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ACQUISITION OF FIJI DISEASE VIRUS BY *PERKINSIELLA SACCHARICIDA* KIRK. AND OBSERVATIONS ON ITS INSTARS

by

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SUMMARY

The first, second and third instars of Perkinsiella saccharicida were the only stages which transmitted Fiji disease virus to healthy Q70 plants after a 48 hour access feed on diseased plants.

A key to distinguish the developmental stages of P. saccharicida was developed with the main distinguishing features being eye colour, and the shape, length and relationship, one to the other, of the fore and hind wing pads.

ACQUISITION OF FIJI DISEASE VIRUS BY PERKINSIELLA SACCHARICIDA KIRK. AND OBSERVATIONS ON ITS INSTARS

OBJECTIVE

The aim of the project was to determine which developmental stages of the planthopper vector could acquire Fiji disease virus (FDV) by feeding on infective plants and to establish a key to identify the instars of Perkinsiella saccharicida Kirk.

REASON FOR PROJECT

Knowledge of requirements for FDV acquisition by P. saccharicida is important in the design of many experiments into Fiji disease because of the obligate requirement for a planthopper vector, and the results could be an important consideration in future modifications to improve the insectary screening technique.

Osborne (personal communication) developed a key for identifying the five instars of P. saccharicida based on the tibial spurs and spines. However, we have found that to use this key the instars have to be anesthetised or destructively sampled and these methods are not practical for our work. Consequently, there was a need to develop a rapid method of identifying the instars.

REVIEW OF RELATED WORK

Mungomery and Bell (1933) reported that nymphs and adults of P. saccharicida, which had been bred on Fiji diseased cane, could transmit the virus although they were apparently unable to acquire it in the adult stage.

Hussain and Hutchinson (1971) stated that P. vitiensis Kirk. planthoppers emerging from eggs laid on healthy plants were apparently unable to subsequently acquire and transmit the virus as nymphs or adults. They concluded that for a planthopper to be able to transmit FDV, the virus must have been present in the egg. The vector biology of P. vitiensis may differ from that of P. saccharicida in this respect.

Chang (1977) demonstrated that FDV could be passed from the viruliferous P. saccharicida planthopper through eggs to the nymphs. He considered that transovarial passage of the virus was more likely than entry to the egg from surrounding plant tissue. Chang also recorded a private communication from Hutchinson that the first instar nymph of this planthopper is the only stage that can acquire the virus.

EXPERIMENTAL DETAILS

Observations on P. saccharicida nymphal stages

In these studies breeding stocks of the planthopper were established by caging 50 females, collected from the field at Eight Mile Plains, on 2-3 month old plants of healthy NCo310 growing in soil in pots in a temperature controlled glasshouse. No tests were carried out to determine whether the female planthoppers were viruliferous.

Sections of leaves containing P. saccharicida oviposition sites were excised and were placed on moistened filter paper in inverted plastic petri dishes. The petri dishes were maintained at room temperature until the planthoppers emerged from the eggs. The first instar nymphs were transferred to small plastic cylinders, covered at one end with a fine terylene gauze, and these were clipped onto the leaves of two healthy plants of NCo310. One NCo310 plant was transferred to the insectary and the other was kept in the laboratory. The temperatures in the insectary ranged from 25°C to 35°C, and the laboratory temperatures ranged from 21°C to 31°C. Observations were made from the 4th January, 1979 to 6th March, 1979.

The observations were repeated in 1980 during February and March with planthoppers, maintained on leaf pieces taken from healthy plants of the variety Pindar, in petri dishes, in the laboratory. The temperatures in the laboratory ranged from 22°C to 31°C.

Observations were made at magnifications of between 10 and 40 times. Photographs of the developmental stages of the leafhopper were taken with an Olympus PM10A photomicrographic system attached to an Olympus 200M-stereo microscope.

Acquisition of Fiji disease virus by P. saccharicida

P. saccharicida planthoppers free of Fiji disease virus were collected in January 1979 from the Rocky Point Mother Plot at Beaudesert, and were allowed to breed on virus-free NCo310, from the same source, in the Pathology Farm insectary. All cages used in the experiment were terylene gauze bags.

After one generation in the insectary, batches of greater than 200 adult hoppers were transferred at intervals to virus-free NCo310 and allowed 24 hours for oviposition before being removed. The first transfer for oviposition was made on the 25th January, 1979, and the final transfer for first instar nymphs was made on the 15th February, 1979.

