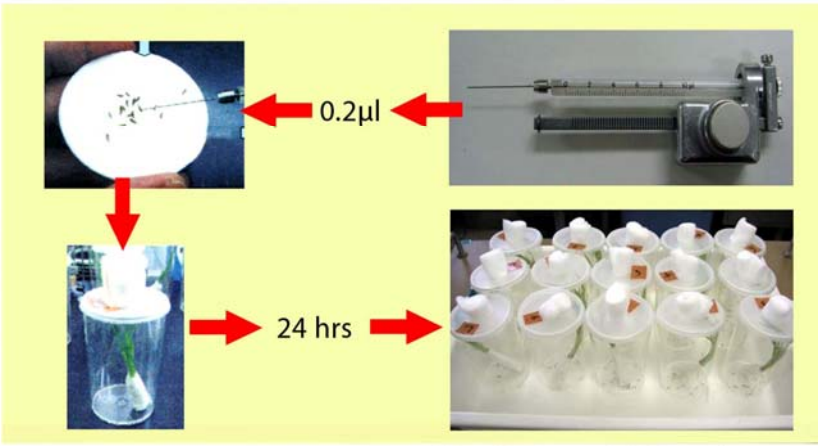
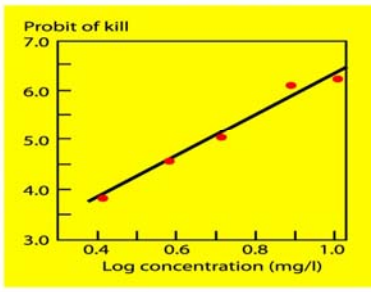
WORKSHOP REPORT

Rice Planthopper Project

Workshop/Training on Toxicology and Insecticide Resistance Monitoring





ADB

27 April - 1 May 2009
IRRI, Philippines

IRRI

8 May 2009

SUMMARY

The Workshop/Training on Toxicology and Insecticide Resistance was held in IRRI, Philippines on 27 April – 1 May, 2009. The twelve participants were mostly junior scientists from NARES who will be involved in the insecticide resistance network.

Dr. K. L. Heong, the principal investigator, formally opened the workshop and introduced Dr. R Zeigler, the IRRI Director General, who delivered the welcome address.

Dr. K.H. Tan, an insect physiologist, provided a refresher lecture on insect biochemistry, physiology, insecticide classification and their modes of action. The metabolic processes provide an understanding of how insecticide affects the insects in general. The appropriate use of insecticides may provide solutions or delay the development of resistance.

The collection and rearing of planthoppers in IRRI, China and Japan was presented by Ms L. Fabellar, Dr. Z. Lu and Dr. M. Matsumura, respectively. According to the speakers, the standardization of insecticide resistance techniques should start from the collection and rearing methods of planthoppers.

Ms. L. Molina, manager of the IRRI analytical service laboratory, presented pointers on safe handling of insecticides in the laboratory. She emphasized the need to know the chemical properties of the insecticide and the safety equipment needed in handling chemicals. She also advised participants to obtain a copy of the material data sheet (MSDS) for easy reference.

Dr. Matsumura, the resource person from Japan, discussed the preparation of insecticide serial dilutions and insecticide treatment by topical application. The participants did the actual topical application from insect collection, anaestization, preparation of insecticide dilutions, preparation of holding cages, data collection and analysis.

Dr. Tan also covered probit analysis designed for quantal response bioassay which estimates the relationship between response(s) and a given stimulus.

Dr. Heong showed participants how to install the the statistical software (PoloPlus) and its application using the data sets from the workshop participants' experiment.

Finally, an insecticide monitoring network was initiated and participans decided on the standard procedure for the bioassay, developed and presented their work plans

Background

The ADB-IRRI Rice Planthopper Project was initiated in 2008. The project will address management of planthopper outbreaks. NARES partners will be involved in monitoring insecticide resistance as a key indicator of pest outbreak.

The workshop-training will provide basic knowledge on insect physiology and toxicology. It will also focus on the standardization of monitoring techniques, which include planthopper collection, rearing, bioassay, data collection and analysis.

Workshop Objectives

1. To understand basic knowledge of insect physiology, toxicology of insecticides, standardization of bioassay techniques, insect rearing, data collection and analysis.

