Monitoring Insecticide Resistance on Planthopper Pests of Rice

(Draft of procedures)

Preparation and Rearing of Test insects

The test insects (figure 1) that will be used for the resistance monitoring network are the brown planthopper (BPH) and the whitebacked planthopper (WBPH). In addition, the smaller brownplanthopper (SBPH) will also be tested in Japan.

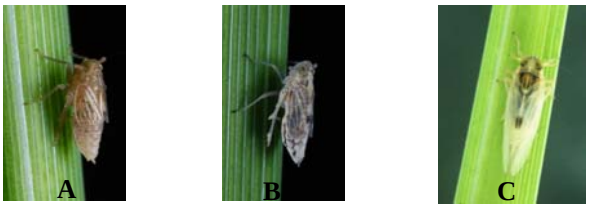

Figure 1: The test insects (A) Adult female brownplanthopper *Nilaparvata lugens (Stal*), (B) Adult female whitebacked planthopper, *Sogatella furcifera* (Horvath), (C) Adult female smaller brown planthopper, *Laodelphax striatellus* (Fallen).

In order to ensure adequate supply of test insects the rearing process needs to be well planned. The insect collection and rearing should provide test insects of uniform ages and sizes that are required for the bioassays.

Collection of Insects

An initial population of about 50 healthy unparasitized adult females or about 100 nymphs is collected from the study fields. Planthopper adults, preferably short-winged, are collected from the base of the rice plants using aspirator (by mouth or suction bulb) and placed into test tubes covered with nylon mesh (Figure 2a). Planthoppers can also be collected from the field using sweep net (Figure 2b).

Figure 2: Collection equipment (A) mouth aspirator and test tube covered with nylon mesh, (B) sweep net.

Preferably collect planthoppers from fields with high and low insecticide application regimes. Record the collection dates and locations of the collection.

The collected planthoppers are transferred into clean potted plants covered with circular or rectangular mylar cages. These cages should be prepared in the research center before going on the collection trip. If the collection site is far, collected insects can be

transferred to rearing cages with clean potted plants. In China and Japan, the collected insects are kept in test tubes with seedlings and small boxes with seedlings, respectively. Collection cages (Figure 3) are labeled with the respective collection dates and locations.

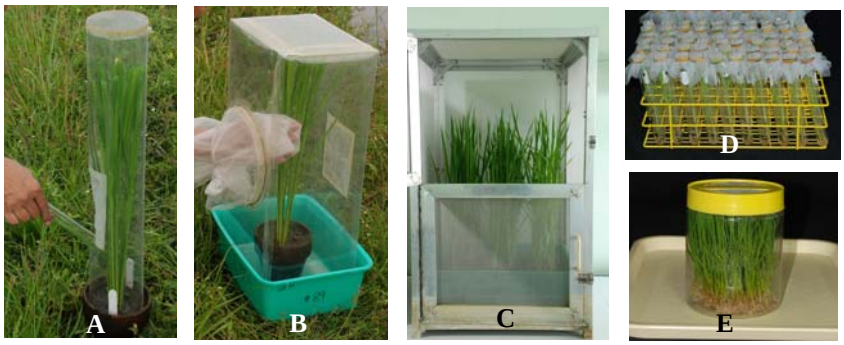

Figure 3: Collection cages (A) potted plant with circular mylar cage, 61 cm high and 10.5 cm in diameter, (B) rectangular mylar cage, measuring 29 cm x 21.5 cm x 56.5 cm, with potted plants (C) rearing cage, measuring 56.5 cm x 56.5 cm x 91.5 cm, with potted plants (D) test tubes with rice seedlings (E) box with rice seedlings.

The insects collected are brought back to the research center and reared in the greenhouse or insectary maintained with a temperature of 26±2°C, 12 hour light (tropical countries) and 25°C, 16 hour light (temperate countries).