During the development of the batches of planthoppers the incubation period of the eggs and the duration of the nymphal stages were recorded.

When the batches of planthoppers had developed into the appropriate nymphal stages, they were transferred to cages on the side shoots of decapitated Q70 with Fiji disease. Approximately half of each batch of planthoppers were placed on separate side shoots. The dates on which the acquisition period of each instar occurred are shown in Table 1.

Table 1

The dates for the acquisition period of various developmental stages

Developmental stage	Date placed on diseased Q70	Date placed on Q70 assay plants
1st instar	28.2.79	2.3.79
2nd instar	2.3.79	4.3.79
3rd instar	28.2.79	2.3.79
4th instar	28.2.79	2.3.79
5th instar	1.3.79	3.3.79
Adult	3.3.79	5.3.79

Assay plants of virus-free Q70 were grown from setts obtained from Meringa. The setts were hot-water treated at 50°C for 30 minutes and planted in vermiculite in peat pots. After planting, the Q70 was taken to the old quarantine house at BSES Head Office at Gregory Terrace. At the beginning of the planthopper acquisition period, the assay plants were returned to the Pathology Farm insectary, transferred to soil in polythene bags and caged.

After the acquisition period, groups of five planthoppers, of each developmental stage, were placed on individual assay plants. Thirty assay plants were prepared in this way for each developmental stage. Also, 30 assay plants were caged with no planthoppers to act as controls and 30 assay plants were left uncaged to detect whether there were any free infective leafhoppers in the insectary. The first instar nymphs were initially placed in fine gauze cages and after seven days these cages were replaced with the cages used for the other developmental stages.

The assay plants remained caged for 46 days, then they were sprayed with pyrethrins (Johnson Protector^{R}*), and the cages removed. The plants were then sprayed with endosulfan (Thiodan^{R}) and chlorpyrifos (Lorsban^{R}) to ensure the total kill of the planthoppers and to control night feeding armyworms.

Assay plants were allowed three weeks to recover from being caged and were then carefully inspected for Fiji disease galls.

The acquisition and inoculation of assay plants were carried out in the insectary where temperatures ranged from 25°C to 35°C.

RESULTS

Observations on P. saccharicida nymphal stages

A general description of the nymphal stages of P. saccharicida from the time of hatching until they develop into adults is given in Appendix 1. A summary of those observations, noting features which may be used to distinguish between the instars, is presented in Table 2.

* R = registered trade name

Table 2

Features used to distinguish the five instars of *P. saccharicida*

Developmental stage	Distinguishing features
1st instar	Hind wing pads rounded, light grey-fawn. Fore wing pads poorly developed. Eyes bright maroon.
2nd instar	Hind wing pads rounded, dark grey-black. Fore wing pads poorly developed, tip of pad extends to just cover the anterior edge of the hind wing pads. Eyes maroon.
3rd instar	Hind wing pads rounded, dark grey-black. Tips of fore wing pads cover one-quarter to three-eights of the hind wing pads. Eyes grey-brown with reddish margin.
4th instar	Hind wing pads less rounded with a distinct outline, dark grey-black. Tips of fore wing pads cover half to three-quarters of the hind wing pads. Eyes grey-brown to black.
5th instar	Hind wing pads grey with darker patches. Tip of fore wing pads now extend past the posterior tip of the hind wing pads. Eyes grey-brown to black.

Photographs of the developmental stages noting the distinguishing features are shown in Appendix 2.

Table 3

Mean duration of the developmental stages of P. saccharicida

The mean duration of the developmental stages of P. saccharicida*

Developmental stage	1. Observations January, 1979 individual leafhoppers 21° - 31°C (days)	2. Observations January-February, 1979 batches of leafhoppers 25° - 35°C (days)	3. Observations February-March, 1980 individual leafhoppers 22° - 31°C (days)	4. Observations made by Osborne (1968) 26.6°C (days)
Egg	-	11.5 range 10-14	-	10
1st instar	4.5 sample size 20	4.5 range 4-5	4.5 range 2-8 sample size 30	4.0 range 0 sample size 3
2nd instar	4.5 sample size 20	4.5 range 4-5	3.6 range 3-5 sample size 18	4.3 range 4-6 sample size 6
3rd instar	4.5 sample size 20	4.5 range 4-5	4.1 range 2-6 sample size 15	3.7 range 3-4 sample size 14
4th instar	-	6.7 range 6-7	4.4 range 3-6 sample size 12	4.8 range 4-7 sample size 14
5th instar	-	4.0 range 3-5	6.3 range 5-8 sample size 9	6.4 range 5-8 sample size 14
Adult	-	-	-	75% dead after 25 days

