	"	male	"
h99-8-10	"	"	"	"
h99-8-11	"	"	female	"
h99-8-13	"	"	"	"
h99-8-14	11	26	male	"
h99-8-17	13	July 2	female	"
h99-8-18	"	"	"	"

n stands for nymphs which died prematurely

The results of daily transfers of these leafhoppers to new healthy rice plants are shown in the following table.

Table 23. Results of daily transfers of some infective progeny of the 3rd generation to test plants

Insect No.	June 7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18
h99-1-1	–	–	–	–	–	–	–	–	–	–	–
h99-1-2		–	–	–	–	–	–	–	–	–	–
h99-8-1											
h99-8-2											
h99-8-4											
h99-8-6											
h99-8-7											
h99-8-9											
h99-8-10											
h99-8-11											
h99-8-13											
h99-8-14											
h99-8-17											
h99-8-18											

Insect No.	June 18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29
h99–1–1	–	–	–	–	–	–	–	–	–	–	–
h99–1–2	–	–	–	–	–	+	+	–	+	–	+
h99–8–1		–	–	–	–	–	–	–	–	–	–
h99–8–2		–	–	–	–	–	–	–	–	–	–
h99–8–4			–	–	–	–	–	–	–	–	–
h99–8–6						–	–	–	–	–	–
h99–8–7								–	–	–	–
h99–8–9								–	–	–	–
h99–8–10								–	–	–	–
h99–8–11								–	–	–	–
h99–8–13								–	–	–	–
h99–8–14									–	–	–
h99–8–17											
h99–8–18											

	June 29–30	30–July 1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9
h99–1–1	–	–	–	–	+	–	+	+	+	+
h99–1–2	+	+	+	+	+	+	+	+	+	+
h99–8–1	–	–	–	–	–	–	–	–	–	–
h99–8–2	–	–	–	–	–	+	–	+	+	+
h99–8–4	–	–	–	–	–	–	–	–	–	–
h99–8–6	–	–	–	–	–	–	–	–	–	–
h99–8–7	–	–	–	–	–	–	–	–	–	–
h99–8–9	–	–	–	–	–	–	–	–	–	–
h99–8–10	–	–	–	–	–	–	–	–	+	–
h99–8–11	–	–	–	–	–	–	–	–	–	–
h99–8–13	–	–	–	–	–	–	–	–	–	–
h99–8–14	–	–	–	–	–	–	–	–	–	–
h99–8–17				–	–	–	–	–	–	–
h99–8–18				–	–	–	–	–	–	–

	July 9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20
h99–1–1	+	+	+	+	+	+	–	+	+	+	+
h99–1–2	+	–	+	+	+	+	+	+	+	+	+
h99–8–1	–	+	–	+	+	+	+	–	+	+	+
h99–8–2	+	+	+	+	+	+	+	+	+	+	+
h99–8–4	–	–	–	–	–	–	–	–	–	–	–
h99–8–6	–	–	–	–	+	+	–	+	+	–	+
h99–8–7	–	–	–	–	–	–	+	+	+	+	+
h99–8–9	–	–	–	–	+	+	+	+	+	+	+
h99–8–10	–	+	+	+	+	+	+	+	+	+	+
h99–8–11	+	+	–	+	+	+	+	+	+	+	+
h99–8–13	+	+	+	+	+	+	+	+	+	+	+
h99–8–14	–	–	–	–	–	–	–	–	–	–	–
h99–8–17	–	–	–	–	–	–	–	–	+	+	+
h99–8–18	–	–	–	–	–	–	–	–	–	+	+

Insect No.	July 20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–31
h95–1–1	+	+	+	+	+	−	+	+	+	+	+
h95–1–2	+	+	+	+	+	+	+	−	−	−	+
h95–8–1	+	+	+	+	−	+	−	+	−	−	
h95–8–2	+	+	+	+	+	+	+	−	+	+	+
h95–8–4	−	−	−	−	−	−	−	−	+	−	−
h95–8–6	+	+	−	−	−	−	−	−	−	−	+
h95–8–7	+	+	+	+	+	+	+	+	+	+	+
h95–8–9	+	+	+	+	+	×	+	+	+	+	+
h95–8–10	+	+	+	−	−	−	−	−	+	−	−
h95–8–11	−	+	−								
h95–8–13	+	+	+	+	+	+	+	−	−	−	+
h95–8–14	+	+	−	+	+	+	+	+	+	+	+
h95–8–17	+	+	+	+	+	+	+	+	+	+	−
h95–8–18	×	+	+	+	+	+	+	+	+	−	−

	July 31–Aug. 1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10
h95–1–1	+	+	+	+	+	+	−	−	+	−
h95–1–2	+	+	+	+	+	+	+	−	−	−
h95–8–1										
h95–8–2	+	+	+	+						
h95–8–4	−	−	−							
h95–8–6	−	+	+	+	−					
h95–8–7	+	+	+	+	−	×	+	+	+	−
h99–8–9	−	+	+	+	+	+	+	+	+	−
h99–8–10	−	+	+	+	+	+	+	+	−	−
h95–8–11										
h99–8–13	−	+	−	+	+	+	−	+	−	−
h95–8–14	−									
h95–8–17	+	+	−	−	+	+	+	+	+	+
h95–8–18	−	−								

	Aug. 10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21
h95–1–1	−	−	−								
h99–1–2											
h99–8–1											
h95–8–2											
h95–8–4											
h95–8–6											
h99–8–7	+	+	−	+	−	+	−	−	+	+	−
h95–8–9	+	+	+	+	+	−	+	+	+	+	+
h95–8–10	−	−	−	−	−	−	−	−	−	−	−
h99–8–11											
h95–8–13	−	−	−	−	−	−	−	−	−		
h95–8–14											
h99–8–17	+	−	−	+	−	−	−	−			
h99–8–18											

	Aug. 21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–31	31–1
h99–1–1											
h99–1–2											
h99–8–1											
h99–8–2											
h99–8–4											
h99–8–6											
h99–8–7	+	−	−	−	−	+	−	−	−	−	−
h99–8–9	+	+	+	+	+	+	+	+	+	+	−
h99–8–10	−	−	−	−	−	−	−	−	−	−	
h99–8–11											
h99–8–13											
h99–8–14											
h99–8–17											
h99–8–18											

	Sept. 1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10
h99–1–1									
h99–1–2									
h99–8–1									
h99–8–2									
h99–8–4									
h99–8–6									
h99–8–7									
h99–8–9	+	+	−	−	+	−	+	−	+
h99–8–10									
h99–8–11									
h99–8–13									
h99–8–14									
h99–8–17									
h99–8–18									

As shown above they remained noninfective for 12 to 25 days after hatching and thereafter retained infectivity for 11 to 59 days.

Two infective female leafhoppers, h99–1–1 and h99–8–2 were paired with nonviruliferous males 13 and 9 days, respectively after emergence into the adult stage. From the eggs laid by the former, 45 larvae were lifted immediately on hatching and reared singly on healthy rice plants transferring daily to successive new healthy plants. Five of 19 insects which survived for a sufficiently long period proved to be infective. On the other hand 6 nymphs were likewise lifted from the eggs laid by h99–8–2 transferring singly in the usual manner employed in these experiments. Only 2 of them survived long enough, developing one infective female leafhopper.

Table 24. Infectivity of the progeny of the 4th generation

Insect No.	Date of oviposition	Date of emergence from egg	Sex	Virulence
h99-1-1-2	July 26	Aug. 4	female	noninfective
h09-1-1-3	"	"	"	"
h99-1-1-5	28	6	"	infective
h99-1-1-6	29	7	male	"
h99-1-1-9	Aug. 2	10	"	noninfective
h99-1-1-11	3	11	"	"
h99-1-1-13	4	12	"	"
h99-1-1-14	"	"	female	"
h99-1-1-15	"	"	"	"
h99-1-1-16	"	"	male	infective
h99-1-1-19	5	"	female	noninfective
h99-1-1-21	"	13	male	"
h99-1-1-24	"	"	"	infective
h99-1-1-26	"	"	"	"
h99-1-1-27	"	"	female	noninfective
h99-1-1-29	6	14	male	"
h99-1-1-31	"	"	"	"
h99-1-1-32	"	"	"	"
h99-1-1-41	8	16	"	"
h99-8-2-2	July 28	Aug. 6	"	"
h99-8-2-3	"	"	female	infective

The results of daily transfers of the infective progeny of the 4th generation to successive new healthy rice plants were as follows.

Table 25. Results of daily transfers of the infective progeny of the 4th generation to test plants

Insect No.	Aug. 6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17
h99-1-1-5	−	−	−	−	−	−	−	−	−	−	−
h99-1-1-6		−	−	−	−	−	−	−	−	−	−
h99-1-1-16							−	−	−	−	−
h99-1-1-24								−	−	−	−
h99-1-1-26								−	−	−	−
h99-8-2-3	−	−	−	−	−	−	−	−	−	−	−

Insect No.	Aug. 17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28
h99–1–1–5	–	–	–	–	–	–	–	+	+	×	+
h99–1–1–6	–	–	–	–	–	–	–	–	–	–	–
h99–1–1–16	–	–	–	–	–	–	–	–	–	+	+
h99–1–1–24	–	–	–	–	–	+	+	–	+	+	+
h99–1–1–26	–	–	–	–	–	–	–	–	–	+	+
h99–8–2–3	–	–	–	–	–	–	+	–	–	–	–

	Aug. 28–29	29–30	30–31	31–Sept. 1	1–2	2–3	3–4	4–5	5–6	6–7
h99–1–1–5	+	+	+	–	+	–	–	+	+	+
h99–1–1–6	–	–	–	–	+	–	–	+	+	+
h99–1–1–16	+	+	–	+	+	–	–	–	–	–
h99–1–1–24	+	+	+	+	+	×	×	–	+	–
h99–1–1–26	+	+	+	+	+	+	+	+	+	+
h99–8–2–3	–	–	+	–	–	–	–	–	–	–

	Sept. 7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18
h99–1–1–5	–	–	+	–	+	+	–	–	–	+	–
h99–1–1–6	+	+	+	+	+	+	–	+	–	–	×
h99–1–1–16	–	–	–	–	–	–	–	–	–	–	–
h99–1–1–24	–	–	–	–	–	–	–	–	–	–	–
h99–1–1–26	+	+	–	–	–	+	+	+	×	+	–
h99–8–2–3	–	–	–	–	–	–	–	–	–	–	–

	Sept. 18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29
h99–1–1–5	+	+	–	–	–	+	+	–	–	–	–
h99–1–1–6	+	–	–	+	–	+	+	+	+	+	+
h99–1–1–16	–	–	–	–	–	–	–	–	–	–	–
h99–1–1–24	–	–	–	–	–	–	–	–	–	–	–
h99–1–1–26	+	+	+	–	+	+	–	+	–	–	–
h99–8–2–3	–	–	–	–	–	–	–	–	–	–	–

	Sept. 29–30	30–Oct. 1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9
h99–1–1–5	–	+	+	–						
h99–1–1–6	+	+	+	–	+	–	–	–	–	–
h99–1–1–16										
h99–1–1–24	–	–	–	–	–	–	–	–	–	–
h99–1–1–26	–	–	–	–	–	–	–	–	–	–
h99–8–2–3	–									

	Oct. 9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20
h99–1–1–5											
h99–1–1–6	–	–	–	–	–	–	–	–	–	+	+
h99–1–1–16											
h99–1–1–24											
h99–1–1–26	–										
h99–8–2–3											

	Oct. 20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–31
h99–1–1–5											
h99–1–1–6	+	–	–	+	×	+	–	–	+	–	–
h99–1–1–16											
h99–1–1–24											
h99–1–1–26											
h99–8–2–3											

	Oct. 31–Nov. 1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10
h99–1–1–5										
h99–1–1–6	+	–	+	+	–	–	+	–	+	–
h99–1–1–16										
h99–1–1–24										
h99–1–1–26										
h99–8–2–3										

