Mass rearing of *Cicadulina* leafhoppers

to screen for maize streak virus resistance

Mass rearing of *Cicadulina* leafhoppers to screen for maize streak virus resistance

A manual

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Foreword

Maize streak virus is generally considered the most important and widespread disease of maize grown in sub-Saharan Africa. Occasionally reaching epidemic proportions, it is a chronic problem for small-scale farmers who lack the labor or equipment to ensure that their entire crop is planted early in the season. The later they plant, the greater the risk of virus damage.

IITA's best-known accomplishment in maize improvement has been the development of a practical resistance screening system for large-scale field use against the streak virus. The system has been used to produce a wide range of resistant germplasm fitting the various agroecologies of sub-Saharan Africa.

The system enables the identification of durable, oligogenic resistance. Since streak resistance carries no yield penalty or other undesirable side effects, and a practical screening technique exists, the effort now is to incorporate streak resistance into any variety before it is released in the region. To help achieve this goal, IITA has assisted national research programs to incorporate the screening method for streak resistance into their routine breeding practice.

This booklet carries that assistance one step further. We hope the national programs in the region will use it profitably. We welcome their feedback on the usefulness of this publication.

L. Brader
Director General

Contents

Introduction 1

The success of any program breeding for resistance to diseases and/or insects depends largely on the development of suitable and reliable screening techniques. Such techniques also have to be simple and relatively inexpensive. Field screening under natural infestations is often unsuccessful because the incidence of pests is erratic. To overcome this problem and to minimize the chance of 'escapes' in screening, it is necessary to infest a large number of plants artificially every season. For that purpose, rearing of the target insect is necessary.

Mass rearing of *Cicadulina* leafhoppers was begun at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, in 1976. Over the years, a number of modifications and improvements have been made (Leuschner et al.1980; Soto et al. 1982; Alam 1983; Dabrowski 1983).

This handbook presents the techniques developed and experiences accumulated at IITA over the past 15 years for mass rearing and infestation of *Cicadulina* leafhoppers in screening for resistance to maize streak virus (MSV).

Maize streak virus

Maize streak virus (MSV) is one of the most economically damaging diseases of maize in sub-Saharan Africa. It is found only in Africa and surrounding islands, where it is widely distributed and transmitted by leafhoppers in the genus *Cicadulina*. MSV is found in both forest and savanna zones and in varying altitudes (0-2000 m). Damage to maize from MSV can be insignificant in some years but epidemics of the disease in other years can devastate crops with yield losses of 100% (Fajemisin and Shoyinka 1976). The severity of the disease is usually related to the age of the plant at the time of infection, as well as to the relative susceptibility of the variety. The younger the plant, the greater the severity of symptoms. At IITA, yield losses under artificially induced infestation of four varieties with differing levels of resistance/susceptibility were found to range from 10 to 72%.

Symptoms of MSV consist of broken to almost continuous, narrow, white chlorotic stripes which develop over and along the vein on most of the leaf surface (Fig.1). The density of striping depends on varietal susceptibility. Maize plants are vulnerable to MSV from emergence to tasseling.

Figure 1
Maize plant with streak virus symptoms

Susceptible plants infected at the seedling stage become stunted and may die or produce small and poorly filled ears (Fajemisin et al. 1976; Rossel and Thottappilly 1985).

Twenty-two species of *Cicadulina* leafhoppers have been reported; 18 of these occur in Africa (Webb 1987). Only eight species are known to be vectors of MSV (Table 1).

Morphology

The *Cicadulina* leafhoppers vary in length from 2.2 mm to 3.8 mm (Rose 1978). Coloration of the insects varies but is generally pale to golden yellow. Some species have black markings on the forewings, pronotum, and venter. The dorsal side of the abdomen is usually brown. Most species have a pair of round, brown spots on the frontal margin of the forehead (Ruppel 1965). The female *Cicadulina* is distinguished from the male by its long ovipositor.