* Observations 1, 2 and 3 made at BSES Pathology Farm, Brisbane
Observation 4 made at University of New South Wales, Sydney

Table 3 compares the duration of the instars as recorded when the observations on instars were made in 1979; during the development of batches of planthopper developmental stages for the acquisition experiment; when observations on instars were made in 1980; and those recorded by Osborne (1968).

Acquisition of Fiji disease virus by P. saccharicida

Only three developmental stages of P. saccharicida were found to be able to acquire FDV. These were, in decreasing order of efficiency, the first, second and third instars.

The number of plants developing Fiji disease after five P. saccharicida planthoppers of each of the developmental stages were caged on 30 assay plants and the minimum possible percentage of infective planthoppers are shown in Table 4.

None of the caged or uncaged control plants became infected with Fiji disease.

Table 4

Number of assay plants infected with Fiji disease and minimum % of infective planthoppers

Developmental stage	Assay plants with Fiji disease/total no. plants	*Minimum % of infective planthoppers
1st instar	15/30	10.0
2nd instar	6/30	4.0
3rd instar	2/30	1.3
4th instar	0/30	-
5th instar	0/30	-
Adults	0/30	-
Control-caged	0/30	-
Control-uncaged	0/30	-

* The minimum possible percentage of infective planthoppers was calculated assuming only one of the five planthoppers on each plant was infective.

DISCUSSION

From the observations made on the P. saccharicida nymphs, the various instars can be distinguished simply and accurately by the eye colour and the shape, length and relationship, one to the other, of the fore and hind wing pads.

The results of this experiment suggest that only the first, second and third instars of P. saccharicida can acquire Fiji disease virus. However, one weakness of this experiment was that the developmental stage of the planthoppers was only checked as they came off the Q70 acquisition plant. It was possible that first instars were inadvertently transferred to the acquisition plant in the batch of second instar insects and that they moulted into second instars during the two-day acquisition period. This places some doubt on the results obtained for second and third instars. However, the positive result for third instars indicates at least that second instars can acquire FDV, based on the durations of that stage in other studies (Table 3). Further studies are required to confirm whether second and third instars can acquire the FDV.

Hussain and Hutchinson (1971) concluded that P. vitiensis planthoppers can not acquire the Fiji disease virus, but that the virus must be present in the egg for the planthopper to become infective. However, the results with P. saccharicida in this experiment show that this species can acquire the virus in the nymphal stages.

ACKNOWLEDGEMENTS

We wish to thank Mr J.L. Lopez for his assistance during these experiments.

RECOMMENDATIONS

Further studies should be undertaken when possible to confirm that second and third instars can acquire FDV. A manuscript summarising the results should be prepared at the conclusion of the proposed studies.

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Mungomery, R.W. and Bell, A.F. (1933). Fiji disease of sugar cane and its transmission. Bull. Bur. Sug. Exp. Stns Qld Divn Path. No. 4.

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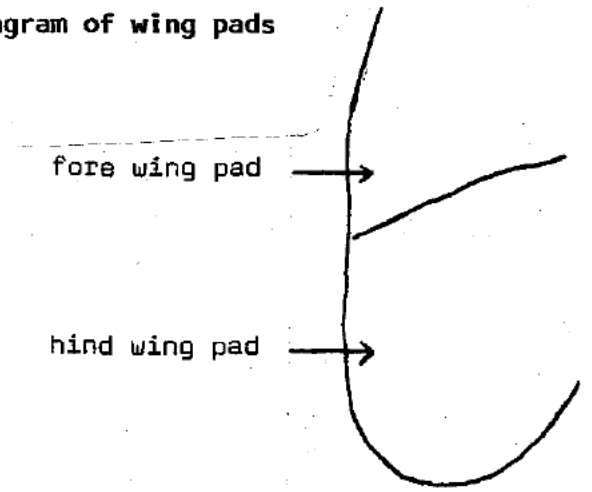
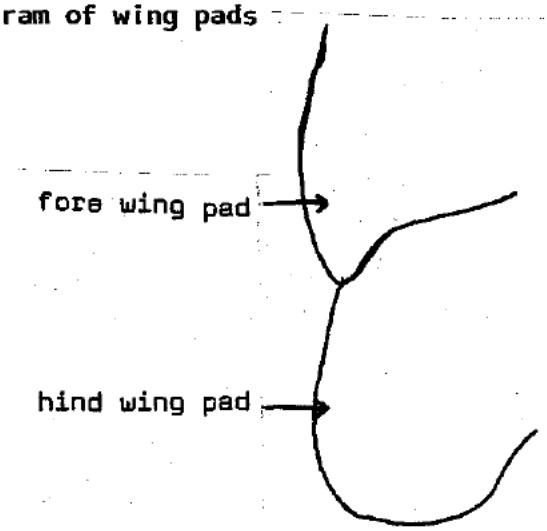
APPENDIX 1