	Nov. 10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21
h99–1–1–5											
h99–1–1–6	–	–	–	–	–	+	–	–	+	–	–
h99–1–1–16											
h99–1–1–24											
h99–1–1–26											
h99–8–2–3											

	Nov. 21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–Dec. 1
h99–1–1–5										
h99–1–1–6	–	–	–	–	–	–	–	–	–	–
h99–1–1–16										
h99–1–1–24										
h99–1–1–26										
h99–8–2–3										

	Dec.	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11
h99–1–1–5											
h99–1–1–6		–	–	–	+	+	+	+	+	+	–
h99–1–1–16											
h99–1–1–24											
h99–1–1–26											
h99–8–2–3											

As shown above these 4th generation progeny became infective 9–25 days after emergence from the eggs and retained infectivity for 8-98 days. It is obvious that certain leafhoppers exhausted the virus during their daily transfer to successive new healthy plants since they remained noninfective for the last 24–33 days before they died. The infective females, h99–1–1–5 and h99-8-2-3 and noninfective ones, h99–1–1–3 and h99–1–1–19 were paired with nonviruliferous males 8 to 11 days after emergence into the adult stage. Except h99-8-2-3 all the insects deposited 17 to 36 eggs. More than 30 nymphs were lifted from the eggs laid by h99–1–1–5 immediately on hatching and transferred singly each to a healthy rice plant enclosed in a glass tube. After 5 days they were transferred daily to successive new healthy rice plants. More than a half of them died prematurely and 11 of the remaining 14 leafhoppers proved to be infective. Thirty-six and seventeen larvae, respectively, were likewise lifted from the eggs deposited by h99–1–1–3 and h99–1–1–19 and bred singly on healthy rice plants in separate glass tubes. Twenty-one and six of them survived for a sufficiently long period producing 18 and 6 infective insects respectively.

Table 26. Infectivity of the progeny of the 5th generation

Insect No.	Date of oviposition	Date of emergence from egg	Sex	Virulence
h99–1–1–5–2	Sept. 8	Sept. 22	female	infective
h99–1–1–5–3	"	"	n	"
h99–1–1–5–5	"	"	n	"
h99–1–1–5–6	"	"	male	"
h99–1–1–5–7	"	"	n	"
h99–1–1–5–8	10	"	n	noninfective
h99–1–1–5–13	"	25	female	infective
h99–1–1–5–18	11	26	n	noninfective

Insect No.	Date of oviposition	Date of emergence from egg	Sex	Virulence
h99-I-I-5-20	Sept. 11	Sept. 26	female	infective
h99-I-I-5-21	"	"	"	noninfective
h99-I-I-5-23	"	"	"	infective
h99-I-I-5-31	13	28	n	"
h99-I-I-5-32	20	Oct. 8	female	"
h99-I-I-5-34	22	9	n	"
h99-I-I-3-1	Sept. 4	Sept. 15	female	"
h99-I-I-3-2	"	16	n	"
h99-I-I-3-4	"	"	male	"
h99-I-I-3-5	"	"	"	"
h99-I-I-3-7	"	"	female	noninfective
h99-I-I-3-8	"	"	male	infective
h99-I-I-3-10	"	"	female	"
h99-I-I-3-11	"	"	male	"
h99-I-I-3-12	"	"	"	noninfective
h99-I-I-3-15	5	17	"	"
h99-I-I-3-19	6	19	"	infective
h99-I-I-3-20	"	20	"	"
h99-I-I-3-21	7	21	n	"
h99-I-I-3-23	"	"	male	"
h99-I-I-3-24	"	"	female	"
h99-I-I-3-26	"	"	male	"
h99-I-I-3-27	8.	23	"	"
h99-I-I-3-29	11	25	"	"
h99-I-I-3-33	"	"	"	"
h99-I-I-3-34	"	27	female	"
h99-I-I-3-36		Oct. 4	"	"
h99-I-I-19-1	Sept. 12	Sept. 28	"	"
h99-I-I-19-2	"	"	male	"
h99-I-I-19-3	"	"	"	"
h99-I-I-19-4	"	"	"	"
h99-I-I-19-5	"	"	"	"
h99-I-I-19-17		Oct. 4	"	"

n stands for nymphs which died prematurely

It is worthy of note that the females h99-I-I-3 and h99-I-I-19 both produced infective progeny notwithstanding the fact that they remained noninfective throughout their entire life. Such a case was also encountered in Experiment 3 as has been already mentioned. This phase of the

problem will be discussed later. The results of daily transfers of the infective progeny of h99–1–1–5 to successive healthy plants are presented in the following table.

Table 27. Results of daily transfer of the infective progeny of the 5th generation to test plants

Insect No.	Sept.	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–Oct. 1	1–2
h99–1–1–5–2		–	–	–	–	–	–	–	–	–	–
h99–1–1–5–3		–	–	–	–	–	–	–	–	–	–
h99–1–1–5–5		–	–	–	–	–	–	–	–	–	–
h99–1–1–5–7		–	–	–	–	–	–	–	–	–	–
h99–1–1–5–13					–	–	–	–	–	–	–
h99–1–1–5–20						–	–	–	–	–	–
h99–1–1–5–23							–	–	–	–	–
h99–1–1–5–31								–	–	–	–
h99–1–1–5–34											

	Oct.	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13
h99–1–1–5–2		–	–	–	–	–	–	–	–	–	–	–
h99–1–1–5–3		–	–	–	–	–	–	–	–	–	+	+
h99–1–1–5–5		–	–	–	–	–	–	–	–	–	–	–
h99–1–1–5–7		–	–	–	–	–	–	–	+	–	+	+
h99–1–1–5–13		–	–	–	–	–	–	–	–	–	+	–
h99–1–1–5–20		–	–	–	–	–	–	–	–	–	–	–
h99–1–1–5–23		–	–	–	–	–	–	–	–	–	–	–
h99–1–1–5–31		–	–	–	–	–	–	–	–	–	–	–
h99–1–1–5–34									–	–	–	–

	Oct.	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24
h99–1–1–5–2		–	–	–	–	–	–	–	–	–	+	+
h99–1–1–5–3		+	–	–	+	+	+	+	+	+	+	+
h99–1–1–5–5		–	–	–	–	+	+	+	+	+	+	+
h99–1–1–5–7		+	–	+	+	+	+	+	+	+	+	+
h99–1–1–5–13		+	+	×	+	+	+	+	+	+	+	+
h99–1–1–5–20		–	–	–	–	–	–	+	×	+	+	+
h99–1–1–5–23		–	–	–	–	–	–	–	–	+	+	+
h99–1–1–5–31		–	–	–	–	–	–	–	+	+	+	+
h99–1–1–5–34		–	–	–	–	–	–	–	–	–	–	–

Insect No.	Oct. 24–25	25–26	26–27	27–28	28–29	29–30	30–31	31–Nov. 1	1–2	2–3
h95–1–1–5–2	+	×	+	−	−	−	+	+	+	+
h95–1–1–5–3	+	+	+	−						
h95–1–1–5–5	+	+	+	+	−					
h95–1–1–5–7	+	−	+	+	−	−	−	+	+	+
h95–1–1–5–13	+	+	+	+	−	−	+	+	+	+
h95–1–1–5–20	+	×	+	+	−	+	−	+	+	+
h95–1–1–5–23	+	+	+	−	−	+	+	−	+	+
h95–1–1–5–31	+	+								
h95–1–1–5–34	−	−	+	+	+	+	−	+	+	

	Nov. 3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14
h95–1–1–5–2	−	+	+	+	+	+	+	+	+	+	+
h95–1–1–5–3											
h95–1–1–5–5											
h95–1–1–5–7	−	+	+	−	−						
h95–1–1–5–13	+	+	+	+	+	+	+	+	+	+	−
h95–1–1–5–20	+	−	−	+	+	+	+	+	+	+	+
h95–1–1–5–23	+	−	+	+	+	−	+	+	+	+	+
h95–1–1–5–31											
h95–1–1–5–34											

	Nov. 14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25
h95–1–1–5–2	+	+	+	+	−	+	+	+	+	+	+
h95–1–1–5–3											
h95–1–1–5–5											
h95–1–1–5–7											
h99–1–1–5–13	+	+	+	+	+	+	+	+	+	+	+
h95–1–1–5–20	+	+	+	+	+	+	+	+	+	×	+
h95–1–1–5–23	+	+	−	+	+	+	+	+	+	+	+
h95–1–1–5–31											
h95–1–1–5–34											

	Nov. 25–26	26–27	27–28	28–29	29–30	30–Dec. 1	1–2	2–3	3–4	4–5
h95–1–1–5–2	+	+	+	+	+	+	−	+	−	+
h99–1–1–5–3										
h99–1–1–5–5										
h99–1–1–5–7										
h99–1–1–5–13	+	+	+	+	+	+	+	+	−	+
h95–1–1–5–20	+	+	+	+	+	+	+	−	+	+
h99–1–1–5–23	+	+	+	+	+	−	+	−	+	+
h99–1–1–5–31										
h95–1–1–5–34										

	Dec. 5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16
h99–1–1–5–2	+	+	+	+	+	+	+	+	+	+	+
h99–1–1–5–3											
h99–1–1–5–5											
h99–1–1–5–7											
h99–1–1–5–13	+	+	+	+	+	+	−	+	−	+	+
h99–1–1–5–20	−	−	−	+	−	−	+	+	+	+	+
h99–1–1–5–23	+	+	+	+	−	+	+				
h99–1–1–5–31											
h99–1–1–5–34											

	Dec. 16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27
h99–1–1–5–2	+	+	+	+	+	+	+	×	+	−	−
h99–1–1–5–3											
h99–1–1–5–5											
h99–1–1–5–7											
h99–1–1–5–13	+	+	−	−	−	−	−	−	+	−	+
h99–1–1–5–20	−	×	−	+	+	−	+	+	+	+	+
h99–1–1–5–23											
h99–1–1–5–31											
h99–1–1–5–34											

	Dec. 27–28	28–29	29–30	30–31	31–Jan. 1	1–2	2–3	3–4	4–5	5–6
h99–1–1–5–2	−	−	−	−	+	−	−	+	−	+
h99–1–1–5–3										
h99–1–1–5–5										
h99–1–1–5–7										
h99–1–1–5–13	+	+	+	×	+	+	−			
h99–1–1–5–20	+	+	+	×	×	+	−	×	+	+
h99–1–1–5–23										
h99–1–1–5–31										
h99–1–1–5–34										

	Jan. 6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17
h99–1–1–5–2	+	+	×	−	−	+	+	−	−	+	+
h99–1–1–5–3											
h99–1–1–5–5											
h99–1–1–5–7											
h99–1–1–5–13											
h99–1–1–5–20	+	+	−	+	×	+	+	+	+	+	+
h99–1–1–5–23											
h99–1–1–5–31											
h99–1–1–5–34											

Insect No.	Jan. 17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25
h99–1–1–5–2	+							
h99–1–1–5–3								
h99–1–1–5–5								
h99–1–1–5–7								
h99–1–1–5–13								
h99–1–1–5–20	+	+	+	+	−	+	+	−
h99–1–1–5–23								
h99–1–1–5–31								
h99–1–1–5–34								

As shown above they became infective 16–30 days after hatching and retained infectivity 83–97 days at most producing infections in 65–73 healthy rice plants. The infective females, h99–1–1–5–2, h99–1–1–5–13 and h99–1–1–5–20, 11 or 12 days after emergence into the adult stage, were paired with nonviruliferous males. Forty-two and ten nymphs were lifted from the eggs deposited by the females, h99–1–1–5–2 and h99–1–1–5–13 respectively, immediately on emergence transferring singly each to a healthy rice plant enclosed in a glass tube. From five days after that they were transferred daily to successive new healthy rice plants. Thirteen and three of them survived for a sufficiently long period to reveal 7 and 3 infective leafhoppers, respectively. Nine larvae were likewise lifted from the eggs laid by the insect, h99–1–1–5–20 and reared singly on healthy rice plants in separate glass tubes. Seven of them surviving long enough proved to be infective.