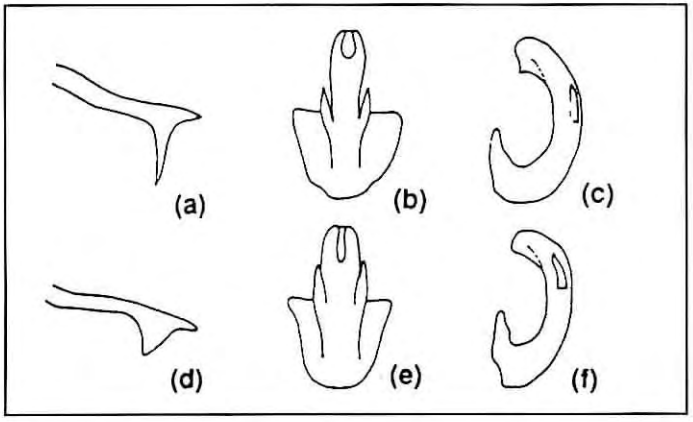
Table 1. Species of *Cicadulina* leafhoppers known to be vectors of maize streak virus in Africa

Species	Selected references
C. mbila (Naudé)	Storey 1925
C. storeyi (= *triangula*) China	Storey 1936
C. bipunctata (Melichar)	Storey 1936
C. latens Fennah	Fennah 1960
C. parazeae Ghauri	Rose 1962
C. arachidis China	Okoth & Dabrowski 1987
C. similis China	Okoth & Dabrowski 1987
C. ghaurii Dabrowski	Dabrowski 1987a

Identification

The identification of *Cicadulina* species is somewhat difficult. Species differ markedly in male genital characters. The shape and size of the aedeagus and the shape of the pygophore processes are useful characters for differentiating between species of *Cicadulina* (Ruppel 1965; van Rensburg 1983; Webb 1987). Figure 2 illustrates genital characters for two of the most common species of *Cicadulina* in Africa: *C. mbila* and *C. storeyi* (= *C. triangula*). Webb (1987) mentioned that care should be taken in distinguishing African species of *Cicadulina* from a leafhopper of the genus *Afrosteles*. Externally, both genera are similar. However, *Afrosteles distans* is slightly larger than *Cicadulina* and it lacks the dark apex of the ovipositor which is present in *Cicadulina*.

Figure 2
***Cicadulina mbila*: (a) pygophore processes; (b) aedeagus, ventral view; (c) aedeagus, lateral view; *C. storeyi:* (d) pygophore processes; (e) aedeagus, ventral view; (f) aedeagus, lateral view.**



Distribution

The distribution patterns of *Cicadulina* leafhoppers also vary considerably across Africa. *C. mbila* and *C. storeyi* (Fig. 3) are widely distributed in Africa. *C. mbila* is the most important vector species (Nielson 1968; Okoth and Dabrowski 1987). Both *C. mbila* and *C. storeyi* are presently being used for mass rearing purposes in various African countries.

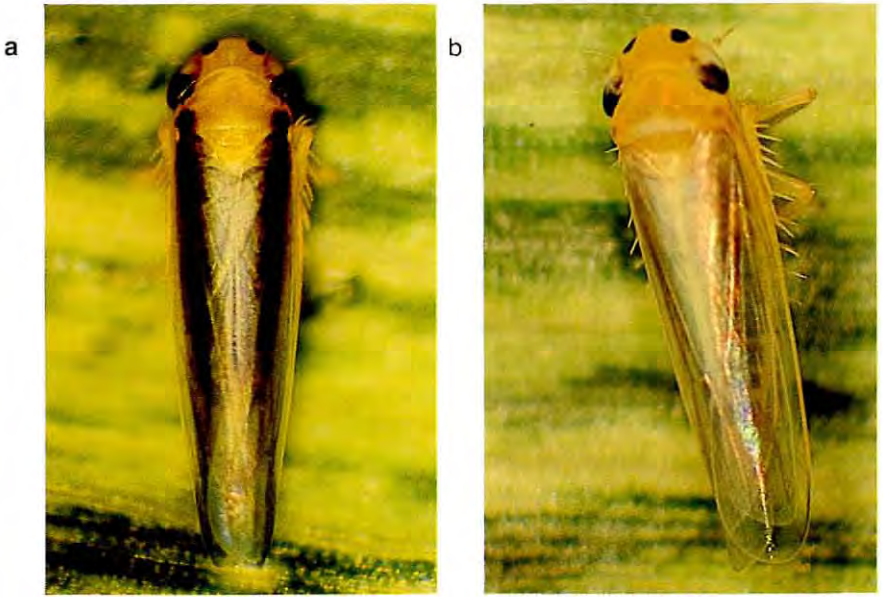


Figure 3
Two dominant species of *Cicadulina*:
(a) *C. mbila*
(b) *C. storeyi* (= *triangula*)