Rearing Methods

a.) Use of aluminum cages

The planthoppers should be reared using a local susceptible rice variety. One month before the collection date, six 10-day-old- seedlings are planted in clay pots 10 cm in diameter. Fifteen days after transplanting, 2 grams of ammonium sulfate fertilizer is applied per pot. One week prior to planthopper collection, the potted plants are cleaned and the outer leaf sheets and/or infested tillers are removed. The potted plants are covered with mylar cages to avoid further infestation of other insect pests.

In the greenhouse or insectary, the adult males and females (at 1:1 ratio) are transferred into the oviposition (egg laying) cages (Figure 4) and labeled with the respective collection dates and locations. The oviposition cages are provided with 35-day-old clean potted plants which are replaced daily to have uniform populations. For insecticide testing, standardized insects are required and this is discussed later.

Figure 4: Aluminum rearing / oviposition cage (56.5 cm x 56.5 cm x 91.5 cm) with aluminum wire mesh on 3 sides, top and doors for ventilation.

Adult hoppers are removed from the oviposition cage and the nymphs are allowed to emerge. Each rearing cage can accommodate six to eight potted plants that can sustain 600-800 hoppers. The standard test insects (1- to 2-day-old female adults) are collected from these daily rearing cages. The plants are replaced every two days (or as needed) and transferred to new rearing cages (labeled with egg collection dates and location).

b.) Use of flexy glass cages

Another rearing method to mass rear planthoppers is the use of seedlings in a transparent flexy glass cage. The three sides of the cage are provided with fine mesh nylon cloth for ventilation. Insects are reared on seedling mats measuring about 22cm x 28cm (Figure 5).

Figure 5: Flexy glass cage (30 cm x 25 cm x 30 cm) with seedling mat.

The seedling mat may be made of 2 layer moistened gauze. About 12 grams seeds for 1 seedling mat are pregerminated for 3 days and sown on 2-layer moistened gauze on flexy glass trays. The mats are watered daily or as needed to keep the seedling mats wet. When the seedlings have grown and the roots are entangled in the gauze, the mats can be transferred into a rearing cage. Fifteen days after sowing, the mats can be fertilized using 1% urea about 2- 3 times or as needed while using the mats. Adult insects are introduced for oviposition and removed after one day. The cage can be inverted and another seedling mat is put into the cage as nymphs emerged. A seedling mat can accommodate 1000-2000 late instar nymphs.

Instead of gauze, peat moss may be used for the seedling mat. About 20 grams seeds for 1 seedling mat are pregerminated for 3-4 days and sown on moistened peat moss on flexy glass trays. The mats (Figure 6) are covered with rectangular flexy glass and are also watered as needed to keep the seedling mats wet.

Figure 6: Seedling mats (A) with germinated seeds and covered with rectangular flexy glass (B) one-week old seedling mat.

One week after seeding, the mats can be transferred into a rearing cage. Adult insects (100-200 pairs) are introduced for oviposition and removed after one week. The nymphs

are allowed to emerge and the seedling mat is replaced weekly until the planthoppers become adults.

Likewise, the rearing cages are placed in an insectary with a controlled temperature of 26±2°C, 12 hour light (tropical countries) and 25°C, 16 hour light (temperate countries).

Planthopper cultures are also protected from infestation of other pest species and natural enemies to maintain a homogenous culture as possible.

A schematic diagram of collection and rearing planthoppers is shown in Figure 7 below.

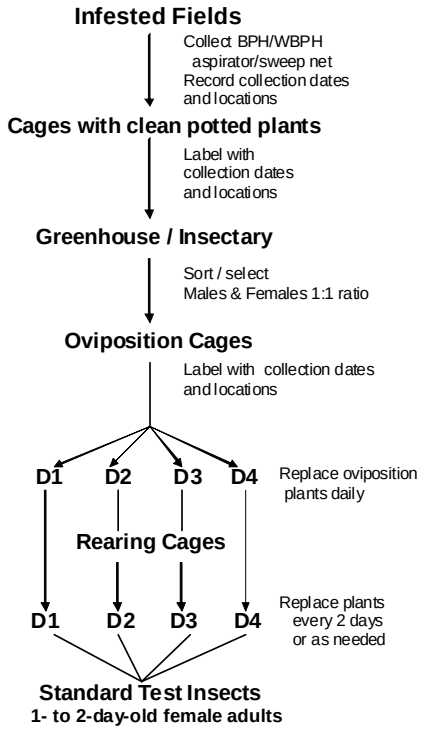

Figure 7. Schematic diagram of collection and rearing planthoppers for insecticide bioassays