Table 28. Infectivity of the progeny of the 6th generation

Insect No.	Date of oviposition	Date of emergence from egg	Sex	Virulence
h99–1–1–5–2–2	Dec. 1	Dec. 13	female	noninfective
h99–1–1–5–2–3	"	14	"	"
h99–1–1–5–2–6	4	16	"	infective
h99–1–1–5–2–7	"	"	male	"
h99–1–1–5–2–10	6	20	"	"
h99–1–1–5–2–11	"	"	female	"
h99–1–1–5–2–13	"	21	n	noninfective
h99–1–1–5–2–14	"	"	male	"
h99–1–1–5–2–21	12	25	female	infective
h99–1–1–5–2–31	15	29	male	"
h99–1–1–5–2–33	16	"	female	"
h99–1–1–5–2–39	Jan. 1	Jan. 13	"	noninfective
h99–1–1–5–2–42	"	"	"	"
h99–1–1–5–13–2	Dec. 2	Dec. 15	male	infective
h99–1–1–5–13–3	"	"	n	"
h99–1–1–5–13–5	"	"	female	"
h99–1–1–5–20–1		Jan. 9	n	infective
h99–1–1–5–20–2		"	n	"
h99–1–1–5–20–4		"	n	"
h99–1–1–5–20–5		"	female	"
h99–1–1–5–20–6		"	"	"
h99–1–1–5–20–8		"	n	"
h99–1–1–5–20–9		"	male	"

n stands for nymphs which died prematurely

The results of daily transfer of the infective offspring of h99-1-1-5-2 and h99-1-1-5-13 to successive new healthy rice plants are shown in the following table.

Table 29. Results of daily transfer of the infective progeny of the 6th generation to test plants

Insect No.	Dec. 15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26
h99-1-1-5-2-6		−	−	−	−	−	−	−	−	−	−
h99-1-1-5-2-7		−	−	−	−	−	−	−	−	−	−
h99-1-1-5-2-10						−	−	−	−	−	−
h99-1-1-5-2-11						−	−	−	−	−	−
h99-1-1-5-2-21											−
h99-1-1-5-2-31											
h99-1-1-5-2-33											
h99-1-1-5-13-2	−	−	−	−	−	−	−	−	−	−	−
h99-1-1-5-13-3	−	−	−	−	−	−	−	−	−	−	−
h99-1-1-5-13-5	−	−	−	−	−	−	−	−	−	−	−

	Dec. 26–27	27–28	28–29	29–30	30–31	31–Jan. 1	1–2	2–3	3–4	4–5
h99-1-1-5-2-6	−	−	−	−	−	−	−	+	−	+
h99-1-1-5-2-7	−	−	−	−	−	−	−	−	−	−
h99-1-1-5-2-10	−	−	−	−	−	−	−	−	−	−
h99-1-1-5-2-11	−	−	−	−	−	−	−	−	−	−
h99-1-1-5-2-21	−	−	−	−	−	−	−	−	−	−
h99-1-1-5-2-31				−	−	−	−	−	−	−
h99-1-1-5-2-33				−	−	−	−	−	−	−
h99-1-1-5-13-2	−	−	−	+	+	+	+	+	−	−
h99-1-1-5-13-3	−	−	+	−	+	+	+	+	+	+
h99-1-1-5-13-5	−	+	+	−	+	−	+	−	−	−

	Jan. 5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16
h99-1-1-5-2-6	+	+	+	+	+	+	+	+	+	+	−
h99-1-1-5-2-7	−	+	+	+	+	+	+	−	+	+	−
h99-1-1-5-2-10	−	+	+	+	+	+	−	+	+	+	+
h99-1-1-5-2-11	−	−	−	+	+	−	+	+	+	−	−
h99-1-1-5-2-21	−	−	+	+	+	+	+	+	+	+	+
h99-1-1-5-2-31	−	−	−	−	−	−	×	−	+	+	×
h99-1-1-5-2-33	−	−	−	−	−	−	−	−	−	−	−
h99-1-1-5-13-2	+	−	+	+	+	+	−	−	+	+	+
h99-1-1-5-13-3	+	+	−	+	+	+	+	+	−	+	−
h99-1-1-5-13-5	+	−	−	+	−	+	+	−	+	+	−

Insect No.	Jan. 16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27
h99-1-1-5-2-6	+	+	+	+	+	+	+	+	+	+	+
h99-1-1-5-2-7	+	+	+	+	+	−	+	−	−	−	+
h99-1-1-5-2-10	+	+	+	+	−	+	−	+	−	+	+
h99-1-1-5-2-11	−	−	+	+	−	+	−	−	−	+	−
h99-1-1-5-2-21	+	+	+	+	+	+	+	+	+	+	+
h99-1-1-5-2-31	+	+	+	+	+	−	+	+	−	+	+
h99-1-1-5-2-33	−	−	−	−	−	−	−	+	×	+	+
h99-1-1-5-13-2	+	+	+	+	+	+	+	+	+	+	+
h99-1-1-5-13-3	+	+	+	+	+	−	+				
h99-1-1-5-13-5	−	+	+	−	−	+	+	+	+	+	−

	Jan. 27–28	28–29	29–30	30–31	31–Feb. 1	1–2	2–3	3–4	4–5	5–6
h99-1-1-5-2-6	+	+	+	+	+	+	+	+	+	+
h99-1-1-5-2-7	+	+	+	+	+	+	+	+	+	+
h99-1-1-5-2-10	+	+	+	−	+	−	−	+	+	+
h99-1-1-5-2-11	+	−	+	+	+	−	+	−	−	−
h99-1-1-5-2-21	+	+	+	×	×	+	−	−	+	+
h99-1-1-5-2-31	−	−	+	×	×	+	+	−	×	+
h99-1-1-5-2-33	−	−	+	−	+	−	−	−	+	+
h99-1-1-5-13-2	+	+	+	+	+	+				
h99-1-1-5-13-3										
h99-1-1-5-13-5	+	+	−	−	+	−	−	−	+	+

	Feb. 6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17
h99-1-1-5-2-6	+	−	+	+	+	+	+	−	+	+	+
h99-1-1-5-2-7	+	+	+	+	+	+	+	+	+	+	+
h99-1-1-5-2-10	+	+	+	+	+	+	+	+	+	+	+
h99-1-1-5-2-11	−	−	−	−	−	+	+	−	−	−	+
h99-1-1-5-2-21	+	+	+	+	+	+	+	+	+	+	+
h99-1-1-5-2-31	−	−	+	−	+	+	+	+	+	+	+
h99-1-1-5-2-33	+	+	+	−	−	+	+	+	+	+	+
h99-1-1-5-13-2											
h99-1-1-5-13-3											
h99-1-1-5-13-5	+	+	+	+	+	+	−	+	−	+	+

	Feb. 17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28
h99-1-1-5-2-6	+	+	−	+	+	+	+	+	+	+	+
h99-1-1-5-2-7	+	+	−	+	−	+	+	+	+	−	−
h99-1-1-5-2-10	+	+	+	+	+	+	+	+	+	+	+
h99-1-1-5-2-11	+	+	−	+	−	+	×	−	+	−	−
h99-1-1-5-2-21	+	+	+	+	+	+	+	+	+	+	+
h99-1-1-5-2-31	+	+	+	+	+	+	+	+	−	+	−
h99-1-1-5-2-33	+	+	+	+	+	+	+	+	+	+	+
h99-1-1-5-13-2											
h99-1-1-5-13-3											
h99-1-1-5-13-5	+	+	+	+							

Insect No.	Feb. 28–Mar. 1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10
h99-1-1-5-2-6	+	×	×	+	+	+	+	+	+	−
h99-1-1-5-2-7	−	+	+	−	+	+	−	+	+	+
h99-1-1-5-2-10	+	+	+	+	−	−	−	−	+	−
h99-1-1-5-2-11	−	+	−	−	−	−	−			
h99-1-1-5-2-21	−	+	+	+	+	+	+	+		
h99-1-1-5-2-31	+	+	−	+	−	−	+	+	+	+
h99-1-1-5-2-33	+	+	+	−	+	+	+	+	+	+
h99-1-1-5-13-2										
h99-1-1-5-13-3										
h99-1-1-5-13-5										

Mar.	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21
h99-1-1-5-2-6	+	+	+	−	+	+	−	−	+	+	+
h99-1-1-5-2-7	+	+									
h99-1-1-5-2-10	−	+	−	−							
h99-1-1-5-2-11											
h99-1-1-5-2-21											
h99-1-1-5-2-31	+	+	+	+	+	+	+				
h99-1-1-5-2-33	+	+	+	+	+	+	−	+	+	−	+
h99-1-1-5-13-2											
h99-1-1-5-13-3											
h99-1-1-5-13-5											

Mar.	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–31	31–1
h99-1-1-5-2-6	+	+	+	−	+	+	+	+	×	−	×
h99-1-1-5-2-7											
h99-1-1-5-2-10											
h99-1-1-5-2-11											
h99-1-1-5-2-21											
h99-1-1-5-2-31											
h99-1-1-5-2-33	+	+	+	+	−	−	+	−	−	+	−
h99-1-1-5-13-2											
h99-1-1-5-13-3											
h99-1-1-5-13-5											

Apr.	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12
h99-1-1-5-2-6	×	−	−	−	+	+	+	−	+	+	+
h99-1-1-5-2-7											
h99-1-1-5-2-10											
h99-1-1-5-2-11											
h99-1-1-5-2-21											
h99-1-1-5-2-31											
h99-1-1-5-2-33	+	+	+	−	+	−	+	×	−	+	+
h99-1-1-5-13-2											
h99-1-1-5-13-3											
h99-1-1-5-13-5											

Insect No.	Apr. 12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23
h99-1-1-5-2-6	−	−	−	−	+	−	−	−	−	−	−
h99-1-1-5-2-7											
h99-1-1-5-2-10											
h99-1-1-5-2-11											
h99-1-1-5-2-21											
h99-1-1-5-2-31											
h99-1-1-5-2-33	+	−	−	+	+	−	−	−	−	−	−
h99-1-1-5-13-2											
h99-1-1-5-13-3											
h99-1-1-5-13-5											

Insect No.	Apr. 23–24	24–25	25–26	26–27	27–28	28–29	29–May 23
h99-1-1-5-2-6	−	+	−	−	−	+	+
h99-1-1-5-2-7							
h99-1-1-5-2-10							
h99-1-1-5-2-11							
h99-1-1-5-2-21							
h99-1-1-5-2-31							
h99-1-1-5-2-33	−	−	−				
h99-1-1-5-13-2							
h99-1-1-5-13-3							
h99-1-1-5-13-5							

A few infective leafhoppers were transferred 5 times a day at one hour intervals to successive new healthy rice plants. Each of them was confined on a healthy plant enclosed in a glass tube during the remaining part of the day. The results of this experiment are shown below.