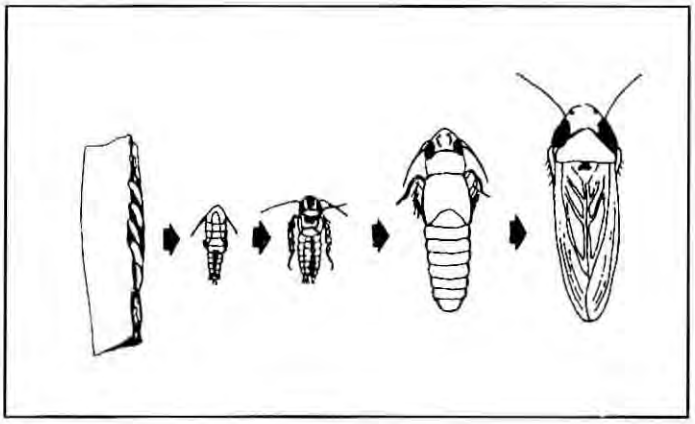


Figure 4
Developmental stages of a leafhopper from egg to adult. There are five nymphal instars; only three are shown.

Life history

Knowledge of an insect's life history is important for a successful mass rearing program. *Cicadulina* species differ in their life history and ability to transmit MSV. The life histories of *C. mbila* and *C. storeyi* have been studied extensively (Table 2). Their developmental periods (egg to adult) (Fig. 4) range between 22 and 45 days, depending on temperature (van der Merwe 1926; van Rensburg 1982). Under 20°C, the developmental period is prolonged. Temperatures over 35°C are detrimental to the insect, especially to *C. mbila*. The suitable temperature for mass rearing *C. mbila* is 25-30°C (van der Merwe 1926; van Rensburg 1982), and for *C. storeyi* 30-35°C (Dabrowski 1985).

Table 2. Biology of two species of *Cicadulina* leafhoppers

Parameters	*C. mbila*	*C. storeyi* (=*triangula*)
Egg incubation period (days)	9-21	7-11
Nymphal developmental period (days)	16-21	15-25
Preoviposition period (days)	3-8	2-7
Longevity of adults (days)	10-50	14-41
Fecundity (mean no. eggs/female)	129	123
Suitable temperature for mass rearing (°C)	25-30	30-35

3 Collecting the leafhoppers

Time

To start a new colony of *Cicadulina* leafhoppers, collect the live leafhoppers from the field. The optimal time for collection of *Cicadulina* leafhoppers is at the end of the rainy season, when leafhopper population density is high and they migrate from old plants to young ones.

Sites

Grass species of the genera *Pennisetum, Digitaria, Eleusine, Brachiaria, Paspalum, Setaria, and Panicum* are preferred by *Cicadulina*; so you should sample them for collection (Rose 1978; Okoth and Dabrowski 1987). In addition, you could sample irrigated wheat and grasses growing near rivers, lakes, or in valley bottoms.

Methods

There are various ways by which you can collect *Cicadulina* leafhoppers from the field, but most suitable is a method that uses a cubical frame made of iron or steel rods,

Figure 5
Iron frame just before covering with dark cotton cloth

covered by a dark green or black cotton cloth with transparent netting or fine mosquito net on one side (Fig. 5) (Dabrowski 1983). The iron frame and the cloth are handy and portable.

To construct a frame, you require four 140-150 cm pieces of iron or steel rod 10-15 mm in diameter. Sharpen the lower ends of the rods so that they can be easily pushed into the soil. Bend the top end of the rod into a short 20-25 cm arm to give corner support. At the sampling site, follow the following steps for collection of *Cicadulina* leafhoppers.

1. Place the iron rods at the four corners of the sampling site in such a way that they will form a cage size 1.25 x 1.25 m. Do not disturb the site.
2. Once the rods are fixed, place the dark cloth quickly over them so that the *Cicadulina* leafhoppers within the cage cannot escape.
3. Enter the cage and disturb the grasses, so that insects within the cage will be attracted to the light on the side with transparent netting (Fig. 6). From there, selectively collect the *Cicadulina* leafhoppers, using a mouth aspirator.
4. Cage the female leafhoppers singly with young millet or maize seedlings, using polyvinyl chloride (PVC) tubes

Figure 6
Entering the collection cage after covering it

(about 8 cm in diameter and 25 to 30 cm long) (Fig. 7). Alternatively release the *Cicadulina* leafhoppers collected into a cage (approx. 40 x 40 x 60 cm) (Fig. 8) with young, potted, insect-free millet or maize plants and transport back to the laboratory or screenhouse.