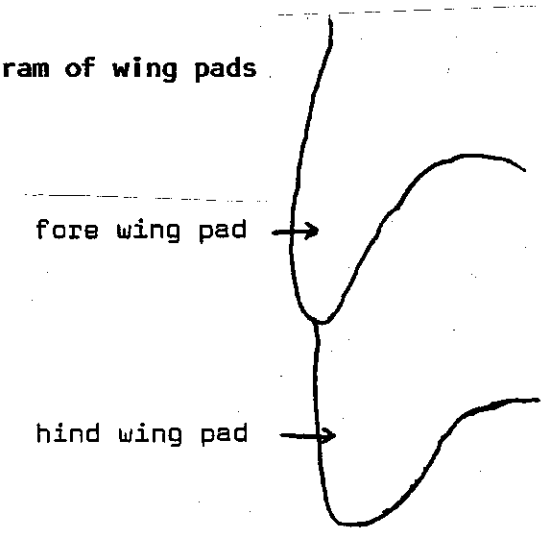
OBSERVATION OF THE FIVE NYMPHAL INSTARS OF P. SACCHARICIDA

Time	Developmental stage	Observations
0	1st instar	The hatching planthopper, still enveloped in a membrane, emerges from the leaf through the wax cap of the oviposition site. Still bound in the membrane, the planthopper is held at right angles to the leaf. In 2-5 minutes, the planthopper frees its body and legs from the membrane.
		The newly-emerged planthopper nymph is pearly white-transparent with dark maroon eyes.
1-2 minutes		Latter abdominal segments change to a very light fawn colour.
10-45 minutes		Dark grey patches appear on thorax.
45-60 minutes		The whole body assumes a metallic grey colour with dark patches occurring in a mosaic pattern on thorax and abdomen.
2 hours		Demarcation of abdominal segments become evident, especially the two anterior segments. Abdomen contracts, chitin becomes shiny.
5 hours		Grey speckling appears on legs, exoskeleton shows a tinge of light brown. Posteria segments of abdomen dark grey.
		Length - 0.815 mm (sample size 2)
1-2 days		Length - 0.97 (sample size 8)
2-3 days		Length - 1.07 mm (sample size 5)
3-4 days		Length - 1.10 mm (sample size 6)
4-5 days	2nd instar	Hind wing pads on the metathoracic segment are rounded and dark grey-black.
		Tip of the fore wing pads on the meta-thoracic segment extends to just cover the anterior edge of the hind wing pads.

Time	Developmental stage	Observations
4-5 days	2nd instar	**Diagram of wing pads**  - dark brown patches scattered over the whole exoskeleton, anterior three segments dark brown to black. - eyes lighter maroon - claspers quite noticeable Length - 1.17 mm (sample size 11).
5-6 days	2nd instar	Length - 1.23 mm (Sample size 11).
6-7 days		Body a lighter brown colour, hind wing pads still distinctive but also a lighter brown. Length - 1.35 mm (sample size 10).
7-8 days	3rd instar	Hind wing pads rounded, dark grey-black. Tips of fore wing pads cover one-quarter to three-eights of the hind wing pads. **Diagram of wing pads** 

Time	Developmental stage	Observations
7-8 days	3rd instar	Body colour generally a yellow-grey mottle on a white background, with dark brown-black patches. A large black circular spot from which hairs protrude is present on the extremities of each abdominal segment. Slightly forward and above these spots there is a smaller circular black spot. Ventral side of abdomen is generally yellow-white. The eyes are now grey-brown with a reddish margin. Length - 1.44 mm (sample size 13).
9-10 days		Length - 1.61 mm (sample size 15).
10-11 days		Length - 1.79 mm (sample size 10).
11-12 days		Length - 1.94 mm (sample size 5).
12-13 days	4th instar	Hind wing pads less rounded with a distinct outline, dark grey-black. Tip of fore wing pads cover half to three-quarters of the hind wing pad.