Table 30. Results of transfer of single infective leafhoppers to new test plants at one hour intervals

	Mar. 6					Mar. 7					Mar. 8					Mar. 9				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
h99-1-1-5-2-7	−	−	−	−	−	+	+	+	−	−	+	+	+	−	−	+	−	−	−	−
h99-1-1-5-2-10	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	+	−	−
h99-1-1-5-2-31	−	+	+	−	−	−	+	+	+	−	+	+	+	−	−	+	+	+	+	+

	Mar. 10					Mar. 11					Mar. 12					Mar. 13				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
h99-1-1-5-2-7	+	+	+	−	−	+	+	+	−	+										
h99-1-1-5-2-10	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−
h99-1-1-5-2-31	−	+	+	+	+	+	+	+	−	−	−	+	−	+	+	+	−	+	−	−

	Mar. 14					Mar. 15				
	1	2	3	4	5	1	2	3	4	5
h99-1-1-5-2-7										
h99-1-1-5-2-10										
h99-1-1-5-2-31	+	+	+	−	−	−	+	+	+	+

	Jan. 26					Jan. 27					Jan. 28					Jan. 29				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
h99-1-1-5-13-2	−	+	+	+	+	−	+	+	−	−	+	+	+	+	+	+	+	+		
h99-1-1-5-13-5	−	−	×	×	−	−	−	−	+	−	−	+	−	−	−	+	+	−		

	Jan. 30					Jan. 31				
	1	2	3	4	5	1	2	3	4	5
h99-1-1-5-13-2	+	−	+	−		−	+			
h99-1-1-5-13-5	−	−	−	−	−	+	+			

As shown in Table 29 the leafhoppers of the 6th generation became infective 12–25 days after emergence from eggs and retained infectivity for 26–118 days, mostly 53–65 days producing infections in 21–84 healthy rice plants. Four infective females among them, h99-1-1-5-2-6, h99-1-1-5-2-11, h99-1-1-5-2-21 and h99-1-1-5-2-33, five to eleven days after emergence into the adult stage were paired with nonviruliferous males but only the first mentioned one laid a few normal and several abortive eggs while the others deposited no eggs. On March 16 only one nymph of the 7th generation immediately on hatching was lifted from an egg laid by h99-1-1-5-2-6 and confined on a healthy rice plant in a glass tube, which subsequently developed the signs of infection. Thus in this experiment the virus has been transmitted through the eggs of the leafhoppers to the progeny of the 7th generation. It is worthy of note that h99-1 and its progeny, or 27 leafhoppers in 5 generations, derived from one viruliferous egg have produced infections in more than one thousand rice plants when they were confined singly on a healthy plant for one day and transferred daily to successive new healthy plants.

It is evident that the virus has practically not been reduced in virulence as a result of its prolonged retention by insect carriers through several generations as shown in the following table.

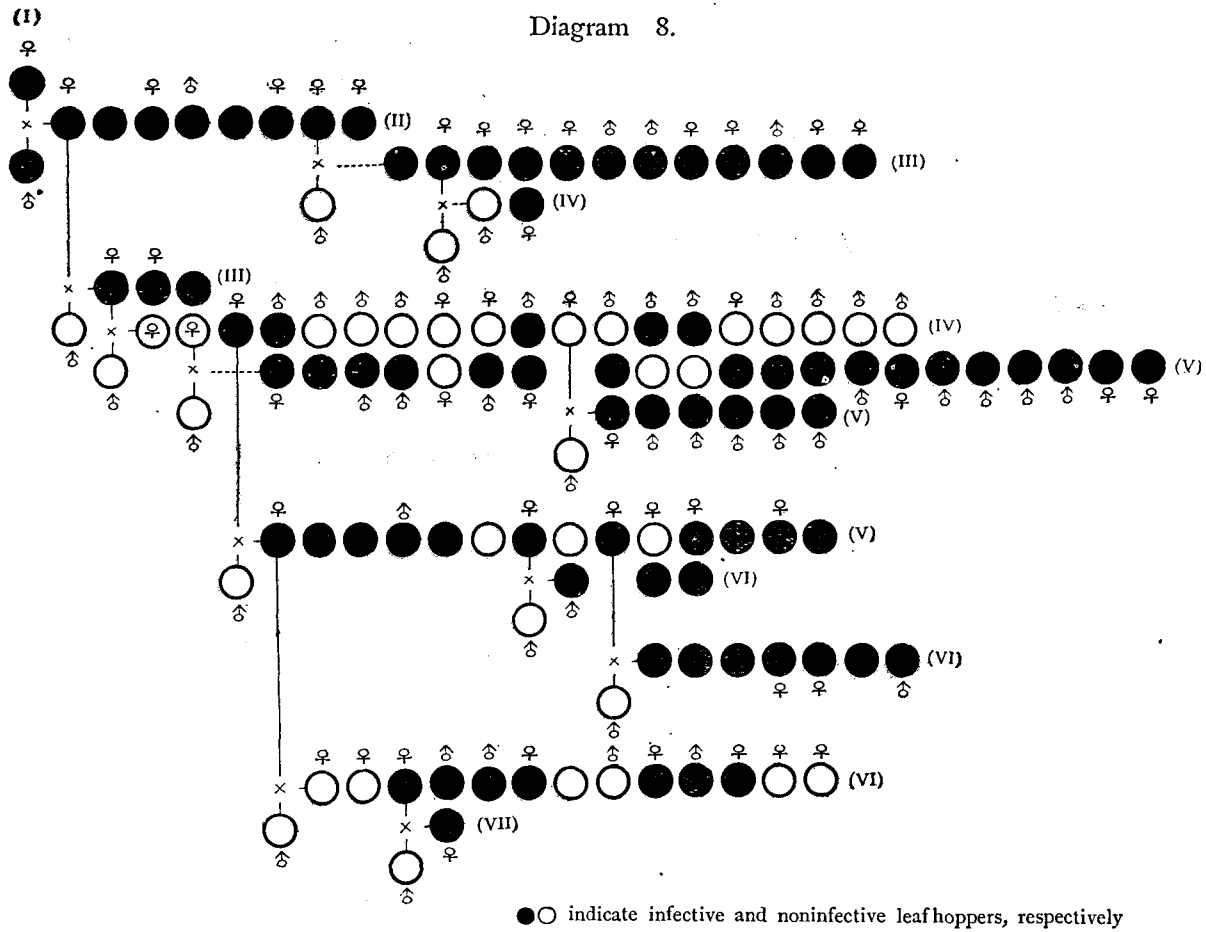
Table 31. Relative infectivity of leafhoppers in successive generations

Insect No.	Duration of life (in days)	Noninfective period after hatching	No. of infections produced	Percentage of infection
h99-1	96 (Apr.–July)	11 days	29	30%
h99-8	59 (Apr.–June)	12	31	52
h99-1-1	67 (June–Aug.)	26	32	48
h99-1-2	63 (")	15	39	62
h99-8-1	41 (June–July)	21	14	67
h99-8-2	46 (June-Aug.)	15	29	63
h99-8-4	44 (")	38	1	2
h99-8-6	43 (")	20	11	28
h99-8-7	68 (")	20	31	46
h99-8-9	77 (June–Sept.)	18	50	65
h99-8-10	67 (June–Aug.)	12	22	33
h99-8-11	28 (June–July)	14	11	39
h99-8-13	55 (June–Aug.)	14	24	44
h99-8-14	36 (")	24	10	28
h99-8-17	47 (July–Aug.)	15	23	49
h99-8-18	31 (")	16	10	32
h99-1-1-5	58 (Aug.–Oct.)	18	20	34
h99-1-1-6	126 (Aug.–Dec.)	25	42	33
h99-1-1-16	48 (Aug.–Sept.)	14	6	13
h99-1-1-24	57 (Aug.–Oct.)	9	11	19
h99-1-1-26	58 (")	13	24	41
h99-8-2-3	55 (Aug.–Sept.)	17	2	4
h99-1-1-5-2	118 (Sept.–Jan.)	30	65	55
h99-1-1-5-3	36 (Sept.–Oct.)	19	14	39
h99-1-1-5-5	37 (")	25	11	30
h99-1-1-5-7	47 (Sept.–Nov.)	17	21	44
h99-1-1-5-13	100 (Sept.–Jan.)	16	67	67
h99-1-1-5-20	121 (")	23	73	60

Insect No.	Duration of life (in days)	Noninfective period after hatching	No. of infections produced	Percentage of infection
h99-1-1-5-23	76 (Sept.-Dec.)	24 days	43	57%
h99-1-1-5-31	28 (Sept.-Oct.)	22	6	21
h99-1-1-5-34	34 (Sept.-Nov.)	17	6	18
h99-1-1-5-2-6	135 (Dec.-Apr.)	17	84	62
h99-1-1-5-2-7	86 (Dec.-Mar.)	21	52	60
h99-1-1-5-2-10	84 (")	17	52	62
h99-1-1-5-2-11	77 (")	19	23	30
h99-1-1-5-2-21	73 (")	13	55	75
h99-1-1-5-2-31	78 (")	15	46	59
h99-1-1-5-2-33	118 (Dec.-Apr.)	25	61	52
h99-1-1-5-13-2	49 (Dec.-Feb.)	14	30	61
h99-1-1-5-13-3	39 (Dec.-Jan.)	13	21	54
h99-1-1-5-13-5	68 (Dec.-Feb.)	12	35	51

The results of Experiment 5 are shown summarized in the following diagrams.

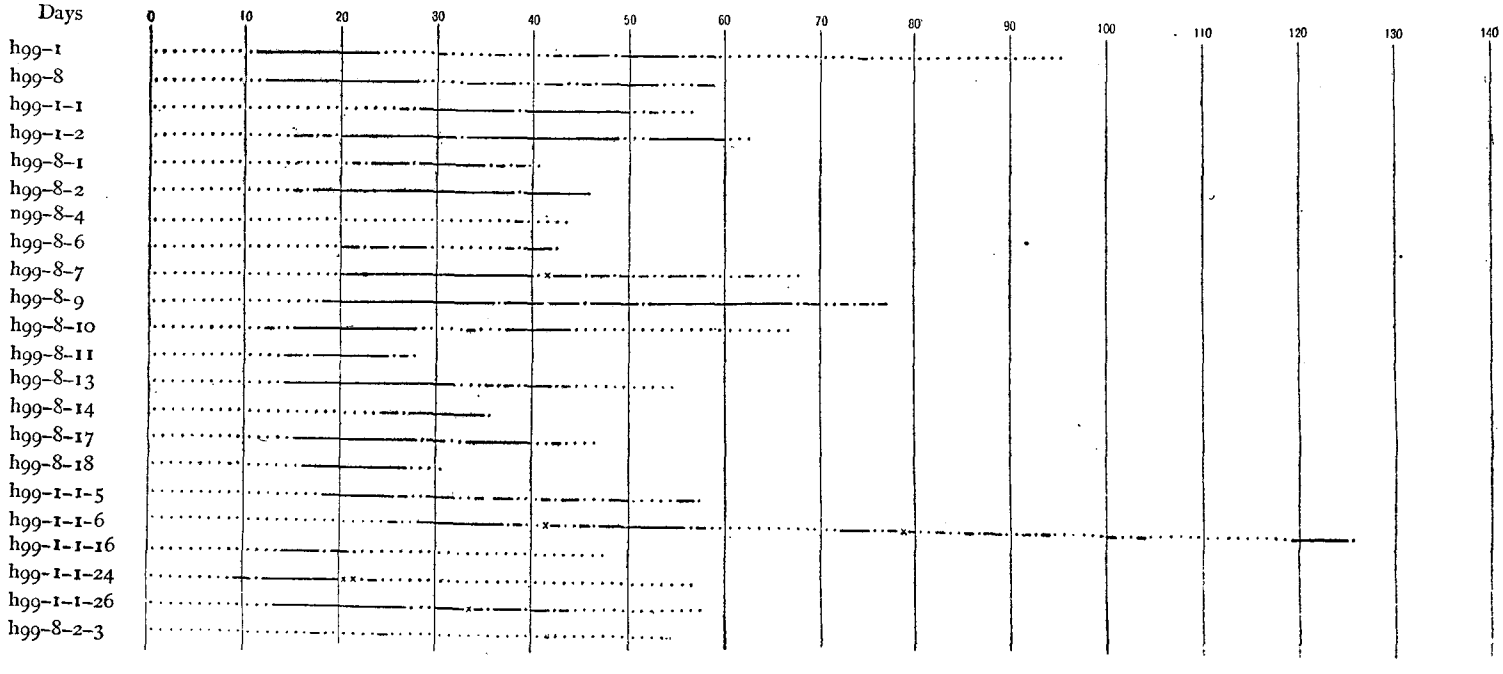
Diagram 8.