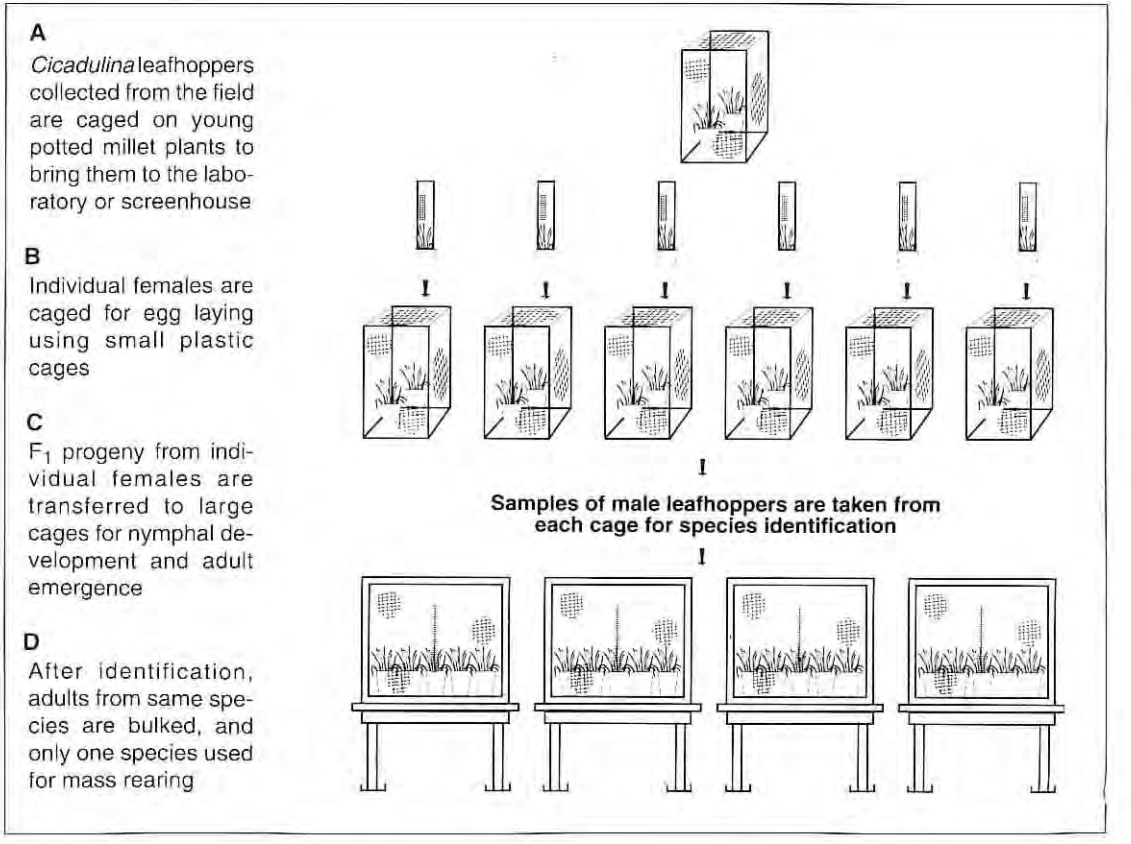
Figure 7
Polyvinyl chloride (PVC) tube used to confine single leafhoppers to obtain progeny or to carry out virus transmission tests

Figure 8
Wooden cage used to confine nymphs when initiating a *Cicadulina* colony

Initiating the colonies

1. Cage about 150-200 female leafhoppers singly on young potted-millet plants, using PVC tube cages. You can also use plastic bottles, or soft drink or mineral water bottles. Cover one side and the top opening of the cage with fine netting.
2. The gravid females will lay eggs, which will hatch in about one week. First allow the nymphs to feed inside the cages. It is important to ensure the young nymphs have access to fresh millet or maize plants for feeding; thus, transfer the nymphs to wooden or metal framed cages (approx. 40 x 40 x 60 cm) covered with fine netting. Do not place nymphs originating from different females together in the same cage, to avoid mixing different species.