Standardization of Test Insects

Insecticide bioassays in the laboratory need to have consistent results. This requires standardization of the test insects to be used for each treatment. Age, sex and physiological condition of the insects affect its susceptibility to insecticides. Thus, after collection, the second to fifth generation planthoppers should be used for the bioassay.

Newly emerged adult insects are generally more susceptible. Thus, 1-day-old to 2-day-old adults should be used in insecticide bioassays. To have approximately the same insect age, adults of the same sizes are collected from daily oviposition cages.

Both the brachypterous and macropterous adult female planthoppers can be used for insecticide treatments.

Prior to insecticide treatment, a batch of 20 test insects should be anaesthetized using CO_2. The fresh weight of the insects should be determined and then should be dried in an oven set at 70^{o}C for 36 hours to obtain the dry weights.

Estimating the Median Lethal Dose (LD_{50}) of Insecticides

The median lethal dose (LD_{50}) of insecticides is an accurate assessment of the comparative toxicity of the insecticides. The lower the LD_{50} values, the higher the toxicity or potency of the insecticide. It also quantifies the tolerance of an insect population to an insecticide treatment. In order to estimate and compare the toxicities accurately, there is a need to standardized methods.

Preparation of stock solutions

Technical grade (95% - 99% pure) insecticides are used for the laboratory tests. The active ingredient (a.i.) of the insecticides varies so a 100% stock solution (SS) is prepared using the correction factor (CF) below.

CF = 100% / %a.i. of the insecticides

For a technical insecticide with 99.5% a.i., CF = 100% / 99.5% = 1.005.

Given the CF, compute the weight of the technical insecticide needed to prepare the desired volume and concentration using the formula:

Concentration of insecticides x Volume x CF

To prepare 2.5 ml of 10000 ug/ml SS, the weight of insecticide needed will be

10000 ug/ml x 2.5 ml x 1.005 = 25,125 ug = 25.125 mg = 0.025 g.

Weigh 0.025g of technical grade insecticide in a 6 ml screw cap vial using the analytical weighing balance (Figure 8) and add 2.5 ml of technical grade acetone.

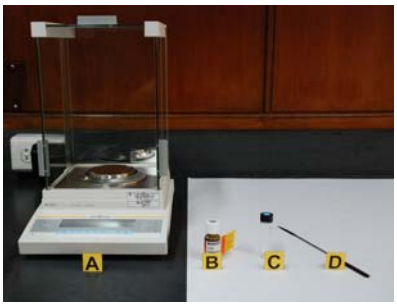

Figure 8: Materials for preparation of stock solution (A) analytical weighing balance, (B) technical grade insecticide, (C) 6 ml screw cap vial, (D) spatula.

Preparation of insecticide concentrations for tests

The insecticide concentrations (at least 5) should give a range of 5-95% insect mortality based on a preliminary unreplicated test. The serial dilution starts from the highest to the lowest concentration

From the SS, prepare serial dilutions using the equation $C_1V_1 = C_2V_2$, where:

C_1 = initial concentration
V_1 = initial volume
C_2 = Final concentration
V_2 = final volume

To prepare 2 ml of 5000ug/ml from 10000 ug/ml SS, the volume needed using the formula above will be: (10000ug/ml) (x) = (5000 ug/ml) (2 ml) = 10000x =10000

x = 1 ml SS + 1 ml acetone

Continue the serial dilutions using the above equation or do a 1:1 dilution of 10-12 concentrations (Figure 9). Secure the cap of the vials with parafilm to minimize evaporation. Store the prepared insecticide dilutions in a refrigerator (4^{o}C) or freezer (preferably -20^{o}C). Replace and dispose properly the pipettor tips after preparation of an insecticide.