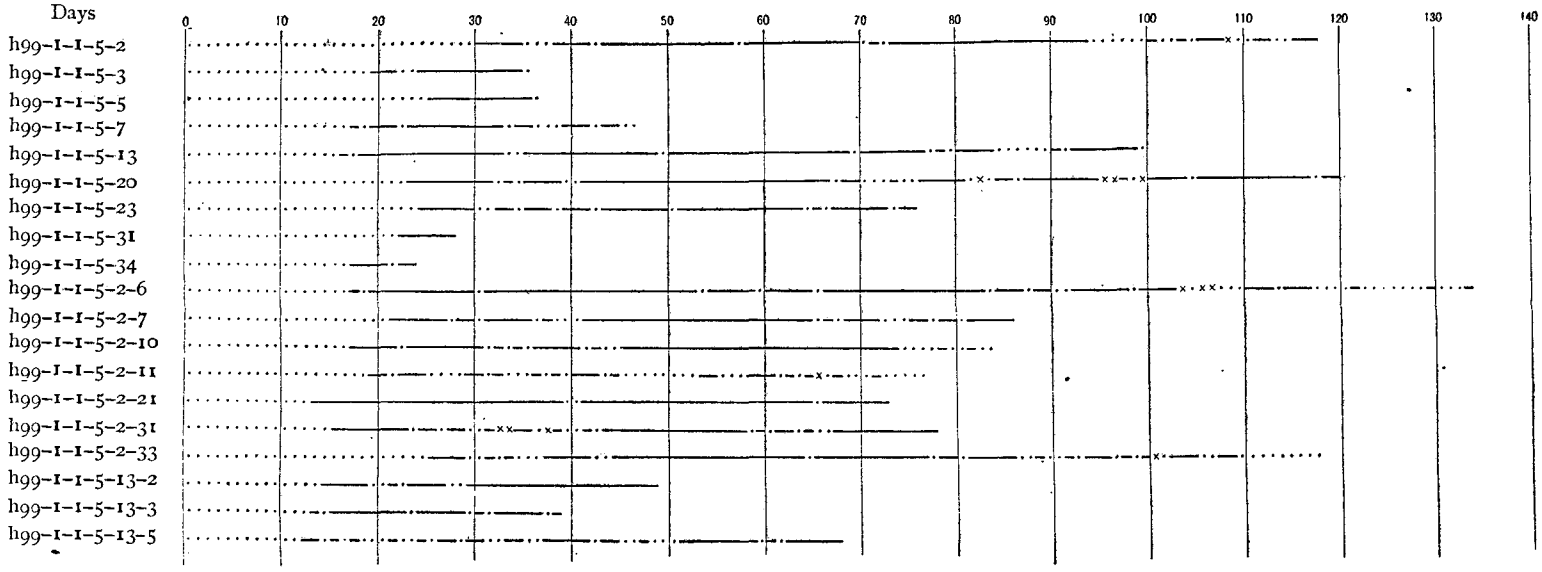


●○ indicate infective and noninfective leafhoppers, respectively

Diagram 9.

Results of daily transfers of infective progeny of h99 to test plants





..... noninfective period (each dot represents one day)

——— infective period

× × × × unknown because the test plants were killed

In the previous paper it was reported that the majority of the offspring from infective parents and some of those from the crosses between infective females and nonviruliferous males proved to be infective whereas the progeny from matings between nonviruliferous females and infective males were entirely free from virus. The experimental results presented in this paper appear to furnish additional evidence to support the former findings. The point becomes more evident from the following table.

Table 32. Infectivity of the progeny from crosses between infective and nonviruliferous leafhoppers

Date of mating	Parents	No. of progeny tested	No. of infective progeny	Percent. of infective progeny
Cross infective female × infective male (♀ × ♂)				
From the data previously reported		34	31	
July 7 '36	f199 × ♂	6	3	
Jan. 13 '37	g9 × ♂	6	4	
Apr. 7 '38	h99 × ♂	8	8	
		54	46	85
Cross infective female × nonviruliferous male (♀ × ♂)				
From the data previously reported		188	105	
Jan. 28 '34	f27 × ♂	10	9	
Aug. 20 '34	f27-2 × ♂	15	15	
Apr. 23 '36	f187 × ♂	4	2	
June 15 '36	f187-11 × ♂	21	14	
Aug. 24 '36	f196-6 × ♂	10	1	
Aug. 2 '37	h66 × ♂	5	3	
Oct. 4 '37	h66-5 × ♂	12	2	
Nov. 23 '37	h66-5-6 × ♂	11	10	
May 18 '38	h99-1 × ♂	3	3	
July 26 '38	h99-1-1 × ♂	19	5	
Sept. 6 '38	h99-1-1-5 × ♂	14	11	
Nov. 30 '38	h99-1-1-5-2 × ♂	13	7	
" "	h99-1-1-5-13 × ♂	3	3	

Date of mating	Parents	No. of progeny tested	No. of infective progeny	Percent. of infective progeny
May 28 '38	h99-8 × ♂	12	12	
July 26 '38	h99-8-2 × ♂	2	1	
Feb. 19 '39	h99-1-1-5-2-6 × ♂	1	1	
		350	211	60
Cross nonviruliferous female × infective male (♀ × ♂)				
From the data previously reported		144	0	
Jan. 25 '38	♀ × h66-5-6-1	35	0	
		179	0	0

As shown above 50 to 100, or an average of 85 per cent of the progeny from infective parents and 0 to 100, or an average of 60 per cent of those derived from crosses between infective females and nonviruliferous males proved to be infective while matings between nonviruliferous females and infective males produced offspring which were entirely free from virus. Inasmuch as the virus is transmitted only through the female line it is rather difficult to explain the greater infective capacity of the progeny from infective parents as compared with that of progeny from matings between infective females and nonviruliferous males. In explanation of this it has been assumed that there exists some kind of affinity between the virus and certain individuals of the leafhopper, which is a hereditary character in the latter represented by a dominat factor or factors and that the virus is incapable of multiplying in the leafhopper lacking that affinity. On this assumption it is probable that the progeny of infective parents should have more affinity with the virus than progeny from crosses between infective females and nonviruliferous males. In either case the virus is probably transmitted to progeny in nearly equal numbers but it is incapable of multiplying in the leafhopper lacking affinity, which should be naturally fewer in number in the progeny from parents both of whom are infective.

2) Affinity of the leafhopper with the virus

As already reported only a small proportion of the progeny of nonviruliferous leafhoppers became infective when reared on dwarf-diseased rice plants. March 30, 1928, fifty virus-free leafhoppers were introduced

into 2 insect-proof cages enclosing dwarf diseased rice plants in the green house. Their progeny reared on affected rice plants were tested individually for their infectivity after they had emerged into adults. Eleven of 87 leafhoppers tested or 13 per cent acquired and transmitted the virus. This experimnnt was carried out in Tottori College of Agriculture in Tottori as stated before. Subsequent to 1934 a series of similar experiments were performed in the Faculty of Agriculture, Hokkaido Imperial University in Sapporo. The results of experiments are summarized in the following table.

Table 33. Infectivity of the progeny of nonviruliferous leafhoppers reared on dwarf-diseased rice plants

Duration of experiment	Origin of leafhopper	No. of offspring tested	No. of infective offspring
May-July '34	*Supplied by Prof. WATANABE in Utsunomiya	46	3
Apr.-Oct. '35	*Progeny of the above	49	16
July-Oct. '35	*Supplied anew by Prof. WATANABE	28	0
Apr.-July '37	*Collected by the writer in Utsunomiya	36	1
		159	20 (or 13%)

*All the insects were tested individually for their infectivity and nonviruliferous ones only were used for experiment.

In parallel to these experiments the progeny from crosses, infective female × nonviruliferous male and infective female × infective male, which had been reared separately on dwarf-diseased rice plants were tested individually for their infective capacity.

June 28, 1934 the offspring from a cross between an infective female and a nonviruliferous male were introduced into an insect-proof cage enclosing dwarf diseased rice plants. As they and their progeny completed the nymphal stages males only, as a rule, were tested individually for their infectivity, excluding females in order to avoid egg deposition in the test plants. The results of this experiment are presented in the following table.

Table 34. Infectivity of the progeny from infective female × nonviruliferous male, reared on dwarf diseased rice plants

Duration of experiment	Generation	No. of insects tested	No. of infective insects
Aug.-Oct. '34	2nd	50	47
Jan.-Mar. '35	3rd	81	33
Apr.-May '35	4th	27	25
July-Sept. '35	5th	19	15
Apr.-May '36	7th	40	28
		217	148 (68%)

Feb. 25, 1936 an infective female leafhopper which had been paired with an infective male was placed in an insect-proof cage enclosing dwarf diseased rice plants and its offspring were continuously cultured on affected rice plants. As they emerged into the adult stage males only were tested individually for their infectivity. The experimental results are shown in the following table.

Table 35. Infectivity of the progeny of infective parents, reared on dwarf-diseased rice plants

Duration of experiment	Generation	No. of insects tested	No. of infective insects
Apr.-June '36	2nd	12	12
June-Aug. '36	3rd	56	53
Sept.-Jan. '36	4th	21	17
		89	82 (92%)

As shown above when the progeny (1) of nonviruliferous parents, (2) of a cross between infective female and nonviruliferous male, and (3) of infective parents had been reared on dwarf diseased rice plants, 13, 68, and 92 per cent of the leafhoppers, respectively acquired and transmitted the virus. In the latter 2 cases it is probable that the virus was transmitted through eggs to the progeny since infective females paired with nonviruliferous or infective males would produce an average of 60 or 85 infective leafhoppers, respectively in their progeny as already stated. To explain

the fact that an infective female paired with an infective male would produce more infective offspring than one mated with a nonviruliferous male it seems necessary to postulate that affinity of the leafhopper to the virus is an inherent character. In order to demonstrate this point more definitely the following experiments have been conducted.

June 17, 1937, two nonviruliferous female leafhoppers were paired with infective males and their progeny were bred on dwarf-diseased rice plants. After they had completed their nymphal stages males only were tested individually for their infective capacity. A similar experiment was also started on Jan. 25, 1938. The results of the experiments are presented in the following table.

Table 36. Infectivity of the progeny from nonviruliferous females × infective males, reared on dwarf-diseased rice plants

Duration of experiment	No. of insects tested	No. of infective insects
Aug.-Sept. '37	12	8
Mar.-May '38	27	11
	39	19 (49%)

The leafhoppers bred from a nonviruliferous female paired with an infective male must be free from virus as already stated but they have, as shown in these experiments, a greater ability to acquire and transmit the virus as compared with those derived from nonviruliferous parents. These findings present additional evidence to support the hypothesis that the affinity of the leafhopper with the virus is a hereditary character.

3) Length of the incubation period of virus within the insect body

Although only a small proportion of nonviruliferous leafhoppers acquired and transmitted the virus after they had been fed on dwarf-diseased rice plants, the previously virus-free leafhoppers which had been bred from nonviruliferous females paired with infective males became infective more frequently. An investigation therefore was undertaken to determine the length of incubation period of the virus within the bodies of these insects.

Jan. 25, 1938, a nonviruliferous female leafhopper after pairing with an infective male was introduced into an insect-proof cage enclosing healthy rice plants and its progeny were reared on healthy plants. The nymphs

in the 4th instar were fed for periods of 1, 3 and 7 days on a dwarf-diseased rice plant and were then tested individually for their infective capacity. The results are shown in the following table.

Table 37. "Infection" of leafhoppers with the virus by feeding for different periods on diseased rice plants

Date	Duration of feeding on diseased plants	No. of insects tested	No. of infective insects
Mar. 23	1 day	14	0
25	3 days	11	1
29	7 days	7	2

As is evident from Table 37, although the experiment was on a rather small scale, leafhoppers fed on affected plants for more than 3 days may be able to acquire and transmit the virus. Accordingly nymphs in the 4th instar and recently molted leafhoppers were fed on dwarf-diseased rice plants for 3 or 5 days and then transferred singly to successive healthy rice plants at intervals of 10 days during their subsequent life.