Figure 9
Procedure used to start a new colony of *Cicadulina* leafhoppers for mass rearing



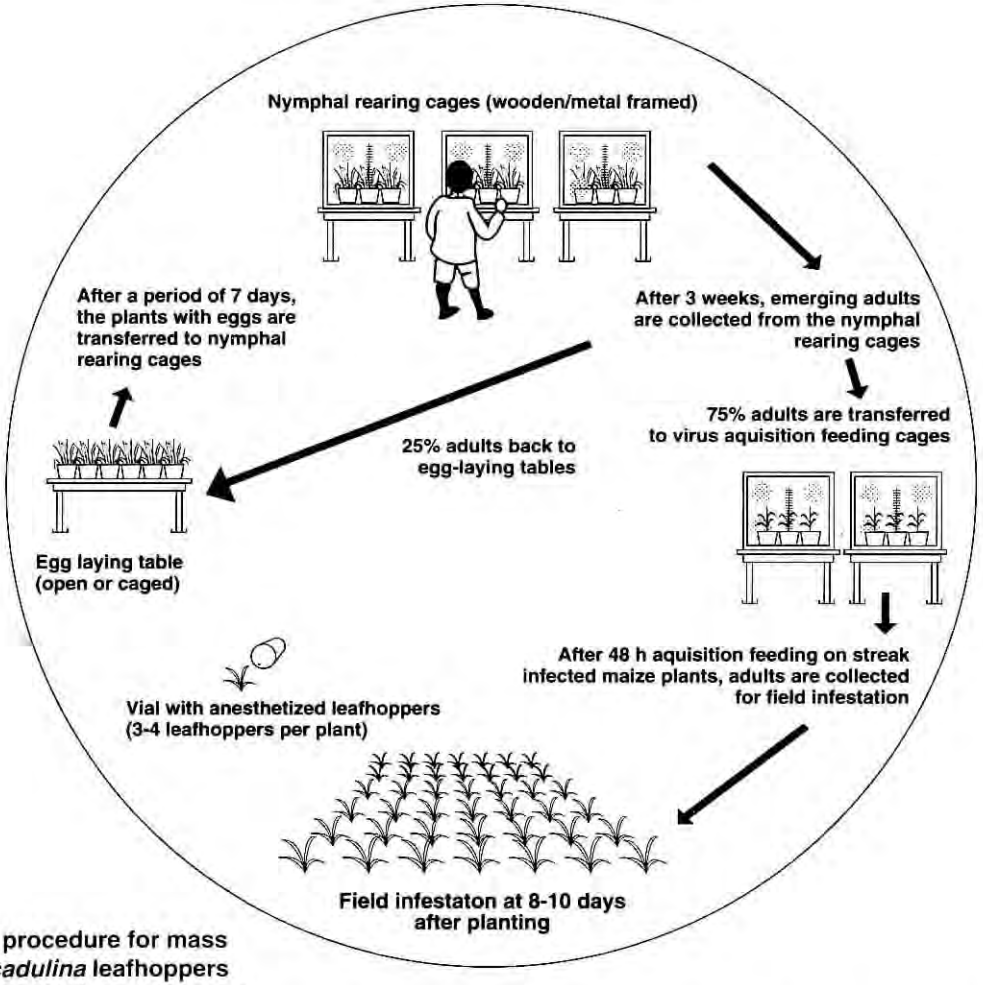


Figure 10
Schematic procedure for mass rearing *Cicadulina* leafhoppers for maize streak virus resistance screening

3. When the nymphs become adults, collect samples of males separately from each cage for species identification, and keep the samples in 75% alcohol (ethanol). Before identification, place the specimens in 10% potassium hydroxide (KOH) solution for 24 hours. Take out a specimen and place it under a dissecting microscope. Dissect the last abdominal segment to look for the male genitalia—the pygophore processes and aedeagus. See Figure 2 for an illustration of the genitalia of two common *Cicadulina* species. Follow Webb's (1987) key for identification of other species.

4. Once species identification is completed, put together adults belonging to the same species and release them into larger cages to build up the population (Fig. 9). Use only one species for mass rearing.

Select *Cicadulina* species with a high reproductive potential and high transmission ability of MSV for mass rearing and resistance screening. As explained, this would mean you use either *C. mbila* or *C. storeyi* for rearing. An added advantage is that ample information and experience exist on mass rearing these two species in various African countries.

The standard procedure for mass rearing of *Cicadulina* leafhoppers at IITA is illustrated in Figure 10. The various steps involved are described next.

Oviposition

The best host for egg laying by *Cicadulina* females is pearl millet, *Pennisetum americanum* (= *typhoides*). Up to 220 eggs per female have been obtained at IITA on this host (Dabrowski 1987b). Millet has the additional advantage over maize of tolerating large leafhopper populations without suffering severe damage. Use potted 14-day old plants for oviposition.