2. To involve junior scientists from NARES partners in an international insecticide resistance network that will conduct continuous monitoring to avoid or slow down insecticide resistance development.

Group Photo

Resource persons

M. Matsumura	National Agricultural Research Center for Kyushu Okinawa Region National Agriculture and Food Research Organization, Kumamoto, Japan.
L. Molina	Asst. Manager II, Analytical Service Laboratory, IRRI
K. H. Tan	Professor of insect physiology (retired) Currently Company Chairman of Nestle Products, Malaysia
Zhongxian Lu	Institute of Plant Protection, Zhejiang Academy of Agricultural Sciences, Hangzhou, China

Participants

China

Yan Ling	Institute of Plant Protection, Guangxi Academy of Agricultural Sciences, Nanning.
Yalin Bian	College of Life Sciences, China Jiliang University, Hangzhou.
Guihua Chen	Plant Protection Station, Jinhua, Zhejiang
Facheng Zhang	Plant Protection Station, Jinhua, Zhejiang.

Thailand

Wantana Sriratanasak	Bureau of Rice Research and Development, Rice Department, Bangkhen
Sukanya Tepundung	Bureau of Rice Research and Development, Rice Department, Bangkhen
Chairat Channu	Chainat Rice Research Center, Bureau of Rice Research and Development, Rice Department, Bangkhen

Vietnam

Pham Van Tuong	Pesticides Control Center for South Vietnam, PPD Ho Chi Minh City
Nguyen Pham Hung	Pesticides Control Center for North Vietnam, PPD Hanoi
Le Van Thiet	Southern Regional Plant Protection Center, Long Dinh

Malaysia
Maisarah Mohamad Saad MARDI Alor Setar

Wan Khairul MARDI Serdang.

Philippines
Carlos Garcia

IRRI
J.L. Catindig
L.T. Fabellar
K. L. Heong
S. Villareal

Workshop Outputs

The participants agreed on the standardization of preparation/rearing of test insect, preparation of insecticide dilutions, bioassay techniques, data collection and analysis.

1. Protocol

Preparation/Rearing of test insects

1. Test Insects
   - BPH
   - WBP
   - SBPH

2. Insect collection

   a. methods
   - Aspirator (by mouth or suction bulb depending on the user)
   - Sweep net

   b. No of insects/site = $\geq$ 50 healthy unparasitized adult females or
   = $\geq$ 100 nymphs
   - Remove other insects/predators
   - Collect from fields with high and low insecticide application

   c. Cages (depend on the country)
   - clean potted plants with circular/rectangular mylar cage
   - rearing cage with clean potted plants
   - test tubes with seedlings
   - small box with seedlings

3. Rearing test insects

a. Variety – any local susceptible variety

b. Seed soaking/germination duration (depend on the temperature of the country)

c. Rearing Method

1. Potted plants = use clean 35 day-old plants after sowing
   - Remove outer leaf sheaths
   - Remove infested tillers/leaves
   - Remove other pests & natural enemies
   - Isolate for a week to prevent other insect infestation

2. Seedling box
   Age of seedling mat - 14 DAS (tropical countries)
   - 5-7 DAS (temperate countries)
3. Rearing room
   Temperature = 25^{o}C – 30^{o}C
   Illumination = 16/8 (Japan);12/12(Tropical countries)

Standardization of test insects

1. Generation = 2-5 generation after collection
2. Age = 1-2 day old adults (macropterous or brachypterous)
3. Sex = female
4. Size = uniform

Preparation of stock solutions

- Use technical grade (95% -99.9%)insecticides

- Prepare a 100% stock solution (SS)
  using the correction factor (CF)
  CF = 100% / % a.i. of the insecticide

- Prepare 1% SS

- Weigh 25.125 mg or 0.025g of technical grade insecticide using the analytical weighing balance.

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

Preparation of different insecticide concentrations

- From the SS, prepare serial dilutions (1:1) using the equation:
  C1V1 = C2V2

C1 = initial concentration
V1 = initial volume
C2 = Final concentration
V2 = final volume

- Prepare 2 ml of 5000ug/ml from 10000 ug/ml SS

  (10000 ug/ml) (x) = (5000 ug/ml) x (2 ml)

  10000x = 10000

  x = 1 ml SS + 1 ml acetone

- Start the dilutions from the highest to the lowest concentration.