The first experiment was started on Sept. 10, 1938 with 33 leafhoppers which were allowed to feed for 3 days on diseased rice plants. Excluding 14 individuals which died within 30 days, 3 of 19 leafhoppers produced infections in healthy plants. The second experiment was begun Sept. 14 with 18 leafhoppers which were also allowed to feed for 3 days on affected plants. Out of 13 surviving for a sufficiently long period 3 became infective. On Oct. 8 the third experiment was started with 24 leafhoppers fed for 5 days on diseased rice plants. Four of them died prematurely and 11 of the remainder proved to be infective. The fourth experiment was started on Mar. 11, 1939. Twelve and forty-eight leafhoppers were allowed to feed for 3 and 5 days, respectively on affected rice plants. Out of 11 of the former leafhoppers and 40 of the latter which survived more than 30 days one each insect became infective.

Summarizing these experiments, 7 of 40 leafhoppers fed for 3 days on diseased rice plants and 12 out of 60 insects fed for 5 days acquired and transmitted the virus as shown in the following table.

Table 38. "Infection" of leafhoppers with the virus by feeding for 3 or 5 days on diseased plants

Date	Duration of feeding on diseased plants	No. of insects tested	No. of insects surviving for more than 30 days	No. of infective insects
Sept. 10 '38	3 days	33	19	3
Sept. 14 '38	3	18	13	3
Oct. 8 '38	5	24	20	11
Mar. 11 '36	3	12	11	1
" "	5	48	40	1

The length of the incubation period of virus within the bodies of these leafhoppers is shown in the following table.

Table 39. Length of incubation period of virus in the insect

No. of insect	Duration of feeding on diseased plants	Intervals of feeding on healthy plants							Temperature
		IX 13–23	23–X 3	3–13	13–23	23–XI 2	2–12	12–22	13–30°C
No. 5 (♀)	3 days	−	−	−	−	−	+		
No. 8 (♀)	"	−	−	−	−	−	−	+	
No. 10 (♀)	"	−	−	+	×	+	−		
		IX 17–27	27–X 7	7–17	17–27	27–XI 6	6–16		13–30°C
No. 42 (♀)	3 days	−	−	−	+	×	+		
No. 46 (♀)	"	−	×	−	+	+	−		
No. 50 (♂)	"	−	×	×	+	+	+		
		X 13–23	23–XI 2	2–12	12–22	22–XII 2	2–12	12–22	15–30°C
No. 54 (♂)	5 days	−	−	−	+	+	+	−	
No. 60 (♀)	"	−	−	×	+	+			
No. 61 (♂)	"	−	−	−	−	−	+		
No. 62 (♂)	"	−	−	−	−	+	+	+	
No. 65 (♀)	"	−	−	−	+	+			
No. 66 (♂)	"	−	−	+	+				
No. 67 (♀)	"	−	−	−	+	+	+		
No. 68 (♂)	"	−	−	−	+	+			

No. of insect	Duration of feeding on diseased plants	Intervals of feeding on healthy plants							Temperature
		X 13–23	23–XI 2	2–12	12–22	22–XI 2	2–12	12–22	15–30°C
No. 70 (♀)	5 days	−	+	×	+	+	+	−	
No. 73 (♂)	"	−	+	+	+	+	+	+	
No. 75 (n)	"	−	−	−	+				
		III 14–24	24–IV 3	3–13	13–23	23–V 3			15–35°C
No. 79 (♂)	3 days	−	−	−	+	+			
		III 16–26	26–IV 5	5–15	15–25				15–35°C
No. 106(♂)	5 days	−	+	+	+				

The sign (+) indicates infection, (−) no infection and (×) test plant died

As shown in Table 39, no effect whatever was produced in the plants upon which the insects passed the first 10 to 60 days after feeding on diseased plants. In other words the insects were not immediately infective after feeding on affected plants but a period of 10–73 days had to elapse before they could transmit the virus; that is to say, the incubation period of the virus within the insect body was 10–25 days at the minimum, 60–73 days at the maximum and mostly 30 to 45 days.

4) Discussion and conclusions

The question as to whether or not a virus multiplies within the insect carrier is considered to have some significance in connection with the nature of virus. It has been assumed that several plant viruses such as those causing curly top of beet (CARSNER and STAHL 1924), aster yellows (KUNKEL 1926), maize streak (STOREY 1928) and rice dwarf disease (FUKUSHI 1934) would multiply in the insect vectors. The assumption is supported principally by the evidence that these viruses seem to require the so-called incubation period in the insect and that some virulifeous insects remain infective for long periods of time without having renewed access to a source of the virus.

As to the relation of curly top of beet to its insect vector, *Eutettix tenellus*, CARSNER (1919) stated that infective leafhoppers after being kept on *Atriplex polycarpa* and *Rumex crispus* both of which are apparently

immune to curly top, retained their infective capacity for a period of 58 and 111 days, respectively. STAHL and CARSNER (1923) reported that when a leafhopper was once able to transmit curly top this ability was apparently never lost. SEVERIN (1924) also found that the leafhoppers which acquired the virus retained their infectivity during all of the nymphal stages and during the entire adult life even when each insect was provided with a healthy beet daily. KUNKEL (1926) working with aster yellows, showed that certain individuals of *Cicadula sexnotata* carried the virus for more than 100 days and that insects confined on rye plants which are immune to yellows have been shown to retain it for at least 2 months. STOREY (1928) stated that *Cicadulina mbila* retained the power to transmit the virus of maize streak through a life as long as 111 or 116 days, when single leafhoppers were transferred daily to new healthy plants. In dwarf disease of rice plant the writer (1933, 34) found that the virus might be transmitted through the egg of its insect carrier, *Nephotettix apicalis cincticeps* and that some insects emerging from the viruliferous eggs retained infectivity during their entire life or for a period of 53 to 97 days without any access to a source of virus. In some cases a period up to 18 days had to elapse before the nymphs emerging from viruliferous eggs became capable of producing infections in healthy plants. It is not unreasonable that these findings led some investigators to hold the view that these viruses might multiply in insect carriers. Recently, however, FREITAG (1936) and BENNETT and WALLACE (1938) suggested that the curly top virus does not multiply in the beet leafhopper. According to FREITAG there was a gradual decrease in the percentage of beets infected by the leafhopper when the insects were transferred daily to a healthy beet and many of the infective leafhoppers apparently lost the capacity to produce infection during late adult life. Infective insects confined on curly-top immune sweet corn showed a gradual loss in their infective capacity. He found that the length of time leafhoppers remained infective depended upon the length of time they had fed upon the source of the virus. Leafhoppers which had fed for short periods on curly-top beets probably accumulated less virus and consequently produced fewer infections than those which fed for long periods. Hence he regards that the leafhoppers are merely internal mechanical carriers. BENNETT and WALLACE also showed that when infective leafhoppers were transferred at frequent intervals to beets or were confined to plants extremely resistant or immune to curly top their virus content decreased with time.

The data presented in this paper give evidence to support the assump-

tion that a multiplication of the virus of rice dwarf disease occurs in the leafhopper. Two cases each have been secured of transmission of the virus through eggs of the leafhopper to the progeny of the 3rd and 4th generations as well as one case to the 7th generation. In experiment No. 1, described above, the virus was retained for at least 147 days within the insect bodies through 2 generations, from f27–2 to f27–2–14. In experiment No. 3 it was likewise retained for 176 days through 3 generations from h66–5 to h66–5–6–11. The most striking case of prolonged retention of the virus by leafhoppers was obtained in experiment No. 5. In this case the virus has been transmitted through the egg to the progeny of the 7th generation and retained within insect bodies for at least 374 days through 5 generations from h99–1 of the 2nd generation to h99–1–1–5–2–6 of the 6th generation. Furthermore 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 healthy rice plants on which they had been confined singly for one day. In this experiment infections were produced in more than one thousand rice plants by 27 leafhoppers of 5 generations derived from one viruliferous egg, when they were confined singly on a rice plant for one day and transferred daily to successive new healthy plants. Since the amount of virus originally contained in one egg must be extremely small it seems necessary to assume the multiplication of the virus in the leafhopper in order to explain these experimental results.

There was a delay of 7 to 38 days or an average of 19 days in the development of the infective capacity of the nymphs emerging from viruliferous eggs. This cannot be attributed to the fact that the amount of virus inoculated by a nymph does not attain to the minimum dose to cause infection in a healthy rice plant, since several nymphs became infective a few days after emergence from the egg, as already shown in a previous paper, whereas some nymphs remained noninfective during all the nymphal stages and even after they had emerged into adults. For example the leafhopper f187–11 remained noninfective for 34 days after emergence from the egg or 4 days after it had completed the last molting, as shown in Table 6. For the leafhopper, h99–1–1–6 in Table 25, the noninfective period after hatching lasted for 25 days or up to 7 days after emergence into the adult stage. When such long noninfective periods had elapsed some leafhoppers retained their infective capacity for considerable periods of time. As shown in Table 27, the leafhopper, h99–1–1–5–2 remained noninfective

for 30 days after hatching and thereafter produced infections in 65 healthy rice plants on each of which it had been confined for one day retaining the infectivity for 88 days. A good many similar examples are found in Tables 4, 6, 11, 23, 25, 27, 29 and 31. A period of 18 to 34 days is considered too long to represent the time required for the virus to migrate through body fluids or tissues to reach a point where it may be injected into the plant by the feeding of the leafhoppers. It is more likely that such a long latent period after hatching may represent the time required for the virus to multiply within the insect.

As previously stated considerable variation was observed in the infectivity of different leafhoppers. Some of them infected plants consistently on consecutive days while others only at great intervals as shown in Diagram 9. A striking example was found in the leafhopper, h99-1-1-6 which after having acquired the infectivity often failed to infect rice plants. It had 2 specially long noninfective periods which lasted for 14 and 15 days, respectively. It is highly probable that this is due to the temporary exhaustion of virus in the salivary glands or the anterior portion of the alimentary canal and that the infections subsequently produced by this leafhopper are attributable to the virus which multiplied within the insect body. It cannot be denied that the infective capacity of some leafhoppers was apparently reduced or lost especially during late adult life. Other insects, however, were shown to retain the infectivity as long as they lived.

It is worthy of note that there were, in the progeny of infective females, a few viruliferous female leafhoppers which proved to be noninfective during their entire life but produced infective progeny as shown in experiments Nos. 3 and 5. On this point some explanations seem to be necessary. When a low temperature prevailed in the green house, a much prolonged incubation period was usually required for the virus in the rice plant. On the other hand most female leafhoppers would show a tendency not to mate readily with males as they approached old age. Accordingly in experiment No. 3, certain females of the 3rd generation which had been reared during the autumn were allowed to mate with nonviruliferous males without waiting for their betraying infectivity. At that time the temperatures in the green house were low as the heating system was not yet working. These leafhoppers eventually proved to be noninfective but some individuals of their offspring produced infections in test plants. This admits the following interpretation. Under low temperature conditions which retard the manifestation of the disease in the rice plant, the multiplication of virus may be notably inhibited not only in the rice plant but also within the

bodies of the insect carriers. 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 merely in ovarian tubules to give rise to viruliferous eggs. Admittedly the latter individuals will prove to be non-infective but may produce infective progeny. Such cases were encountered in experiment No. 5, where 2 females of the 4th generation produced infective progeny notwithstanding that they remained noninfective during their entire life. These female leafhoppers were reared during a period from early August to early September when the temperatures in the green house varied from 15° to 38°C. and mostly 20° to 35°C. In this case it is hardly possible that low temperatures retarded the multiplication of virus in the leafhopper. It is conceivable, however, that some other factor or factors might have hindered the multiplication of the virus in the leafhopper since infective individuals were particularly few in the 4th generation as is shown in Diagram 8.