For egg laying, keep adults on open oviposition tables (0.75 m high) (Fig. 11) or in metal framed cages (1.25 x 1.25

Figure 11
Open table for egg-laying by leafhoppers on millet plants

Figure 12
Nymphal rearing cage

x 1.50 m) covered with fine insect proof mesh (Fig. 12). Sew the mesh to form a cage that will fit over the metal frame; a zipper on one side of the mesh cage allows easy access to the plants and insects inside. Place cages over tables about 0.75 m high. Our experience at IITA is that adults kept on open tables do not escape from the screenhouse. This could be partly a result of adaptation to rearing conditions (i.e., selection for short-distance flyers), likely to occur in any large colony. We thus recommend using a combination of both methods (open tables and close cages) for oviposition until you obtain a large enough colony of leafhoppers adapted to open tables.

After a one-week oviposition period, transfer the potted plants to nymphal rearing cages (1.25 x 1.25 x 1.50 m) for egg hatching and nymphal development (Fig. 12). Add fresh plants to the tables, so that females can continue laying eggs. Collect the adults and release them onto oviposition tables at least every 3-4 weeks. The duration of the oviposition period might vary, according to species and environmental conditions. Therefore, carry out experiments to determine the most appropriate length of time for any given location. This period, however, should not be very long, so as to avoid a correspondingly long nymphal emergence period that will result in a mixture of nymphs of different ages. Plant millet weekly to ensure availability of host plants for rearing.

Nymphal rearing

Once eggs hatch, nymphs will start to feed on the millet plants. Add fresh plants to the cages regularly to ensure an adequate supply of food. Cut the older plants, on which nymphs have been feeding, with a sharp knife, and shake gently to dislodge the nymphs onto the fresh plants. Nymphs will take about 3 weeks to become adults.

A nymphal rearing cage can hold 20-24 pots. However, it is advisable to reduce this number to 16 during the rainy season when high relative humidity prevails. This will ensure air flow and will help reduce problems of fungal growth on the millet plants.

Collecting adults

Emergence of all the adults in a nymphal rearing cage should ideally occur within one week. Collect the adults by covering the cage with a dark cotton cloth, leaving a small portion of the cage uncovered (Fig. 13). The leafhoppers will respond to the light and move to the portion of the cage left uncovered. A person can then move into the cage and

Figure 13
The cage is covered with dark cloth prior to collection of leafhoppers

use a mouth aspirator or a modified vacuum cleaner (200-500 W) for collection. The rubber tube of the vacuum cleaner is attached to a thick rubber tube (15 cm long x 6 cm diam) and the latter connected to a small plastic collecting vial (9 cm long x 5 cm diam) (Fig. 14). The collecting vial should have one end covered by fine mesh; the other end should be a narrow tube that will be used to collect the insects (see inset in Fig. 14). After you have collected them, transfer leafhoppers to virus acquisition feeding cages or to oviposition tables, according to your rearing needs at that time.

Figure 14
Attachment of thick rubber tube and *Cicadulina* collecting vial to vacuum cleaner for collection of leafhoppers. Inset: leafhopper collecting vial

5 Using *Cicadulina* in resistance screening

Obtaining virus-carrying leafhoppers

Use young potted MSV-infected maize plants of a streak-susceptible variety for virus acquisition feeding. Plant maize on a regular basis and infect it with MSV, 7-10 days before it is needed. Keep the plants in a cage the same size as the nymphal rearing cages. A period of 48 h is optimal for virus acquisition. After this period, collect the viruliferous leafhoppers again, using the vacuum cleaner, and transfer to leafhopper dispensing vials (9 cm long x 5 cm diam) for transport to the field.

Infesting the field

The leafhopper dispensing vial is made of PVC, covered with fine mesh at one end and with a plastic lid at the other. The plastic lid of the dispensing vial is removable, to allow transfer of leafhoppers from the collecting vial into it. The lid has a small orifice (3 mm diam) to allow the leafhoppers to pass when they are dispensed. This orifice is covered with a small plug of cotton wool or grass, to prevent the leafhoppers from escaping during transport.

If the field is far from your rearing facilities, collect the viruliferous leafhoppers from the virus acquisition cage and place them in a smaller cage (approx. 40 x 40 x 60 cm) with a few healthy potted maize or millet plants, so that they can be easily transported to the field. Prior to infesting the field, collect the leafhoppers from the cage with a mouth aspirator and transfer them to leafhopper dispensing vials.