Insecticide Treatment

For countries with no controlled room, bring the test insects to the experimental room and acclimatize for 2 days to avoid condition shock.

When test insects are not enough on testing day, do 1 replication for the 5 doses and do the 2nd replication the next day. Finish the test within the week.

Record the fresh weight of 20 control insects (mg or g). Put the insects in 70^{o}C oven for 48 hours and record the dry weight.

Prepare insecticide dilutions on or before treatment. Keep the prepared dilutions in freezer (-20^{o}C) but should be used within a week.

- Do topical application with a Hamilton Repeating dispenser + microsyringe (10ul).

- Do preliminary unreplicated test to estimate the insecticide concentrations that will give a range of 5-95% insect mortality.

- For the final test use at least five (5) concentrations and a minimum of three (3) replications.

- Use twenty insects per replication. Anaesthetized 10 insects at a time with carbon dioxide (CO_2) for 10-30 seconds**.**

- Transfer anaesthetized insects on a watch glass wrapped with gauze secured by rubber band.

- Apply 0.2 microliter (ul) of the insecticide on the thoracic tergites of test insect.

- Start treatment with all the control insects with analytical reagent acetone followed by the insecticide treatment from the lowest to the highest concentration.

- Transfer treated insects in plastic tumbler cages (with 15 one-week-old seedlings) through a funnel to avoid mechanical damage.

- After an insecticide treatment, replace either the gauze or the gauze covered watch glass to avoid contamination of the new batches of test insects with the previous insecticide.

- Put treated insect in a controlled room with temperature range of 25-30^{o}C, illumination of 16/8 (temperate countries) and 12/12 (tropical countries).

- Use thermohydrograph to monitor the temperature and humidity.

- Observe mortality 24 hrs after treatment. Moribund insects are considered dead. Continue mortality count up to 48 and 72 hrs after treatment in some insecticide group.

- Calculate LD50s using PoloPlus and record as µg/g body weight.

2. Work Plan

	2009											
Activities	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
China												
Workshop				X								
Finalise protocol and send out					X							
Prepare expt lab in country					X							
Prepare rearing system						X						
Collect insects from sites						X	X					
Insect rearing							X	X				
Prepare stock solution/dilutions							X	X				
Conduct topical application								X	X			
Data analysis									X	X		
Malaysia												
Workshop				X								
Finalise protocol and send out					X							
Prepare expt lab in country						X	X					
Prepare rearing system						X	X					
Collect insects from sites							X					
Insect rearing							X	X	X	X	X	
Prepare stock solution/dilutions										X		
Conduct topical application										X	X	
Data analysis											X	X
Follow up workshop												X
Thailand												
Workshop				X								
Finalise protocol and send out					X							
Prepare expt lab in country						X	X					

Prepare rearing system							X					
Collect insects from sites							X	X	X	X		
-Chainat / Nakhon Pathom							X	X	X			
-Chiangmai									X	X		
Insect rearing							X	X	X	X	X	
Prepare stock solution/dilutions								X				
-Technical grades (3)							X					
Conduct topical application									X	X	X	X
Data analysis											X	X
South-Vietnam												
Workshop				X								
Finalise protocol and send out					X							
Prepare expt lab in country					X	X						
Prepare rearing system					X	X	X					
Collect insects from sites							X					
Insect rearing							X	X	X			
Prepare stock solution/dilutions								X	X	X		
Conduct topical application									X	X		
Data analysis											X	X
North-Vietnam												
Workshop				X								
Finalise protocol and send out					X	X						
Prepare expt lab in country					X	X						
Prepare rearing system						X	X					
Collect insects from sites								X	X			
Insect rearing								X	X	X		
Prepare stock solution/dilutions									X	X		
Conduct topical application									X	X	X	
Data analysis											X	X
IRRI												
Workshop				X								
Finalise protocol and send out					X							
Prepare expt lab in country					X	X						
Prepare rearing system						X						
Collect insects from sites						X		X		X		X
Insect rearing						X	X	X	X	X	X	X
Prepare stock solution/dilutions						X	X	X	X	X	X	X
Conduct topical application						X	X	X	X	X	X	X
Data analysis						X	X	X	X	X	X	X