Diagram of wing pads

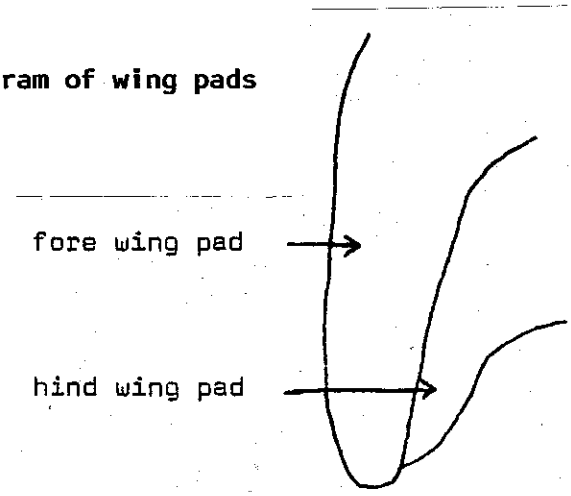


Eyes grey-black with reddish margins. Body dark grey with darker patches. Posterior three abdominal segments much darker than anterior segments. Ventrally, abdomen bright yellow with whitish background. Abdominal hairs and associated spots present.

Length - 2.07 mm (sample size 11).

Time	Developmental stage	Observations
13-14 days		Length - 2.27 mm (sample size 13).
14-15 days		Length - 2.43 mm (sample size 14).
15-16 days		Length - 2.48 mm (sample size 10).
16-17 days	5th instar	Hind wing pads grey with darker brown-black patches. Fore wing pads now extend past the posterior tip of the hind wing pads.

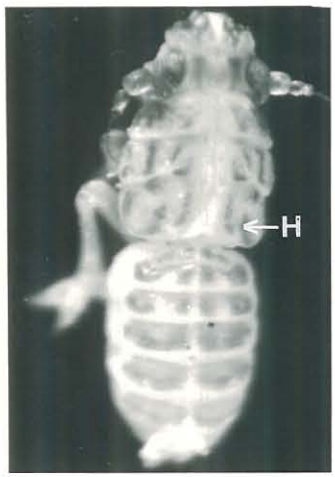
Diagram of wing pads



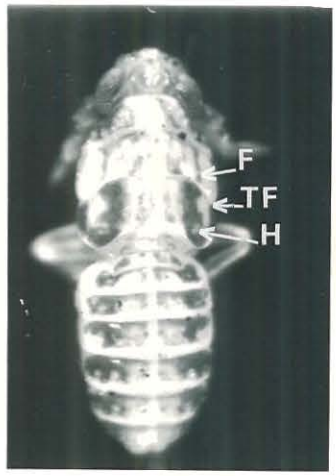
		Eyes grey-black. Wing pad development gives the nymph a bulkier appearance. Abdominal hairs very well developed. Body grey with dark grey to black markings.
		Length - 2.82 mm (sample size 10).
18-19 days		Length - 2.80 mm (sample size 11).
19-20 days		Length - 3.12 mm (sample size 10).
20-21 days		Length - 3.32 mm (sample size 9).
21-22 days		Length - 3.46 mm (sample size 9).
22-23 days		Length - 3.55 mm (sample size 5).
23-24 days	Adult	

APPENDIX 2 : The developmental stages of P. saccharicida

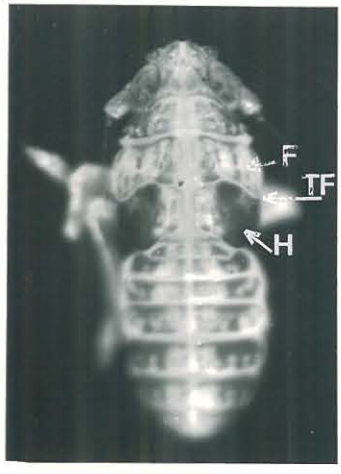
(a) First instar nymph; note that the hind wing pads (H) are light grey.