Considerable variation has been observed by several workers in the infective capacity of different individuals of insect vectors of curly top of beet (CARSNER and STAHL 1924), aster yellows (KUNKEL 1926), maize streak (STOREY 1928) and rice dwarf disease (FUKUSHI 1934). However, STOREY (1932) was the first to demonstrate definitely that strains of unequal ability to transmit a virus might exist within a single species. According to him there are 2 races in the leafhopper, *Cicadulina mbila*, which are on the one hand able, and on the other unable to transmit the virus of maize streak. By the crossing of the pure races he has demonstrated that the ability to transmit this virus is inherited as a simple dominant Mendelian factor, linked with sex. BENNETT and WALLACE (1938) reported that it was possible to produce strains of beet leafhopper lower or higher than normal in ability to transmit the curly-top virus, as a result of selecting and mating leafhoppers in successive generations. In rice dwarf disease the leafhoppers bred from nonviruliferous females which had been paired with infective males were free from virus but they had a greater ability to acquire and transmit the virus as compared with those derived from nonviruliferous parents. In explanation of this it has been assumed that there exists some kind of affinity between the virus and certain individuals of the leafhopper, which is a hereditary character in the latter represented by a dominant factor or factors and that the virus is incapable of multiplying in the leafhopper

lacking affinity. On this assumption it is probable that the progeny from crosses between nonviruliferous females and infective males should have more affinity with the virus than those derived from nonviruliferous parents, because only a small proportion of ordinary nonviruliferous leafhoppers become infective when allowed to feed on affected plants indicating that most of them have no affinity with the virus.

As already stated the virus may be present in some eggs laid by infective females which have been paired with either infective or nonviruliferous males whereas the eggs derived from nonviruliferous females mated with infective males are entirely free from virus. Both the male and female germ cells have approximately equal amount of nuclear material but only the egg has any significant amount of cytoplasm and consequently for an explanation of maternal transmission of virus it must be assumed that the virus is present in the cytoplasm of the egg. In the present study an average of 85 percent of the progeny from infective parents and an average of 60 percent of those derived from crosses between infective females and nonviruliferous males proved to be infective while matings between nonviruliferous females and infective males produced offspring which were entirely free from virus. Inasmuch as the virus is transmitted through only the female line it would be difficult to explain the greater infective capacity of the progeny from infective parents as compared with that of progeny from matings between infective females and nonviruliferous males but for the hypothesis that the affinity of the leafhopper with the virus is a hereditary character. On this assumption the former progeny should have more affinity with the virus than the latter. In either case the virus is probably transmitted to the progeny in nearly equal numbers but it is incapable of multiplying in the individuals lacking affinity, which should be naturally fewer in number among the progeny from parents both of whom are infective.

When the progeny from nonviruliferous parents, from crosses between infective females and nonviruliferous males and from parents both being infective, had been reared on dwarf-diseased rice plants, 12, 68 and 92 percent of the leafhoppers, respectively, acquired and transmitted the virus. In this case the fact that an infective female paired with an infective male would produce more infective offspring than that mated with a nonviruliferous male can be also readily explained on the above assumption.

SMITH and BONCQUET (1915) were the first to report the incubation period of a plant virus in an insect carrier. They found that the beet leafhopper, *Eutettix tenellus* BAKER was not immediately infective after the act of feeding on affected beet but that a period of at least 24 hours, but

not much more, had to elapse before it could produce infection in healthy plants. SEVERIN (1921) found that at high temperatures the minimum incubation period of this virus in the beet leafhopper might be as short as 4 hours. KUNKEL (1926) working with the aster yellows in New York demonstrated that the leafhopper, *Cicadula sexnotata* FALL. was unable to transmit the virus immediately after feeding on affected plants and that a period varying from 10 to 19 days had to elapse before it became infective. He later (1932) showed that this period varied from 17 to 26 days with the virus of the Californian aster yellows. STOREY (1928) reported that the incubation period of maize streak virus in the leafhopper, *Cicadulina mbila* NAUDE was from 6 to 63 hours at 30°C. and 60 to 84 hours at 25°C. An incubation period of 10 to 26 days with an average of 16 days has been shown by HARTZELL (1936) for peach yellows virus in the leafhopper, *Macropsis trimaculata* FITCH. BALD and SAMUEL (1931) showed that in the transmission of spotted wilt of tomatoes there was a delay of 5 to 7 days in the development of the infective capacity of the thrips, *Frankliniella insularis* FRANKLIN. LINFORD (1931, 32), working with yellow spot of pineapple caused by the same virus found that it required an incubation period of approximately 10 days within the thrips, *Thrips tabaci* LINDEMAN. ELZE (1927) and SMITH (1931) have shown that the potato leafroll virus undergoes an incubation period of 24 to 48 hours in the aphid, *Myzus percicae* SULZ. OSBORN (1935, 38) found that the incubation period of pea virus 1 varied 12 to 28 hours in the aphid, *Macrosiphum pisi* KALT. and 12 to 18 hours in *Macrosiphum solanifollii* ASHM. The virus of rice dwarf disease requires an incubation period varying from 10–25 to 60–73 days, and mostly 30–45 days in the leafhopper. This is comparable with the incubation periods of the viruses of peach yellows and aster yellows. It is difficult to conceive that such a long incubation period represents the time for the virus to pass into the mouth parts, alimentary canal, blood, salivary glands and out of the mouth parts in sufficient quantity to produce infection as defined by SEVERIN (1931). Presumably there may be involved a multiplication of virus in the leafhopper.

Briefly stated all the evidence available at present seems to indicate, as stated in the previous paper, that the virus of rice dwarf disease multiplies within the body of the leafhopper and that it is not merely a chemical substance but an ultramicroscopic entity of living nature.

5) Summary

In a continuation of the studies on the relation of the leafhopper, *Nephotettix apicalis cincticeps* UHL. to the virus of dwarf disease of the rice plant, an investigation was undertaken to determine how and to what extent the virus would be passed on to the offspring of leafhopper.

During the past 5 years 2 cases each have been secured of transmission of the virus through eggs of the leafhopper to the progeny of the 3rd and 4th generations as well as one case to that of the 7th generation. In the last case the virus was retained for at least 374 days within the insect bodies through 5 generations. In this case infections have been produced in more than one thousand healthy rice plants by 27 leafhoppers of 5 generations derived from one viruliferous egg, when they were confined singly on a rice plant for one day each and transferred daily to successive new healthy plants. The virus has practically not been reduced in virulence as a result of its retention by insect carriers through several generations. Since the amount of virus originally contained in one egg must be extremely small it seems necessary to assume the multiplication of the virus in the leafhopper for an explanation of these experimental results.

There was a delay of 7 to 38 days with an average of 19 days in the development of the infective capacity of the nymphs emerging from viruliferous eggs. When such noninfective periods had elapsed most leafhoppers retained their infective capacity for considerably long periods of time up to 88 days. It is very likely that such a long noninfective period after hatching from a viruliferous egg may represent the time required for the virus to multiply in the insect.

Considerable variation was observed in the infectivity of different leafhoppers. Some of them infected plants consistently on consecutive days while others did so only at great intervals. In some leafhoppers such noninfective periods lasted for 14 or 15 days. The infective capacity of some leafhoppers was apparently reduced or lost especially in late adult life but other leafhoppers were shown to retain the infectivity as long as they lived.

In the progeny of infective females there were a few viruliferous female leafhoppers which proved to be noninfective during their entire life but produced infective progeny. In such cases, it is assumed, the virus might have been inhibited from multiplication and it localized by chance in ovarian tubules to give rise to viruliferous eggs.

The leafhoppers bred from nonviruliferous females paired with infective

males were free from virus but they had a greater ability to acquire and transmit the virus as compared with those derived from nonviruliferous parents. In explanation of this it has been postulated that there exists some kind of affinity between the virus and certain individuals of the leafhopper, which is a hereditary character in the latter represented by a dominant factor or factors and also that the virus is incapable of multiplying in the leafhopper lacking such affinity.

An average of 85 percent of the offspring of infective parents proved to be infective while an average of 60 percent of those derived from crosses between infective females and nonviruliferous males became infective. For an explanation of the greater infective capacity of the former leafhoppers than the latter the above mentioned assumption is helpful.

When the progeny from nonviruliferous parents, from crosses between infective females and nonviruliferous males, and from parents both of whom were infective had been reared on dwarf-diseased rice plants, 12, 68 and 92 percent, respectively of the leafhoppers acquired and transmitted the virus. In this case the fact that an infective female paired with an infective male would produce more infective offspring than that mated with a nonviruliferous male can be also readily explained on the above assumption.

The virus under consideration requires an incubation period in the leafhopper varying from 10–25 to 60–73 days, mostly 30–45 days. It is difficult to conceive that such a long incubation period represents the time necessary for the virus to migrate through body fluids and tissues of the insect to reach a point where it may be injected into the plant by the feeding of the leafhoppers. Presumably there may be involved a multiplication of virus in the leafhopper.

Briefly stated the data presented in this paper are considered to confirm the writer's former findings indicating that the virus of rice dwarf disease multiplies in the insect carrier.

Postscript

After the manuscript of the present paper had been prepared, I had an opportunity to read through BAWDEN's recent work, "Plant viruses and virus diseases (1939)." In his opinion (v. pp. 74–75), there is no justification for my view that the virus of rice dwarf disease multiplies in its insect carrier. His discussions seem chiefly to rely upon FREITAG's and also BENNETT and WALLACE's studies on the beet curly top which suggest that there is no multiplication of virus in the beet leafhopper.

If the leafhopper, *Nephotettix apicalis cincticeps* merely transmits the virus of rice dwarf disease, as BAWDEN supposes, which it has accumulated feeding upon the source of virus, its virus content or infective capacity should gradually decrease as the virus is passed on to its progeny from generation to generation. However, this is difficult to reconcile with my experimental results as shown in the present paper. As is evident from the following table reprinted from my previous paper, the percentage of infective individuals in the progeny of infective leafhoppers does not necessarily decrease in successive generations.

Transmission of the virus of rice dwarf disease through the eggs of leafhoppers in successive generations

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

In Experiment 4, as shown above, there was a decrease in the percentage of infective leafhoppers in the 3rd generation but a striking increase was shown in the 4th generation. Again in Experiment 5 there was a reduction in the percentage of infective individuals in the 4th generation and a

notable increase in the next generation. Such was also the case for the infective capacity of the individual leafhoppers as measured by the numbers of infections produced in healthy plants on which they were confined singly for one day as shown in diagrams 7 and 9 as well as in table 31 in this paper. I regard this as evidence that this virus multiplies in the leafhopper.

BAWDEN writes, "However, as he also finds that infective leafhoppers often not only fail to transmit to their progeny but themselves cease to be infective, even in favourable conditions, it seems improbable that the virus does multiply." But if we assume that in some leafhoppers with a low virus-content the virus localizes in or migrates to the salivary glands to be injected into the plant by the feeding of the leafhoppers as soon as it multiplies but does not enter the ovarian tubules, the fact that certain infective leafhoppers fail to transmit the virus to their progeny does not contradict the assumption that the virus multiplies in the leafhopper. It is probable that the virus multiplies less readily in some individuals than in others and that the virus content in the former will gradually decrease and ultimately will be exhausted as a result of daily transmission to successive healthy plants.