Alternatively, after collecting the leafhoppers from the virus acquisition cage, transfer them to the dispensing vials and transport these to the field in a cooler. At low temperatures (10 to 12°C) the leafhoppers will survive for several hours. While humidity should be high to prevent dessication, water condensing in the vial will kill the insects. Therefore, cover the inner wall of the vial with absorbent paper (i.e., paper towel or filter paper) to collect the excess moisture resulting from condensation. If the field is close to your rearing facilities, you can take the leafhoppers directly to the field in the dispensing vials.

To ease infestation, anesthetize leafhoppers with carbon dioxide (CO_2) immediately before you dispense them. Carry the CO_2 to the field in a rubber inner tube, to which a thin

Figure 15
Anesthetizing leafhoppers with carbon dioxide (CO_2) in the field before infestation of maize seedlings. Notice the inner tube used to carry CO_2 to the field

rubber hose with a valve is attached (Fig. 15) (Leuschner et al. 1980). The CO_2 immobilizes the leafhoppers, preventing their escape. Next, dispense the insects into the leaf whorl at a rate of 3-4 leafhoppers per plant (Fig. 16). The leafhoppers will become active shortly after release, and they will start feeding on the plant.

If necessary, use CO_2 a second time to inactivate the wakening leafhoppers in the vial. Excessive use of CO_2, however, will kill some insects and result in nonuniform MSV infestations. CO_2 can be bought from commercial establishments, such as soft drink bottling companies, or it can be obtained from fire extinguishers. Carry out field infestations when plants are at the three-leaf stage (approx. 8-10 days after planting) (Fig. 16).

Figure 16
Anesthetized leafhoppers are shaken out of the vial onto a maize seedling (3-4 per plant)

Table 3. MSV visual rating scale

Rating scale	Description	Expression in terms of resistance
0	No symptoms	Immune or escape
1	Very few streaks on leaves	Highly resistant (HR)
2	Light streaking on old leaves, gradually decreasing on young leaves	Resistant (R)
3	Moderate streaking on old and young leaves, slight stunting	Moderately resistant (MR)
4	Severe streaking on about 60-75% of leaf area, plants stunted	Susceptible (S)
5	Severe streaking on more than 75% leaf area, plants severely stunted or dead	Highly susceptible (HS)

Figure 17
A susceptible check variety (center) showing MSV symptoms three weeks after infestation

Figure 18
Reaction to MSV (left to right). Susceptible (5), moderately resistant (3), and resistant (1). Ratings are based on the visual rating scale (Table 3)

Rating for MSV

Viral symptoms will appear in 5-10 days and they will be clearly visible 2-3 weeks after infestation (Fig. 17). The following visual rating scale of 0 to 5 (Table 3) has been developed based on Soto et al. (1982) for evaluating resistance to maize streak virus (Fig. 18).

Selecting for MSV resistance

Selection for MSV resistance is done by first thinning out susceptible plants 3-4 weeks after planting. At flowering, select plants combining adequate levels of resistance (1-3 on the rating scale) with other desirable characters (Efron et al. 1989).

6 Problems related with mass rearing

The host plants used for rearing should be free from other insects, hence they should be grown inside a screenhouse to avoid pest infestation. Insects which might be problematic include whiteflies, aphids, leaf beetles, planthoppers, and lepidopterous larvae. Manual removal of insects is often effective. If an infestation develops, it might be necessary to discard the plant batch or to spray a short-term action insecticide, such as malathion. Spraying should be done outside the screenhouse, and the plants left for 1-2 weeks prior to their use for *Cicadulina* rearing. Large populations of aphids on millet seedlings have been effectively controlled in the past, using a coccinellid beetle, *Chilomenes sulphureus* (IITA 1987).

Mass production of *Cicadulina* leafhoppers may be complicated by:

(a) presence of natural enemies inside the rearing cages;

(b) temperature fluctuation during winter months or at high elevation.

Natural enemies

Like other insects, leafhoppers are attacked by predators and parasitoids. Ants, spiders, and lizards are common predators of *Cicadulina* nymphs and adults. In addition, there is a mirid bug, which is an egg predator. Ants can be kept off from the rearing cages by placing a water pan with water and kerosine or oil under the legs of the tables.

The common parasitoids of *Cicadulina* leafhoppers are a hymenopterous wasp (Dryinidae) and a dipteran fly (Pipunculidae). Sometimes, they can seriously affect mass production. Regular inspections by an entomologist should be carried out. Once it is noticed that a rearing cage has been infested with parasitoids or mirid bugs, it is advisable to discard the insects and, if necessary, also the plants and soil in the cages. The cages should be cleaned thoroughly and left empty for some days. If all the cages are severely infested, then it is advisable to discard the entire colony and start with a new colony as described in Figure 9. Alternatively, 200-300 healthy males and females can be collected from the cages to start a new colony.