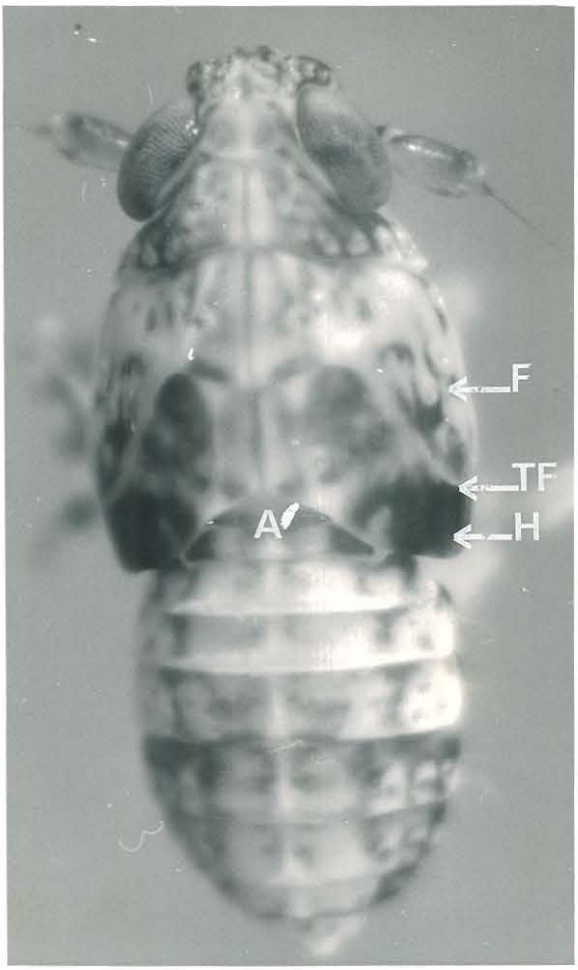
(b) Second instar nymph; note that the hind wing pads (H) are dark grey to black and that the tip (TF) of the fore wing pads (F) extend to just cover the anterior edge of the hind wing pads.



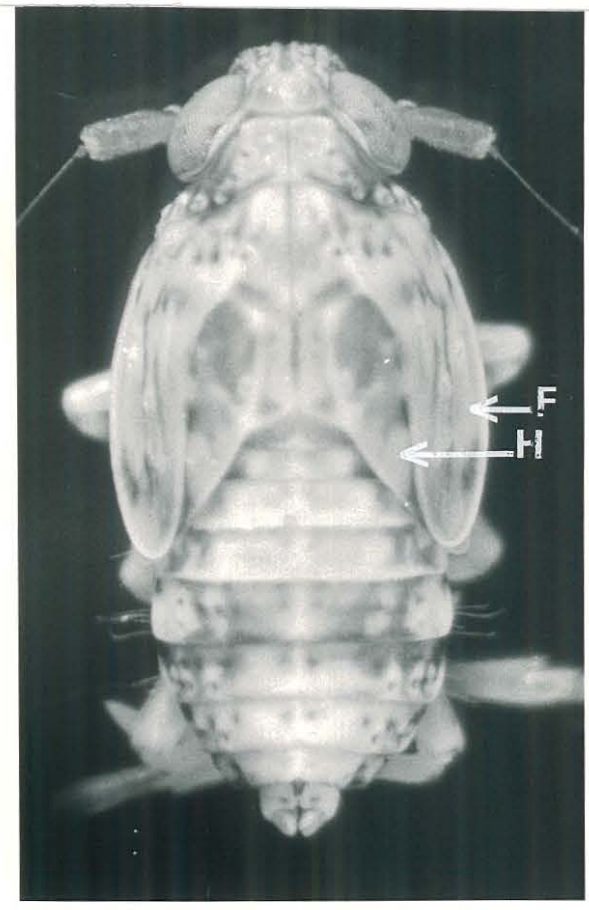
(c) Third instar nymph; note that the tip (TF) of the fore wing pads (F) extends along the edge of the hind wing pads (H) one-quarter to three-eighths of their length.



(d) Fourth instar nymph; note that the tip (TF) of the fore wing pads (F) extend along the edge of the hind wing pads (H) half to three-quarters of their length. Note the distinctive crescent shaped arch (A) between the two hind wing pads.



(e) Fifth instar nymph; note that the fore wing pads (F) are longer than the hind wing pads and the hind wing pads (H) are light grey with dark areas.



(f) Long wing adult

(g) Short wing adult