Figure 9: Materials for preparation of serial dilutions (A) stock solution, (B) 6 ml screw cap vials with label, (C) technical grade acetone, (D) pipettor tips, (E) pipettor, (F) parafilm strips, (G) disposable nitrile gloves, (H) disposable mask.

Preparation of recovery cages with seedlings

Use 7- to 10-day-old rice seedlings (at least 15) of any local susceptible variety. Fold half of the paper towel into three and wrap the roots of the seedlings. Soak the seedlings in water just enough to avoid the seedlings from drying. Put the prepared seedlings inside the clear tumbler cages labeled with the insecticide treatment, dose and replication (Figure10).

Figure 10: Recovery cages preparation (A) 7- to 10-day-old rice seedlings of any local susceptible variety, (B) folded paper towel, (C) wrapping seedling roots with paper towel, (D) 15 rice seedlings wrapped with paper towel and soaked in water, (E) clear tumbler cage (12.5 cm tall, 7 m in dia.) with seedlings and cotton stopper.

Topical Application

The final treatment also used at least 5 concentrations and a minimum of three replications with 20 insects per replication.

Two days before insecticide treatment, transfer the daily rearing cages with 5^{th} instar nymphs to the testing room. This is done to acclimatize the planthoppers to be used for the bioassays.

The treatment starts with all the control insects treated with analytical reagent acetone then followed by the insecticide treatment from the lowest to the highest concentration.

Prior to treatment, the planthoppers, 1- to 2-day-old female adult (BPH or WBPH) are collected from the culture cages using an aspirator. They are confined into a vial with wire mesh screen. Ten insects are collected per vial and anaesthesized with carbon dioxide (CO_2) for 10-30 seconds to facilitate handling during treatment (Figure 11).

Figure 11: Preparation of test insects for topical application (A) collection of 10 female adult planthoppers (1- to 2-day old) into a vial with wire mesh screen cap, (B) anaesthetization with CO_2.

The anaesthetized insects are transferred on a watchglass wrapped with gauze secured by rubber band. Insecticide is applied topically with a Hamilton Repeating Dispenser + 10 ul microsyringe (Figure 12). A 0.2 microliter (ul) of the insecticide is applied on the thoracic region of each test insect.

Figure 12: Topical application (A) anaesthetized planthoppers on watchglass wrapped with gauze, (B) Hamilton repeating dispenser and microsyringe.

The treated insects are transferred in clear tumbler cages through a funnel with the aid of small camel-hair brush to minimize mechanical damage (Figure 13). The cages with treated insects are placed in a controlled room with a temperature range of 25-30^{o}C and 12 hour light (tropical countries) or 16 hour light (temperate countries).

Figure 13: Treated planthoppers are transferred into the clear tumbler cages through a funnel.

After an insecticide treatment either the gauze or the whole watchglass covered with gauze is replaced to avoid contamination of the new batches of test insects with the previous insecticide.

Twenty four hours after treatment, insect mortality is recorded in a data sheet. Moribund insects are considered dead. Continue mortality count up to 48 and 72 hrs after treatment in some insecticide group.

Recorded data can be summarized and encoded in an Excel File (Figure 14). From these data, the LD_{50} values are estimated using the PoloPlus probit program (to be described in later chapter) and will be recorded in ug/g body weight of the insect.

Microsoft Excel - quantal response summary data sheet

File Edit View Insert Format Tools Data Window Help

K29

	A	B	C	D	E	F	G
1	Quantal Response Data Summary Sheet						
2	Date						
3	Country/Location						
4	Chemical						
5	Method						
6	Amt Applied						
7	Temperature						
8	Rel. Humidity						
9	Insect						
10	Age						
11	Sex						
12							
13	Dose (ppm)	Total insects	# Dead	Log Dose	% Mortality	Corr. Mortality*	Probit
14–49							
50							
51	*Abbotts = (Po-Pc) / (100-Pc) * 100			where Po = % observed mortality in treated			
52				Pc = % control mortality			
53–56							

Sheet1 Sheet2 Sheet3

Ready

Figure 14: Data recording sheet