"Unfortunately", he states, "no attempt has been made to correlate the length of time *N. apicalis* remains infective, or its ability to transmit the virus to progeny, with the length of time it has fed on the diseased plants, but the feeding times mentioned are days or weeks. It may be that those fed for long periods, so acquiring a high charge of virus, are those that transmit for their whole lives and transmit to their progeny. Similarly, those that feed for short periods may be those that lose their infectivity." In curly top of the beet, the beet-leafhopper is able to pick up the virus from diseased plants in a feeding time of 1 minute and to transmit it to a healthy plant in 4 hours. In rice dwarf disease, however, the leafhopper should be fed on affected plants for more than 3 days to acquire the virus and a long period varying from 10–25 to 60–73 days, mostly 30–45 days has to elapse before it can produce infection in healthy plants. Furthermore, all the leafhoppers which fed on affected plants do not acquire the infective capacity. In this case, therefore, the experiments on this line cannot be performed so readily as in curly top of the beet. However it may be, is it inconceivable that the virus-content of one egg of the 6th generation in Experiment 5, for example, is by no means much greater than the amount of virus which a leafhopper picks up feeding on the diseased plant for a short period? Yet the leafhopper emerging from such an egg, h99-1-1-5-2-6, remained infective for its whole life and

transmitted the virus to its progeny.

"Furthermore, if the virus does multiply in the insect there seems no reason why transmission should be limited to the third generation." As shown in this paper I could transmit the virus through eggs of the leafhopper to the progeny of the 7th generation. It does not mean that transmission is limited to the 7th generation. If I could have extended the experiment further, it might have been possible to transmit the virus to still more successive generations.

"Indeed, an insect born infective would be expected to have a greater chance of producing infective progeny than one which only becomes infective in later life by feeding on infected plants." Hereupon BAWDEN refers to seed-transmission of the bean mosaic stating, "This effect is clearly shown in bean plants suffering from common mosaic. Here the virus is seed transmitted and does multiply in the bean plant, and the amount of seed transmission is greater in plants raised from infected seeds than in those that become infected during the growing period." In bean mosaic the virus does multiply in the bean plant and if there is no multiplication of the virus of rice dwarf disease in the leafhopper, as BAWDEN supposes, then there can be no analogy in these cases. The fact that "the amount of seed transmission is greater in bean plants raised from infected seeds than in those that become infected during the growing period" is explained on the ground of a multiplication of the virus in bean plants after all. As far as BAWDEN denies the multiplication of virus of rice dwarf disease in the leafhopper, it seems irrational to consider that "an insect born infective would be expected to have a greater chance of producing infective progeny than one which only becomes infective in later life by feeding on infected plants."

Finally BAWDEN states, "Some infective nymphs of *N. apicalis* can infect healthy plants immediately they emerge from the eggs, whereas others do so only after a variable waiting period. This at first sight again suggests multiplication of the virus in the insect, for the newly-hatched nymphs may contain too little virus to cause infection. And the waiting period may be the time required for this virus to multiply. But there are other equally probable interpretations. Newly-hatched nymphs may contain different initial charges of virus or the virus they contain may be distributed differently. A nymph born with a high virus content or with virus already in the salivary glands might be expected to transmit immediately, whereas it might be a considerable time before the virus in an insect with a low virus-content entered the salivary gland and the insect could transmit." On this point

I wrote in my previous paper, that there are several possible interpretations: either (1) the newly emerged tiny nymphs may be unable to transfer a dose of the virus sufficient to produce infection in a healthy plant, or (2) some developmental changes or multiplication of the virus may take place in the insect before it is fully infective, or (3) the virus may migrate from the other parts of the insect body to the salivary glands and the anterior portion of the alimentary canal. As stated in the present paper the first mentioned interpretations are improbable because the latent period lasts in certain leafhoppers during all the nymphal stages and even after they reached the adult stage. Indeed, it lasted for more than 30 days in some leafhoppers. This appears to be too long to represent merely the time required for the virus to migrate through body fluids or tissues to reach the salivary glands.

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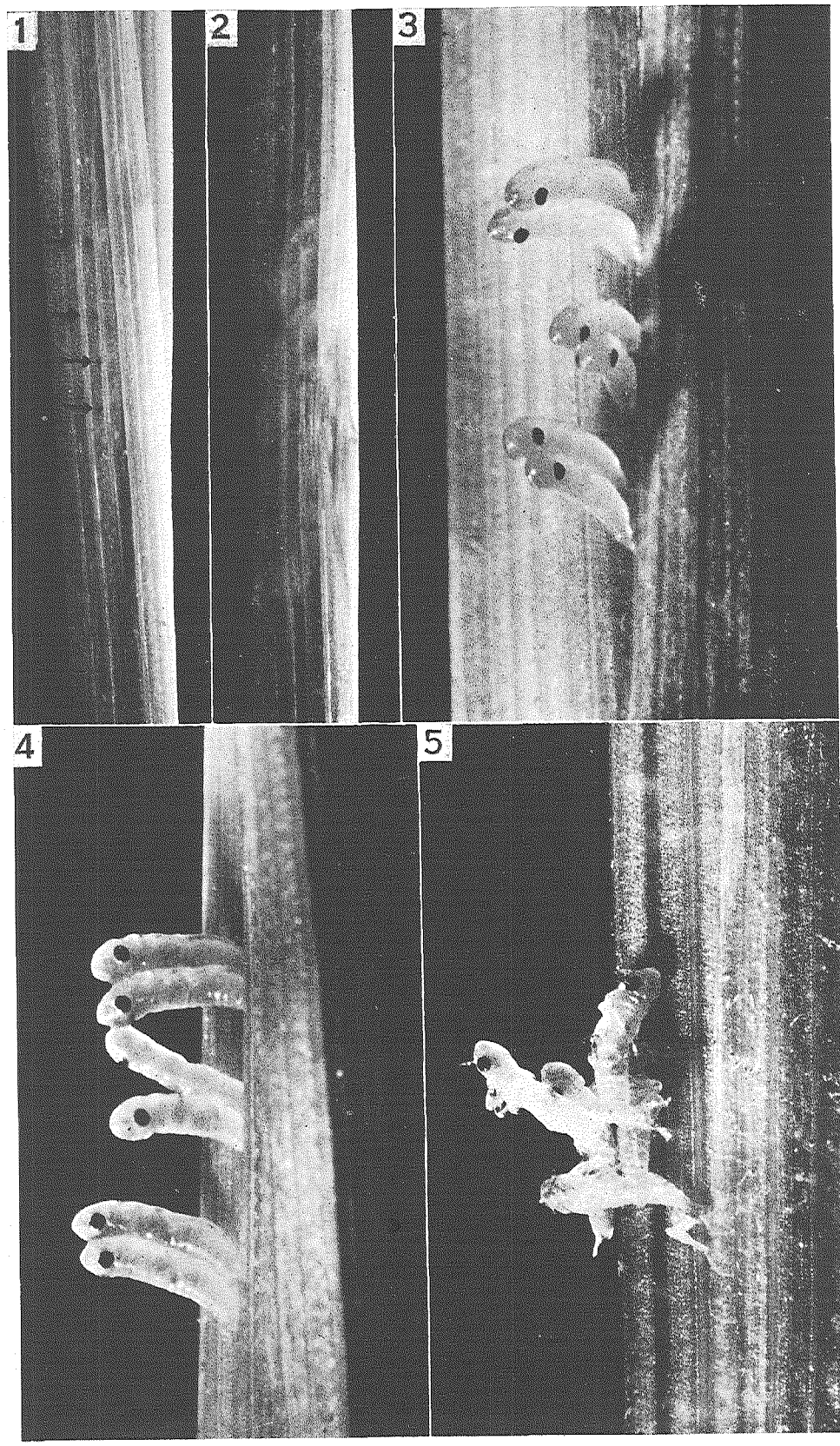
Explanation of Plates IV and V

Figs. 1 and 2. Eggs of *Nephotettix apicalis cincticeps* UHL. deposited in the leafsheaths of the rice plant. Note the location of eyes.

Figs. 3 to 6. Larvae in the process of hatching. Some nymphs are ready to crawl away as shown in Fig. 5. In Fig. 6 are shown the egg-shells shed in the tissues of leafsheath and the cast skins adhering to the surface of the latter. (× ca. 15-20)

Figs. 7 to 9. Larvae of the leafhopper, *Nephotettix apicalis cincticeps* UHL. in different stages, showing a male in Fig. 8 and a female in Fig. 9. (× ca. 10)

Fig. 10. An adult (♀) of the leafhopper. (× ca. 8)



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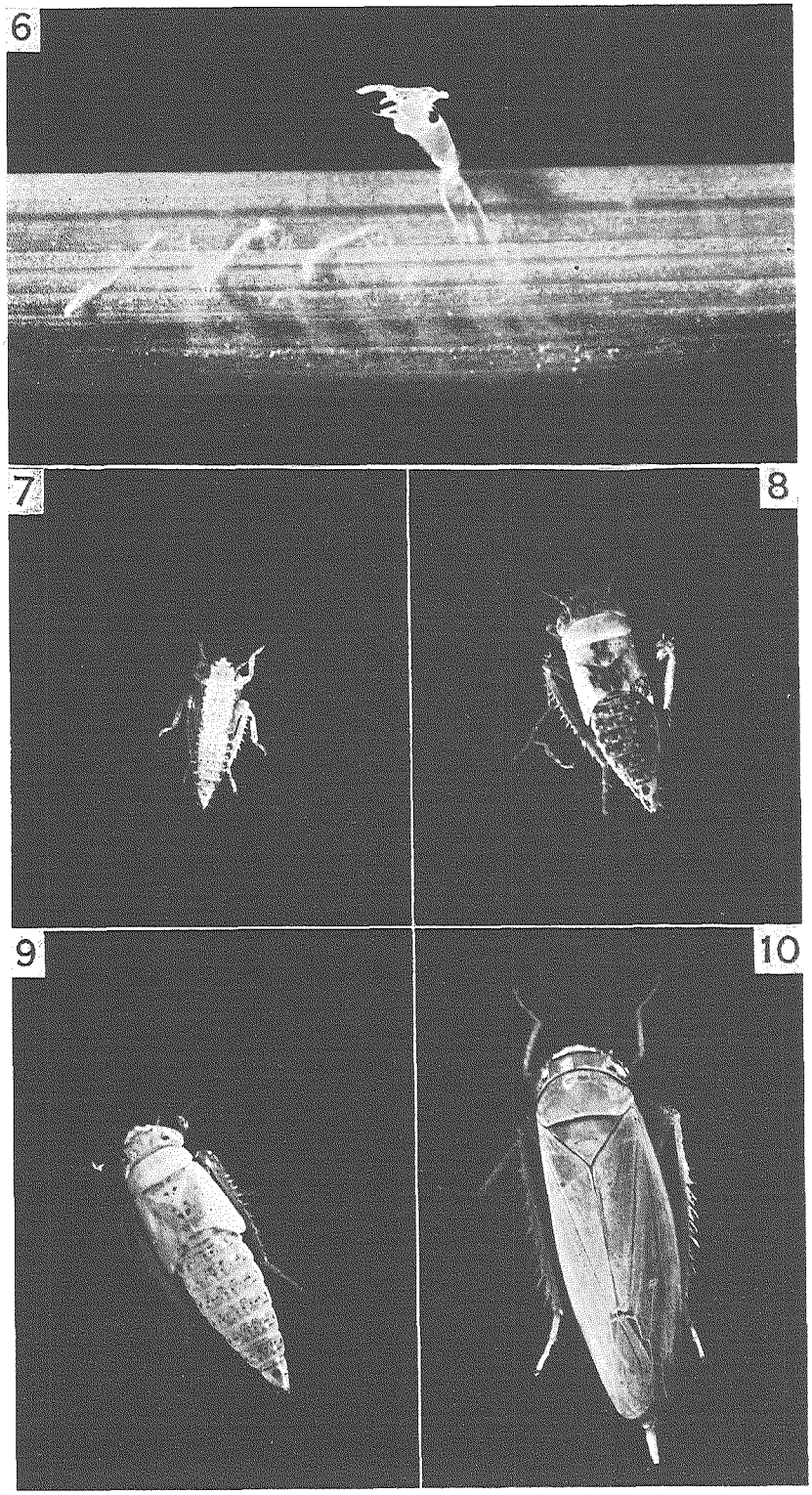

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