Temperature

In countries with severe winter, the temperature fluctuation during the winter months may affect the mass rearing of *Cicadulina* leafhoppers. Where the night temperature drops below 15°C, special arrangements should be made to increase the temperature in the screenhouse. Low temperature will affect the leafhoppers as well as the germination and growth of the host plants. To augment the night temperature in the screenhouse during winter months, the sides of the screenhouse should be covered at night with clear plastic sheeting. During daytime, the plastic sheeting should be rolled and tied up for cross ventilation. In addition, supplementary heating can be obtained from a kerosine burner or an electric heater (where electric power supply is available). The rearing cages can also be covered with plastic sheets at night.

Quality control

Cicadulina leafhoppers belong to two categories: active transmitters of MSV and nonactive transmitters (Storey 1932). This character is genetically controlled. When mass rearing *Cicadulina* leafhoppers, it is important to maintain a high percentage (60-80%)of active transmitters in the colony. This will reduce the number of insects required for infestation of each plant, ultimately resulting in more plants being infested using the same number of leafhoppers. But it will require periodical quality control.

To test the proportion of active transmitters, about 50-100 female leafhoppers should be collected from a nymphal rearing cage and released for 48 h into a small cage with streak-infested maize plants. The females should then be caged singly with 5 to 7-day old potted maize plants susceptible to MSV, using PVC cages (Fig. 7). About 7-10 days later, the number of plants showing streak symptoms should be observed and the percentage of transmission should be calculated. If the percentage of transmission is below 35, a new colony of active transmitters should be initiated, by putting together the progeny of those females that transmitted MSV. Also, plants showing MSV symptoms from the transmission test should be placed together in a cage for egg hatching and nymphal development. Within 3 weeks, the nymphs will develop into adults and the new colony of the leafhoppers should have a higher proportion of active transmitters. The new colony can be used to build up the population of active transmitters. The percentage of active transmitters in the colony should be checked twice a year.

7 Assisting national programs

To meet the needs and circumstances of national programs and private seed companies, modifications to the described techniques might be needed.

Entomologists setting up *Cicadulina* rearing facilities should be able to adapt the techniques developed at IITA to fit their own conditions. IITA scientists are available to assist national programs in developing *Cicadulina* rearing facilities.

The Togolese national program developed leafhopper rearing facilities with assistance from IITA and Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) and modified the infestation method to their needs. The national program of Zaire and CIMMYT's mid-altitude station in Harare, Zimbabwe, were assisted by IITA to develop their rearing facilities. For the latter two countries, modifications were needed to ensure survival of the insect colonies during the cold winter months. IITA is also assisting the national programs of Ghana and Cameroon to develop their leafhopper rearing facilities.

During 1990, IITA offered an intensive two-week training course on *Cicadulina* leafhopper rearing. It was attended by participants from four African countries. This course can be offered again in the future if there is sufficient need or demand. National program scientists should contact IITA to express interest and seek further information.

Figure 19
Trainees listen to IITA scientist (third from left) explaining mass rearing techniques

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About IITA

The goal of the International Institute of Tropical Agriculture (IITA) is to increase the productivity of key food crops and to develop sustainable agricultural systems that can replace bush fallow, or slash-and-burn, cultivation in the humid and subhumid tropics. Crop improvement programs focus on cassava, maize, plantain, cowpea, soybean, and yam. Research findings are shared through international cooperation programs, which include training, information, and germplasm exchange activities.

IITA was founded in 1967. The Federal Government of Nigeria provided a land grant of 1,000 hectares at Ibadan, for a headquarters and experimental farm site, and the Rockefeller and Ford foundations provided financial support. IITA is governed by an international board of trustees. The staff includes about 180 scientists and professionals from some 40 countries, who work at the Ibadan campus and at selected locations in many countries of sub-Saharan Africa.

IITA is one of the nonprofit, international agricultural research centers supported by the Consultative Group on International Agricultural Research (CGIAR). Established in 1971, CGIAR is an association of about 50 countries, international and regional organizations, and private foundations. The World Bank, the Food and Agriculture Organization of the United Nations (FAO), and the United Nations Development Programme (UNDP) are cosponsors of this effort.

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