

***Ecological Engineering to Reduce Rice Crop
Vulnerability to Planthopper Outbreaks***

**Interim Technical Report III: Visit to Vietnamese
ecological engineering research site at Cai Be and
ADB 13th RETA 6489 Review and Planning
Workshop *Reducing Pest and Postharvest losses in
Rice Production* November-December 2009.**

Geoff Gurr

Charles Sturt University, Australia.

ggurr@csu.edu.au

Introduction

As detailed in Interim Technical Reports I and II, rice production in the tropics of Asia is under threat from insect pests, particularly planthoppers. Insecticide- and host plant resistance-based control is faltering. There is a need to restore ecosystem resilience to make biological control better able to contribute to IPM. Ecological engineering (Gurr et al. 2004) provides a framework to strengthen essential pest management ecosystem services that will improve crop health, thus preventing secondary pest outbreaks, like planthoppers. Prospects for this to be done in tropical rice are good (Gurr, 2009).

The utilization of these principles for rice pests is now being explored with the assistance of Professor Geoff Gurr of Charles Sturt University (CSU). Prof Gurr is collaborating with the International Rice Research Institute (IRRI) and other organizations, initially undertaking *ad hoc* trips to the Philippines and China and now under the terms of a Letter of Agreement for the project entitled *Ecological Engineering to Reduce Rice Crop Vulnerability to Planthopper Outbreaks*.

This interim technical report concerns the third trip undertaken by Prof Gurr under the terms of the Letter of Agreement. This visit was to the ecological engineering research site established at Cai Be, Tien Giang Province, Vietnam on 1st December 2009. That visit was part of the ADB 13th RETA 6489 Review and Planning Workshop *Reducing Pest and Postharvest losses in Rice Production* held in Ho Chi Minh City from 30 November to 3 December 2009.

Activities and Recommendations

Visit to Vietnamese ecological engineering research site at Cai Be

Prof Gurr travelled to the ecological engineering research site located at Cai Be in Tien Giang Province on 1st December 2009 accompanied by other participants in the ADB 13th RETA 6489 Review and Planning Workshop *Reducing Pest and Postharvest losses in Rice Production* including local collaborators and Dr LK Heong (International Rice Research Institute, IRRI) and Prof Cheng Jia An (Zhejiang University) (Figure 1).

This was Professor Gurr's second visit to the site and great progress had been made since the first visit in July that year (see Interim Technical Report I). Over the intervening five months, high quality signage providing detail on the use of ecological engineering had been erected (Figure 2). A range of plant species had been established on the main earthen bank separating the rice fields from the adjacent river. Insect monitoring had also commenced in the newly established rice crops.



Figure 1. Participants in the ADB 13th RETA 6489 Review and Planning Workshop *Reducing Pest and Postharvest losses in Rice Production* inspecting the ecological engineering research site located at Cai Be in Tien Giang Province on 1st December 2009. (Photograph: GM Gurr).



Figure 2. Example of the signage at the ecological engineering research site located at Cai Be in Tien Giang Province (Photograph: GM Gurr).

Sticky trapping, yellow water pan traps and a vacuum sampler were demonstrated (Figure 3). Growers had been encouraged to avoid insecticide spraying, particularly in the 40 days after sowing. The 'Escape Strategy' (based on synchronizing rice plantings within a district to occur after a decline in rice plant hopper catches in light traps) had also been employed

The ecological engineering site extended over an area reported to be approximately 30ha and involved 36 farmer households. A control site was located 1km away from the ecological engineering site.

Both Professor Gurr and Dr Huynh (local collaborator) were interviewed by a local TV station, filmed on the ecological engineering site.

Approximately 2km of earth bank was reported to be planted with flowers at the ecological engineering site and, as an indicator of the practicality and potential popularity of the method, an additional 27 km had been similarly planted in nearby areas. These plants had resulted partly from withholding the herbicides normally used to control weeds on the bunds.

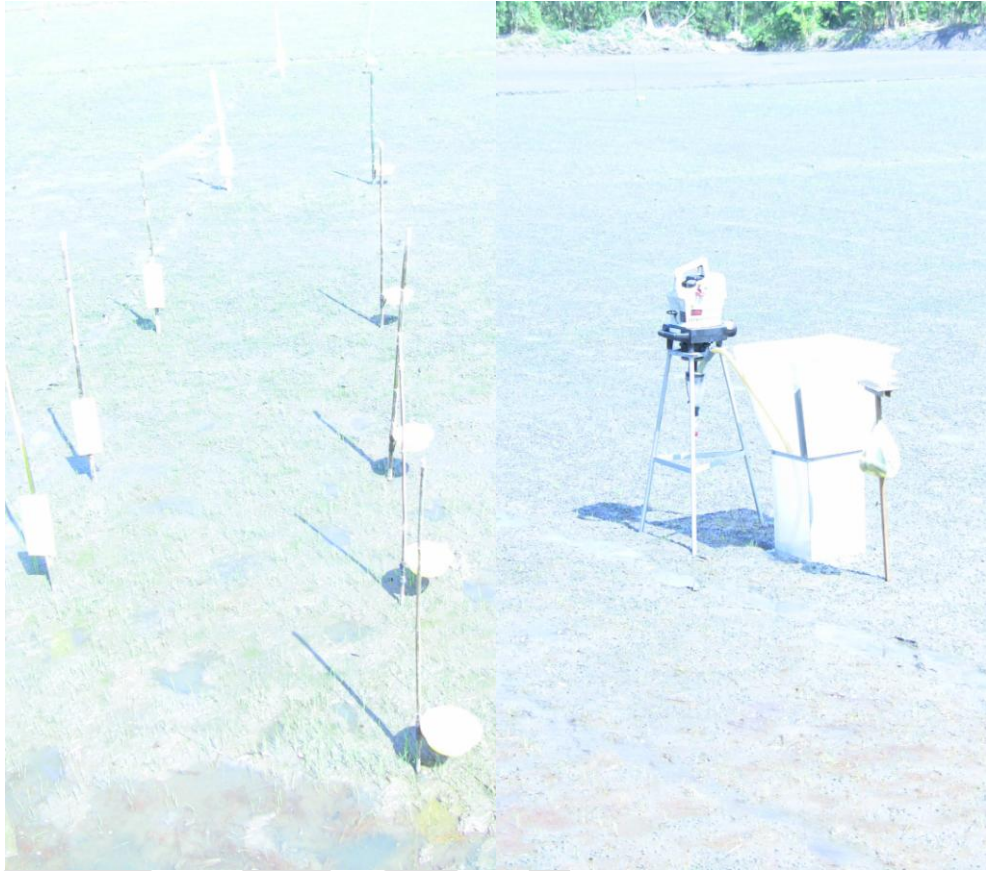


Figure 3. Transect of sticky traps and yellow water pan traps (left) and vacuum sampler (right) at Cai Be in Tien Giang Province (Photographs: GM Gurr).

Lantana, sesame, a yellow composite and an unidentified, prostrate purple flowered plant had been planted in large numbers (Figure 4). Allied to this, discontinuation of herbicide use on the earth bank had led to good levels of understory vegetation in the pre-existing, perennial woody vegetation (Figure 5).



Figure 4. Plants established on earth bank beside rice fields in ecological engineering site at Cai Be in Tien Giang Province. Clockwise from top left: lantana, sesame, a yellow composite and an unidentified, prostrate purple flowered plant (Photographs: GM Gurr).



Figure 5. Understorey vegetation in the perennial woody vegetation on earth bank beside rice fields in ecological engineering site at Cai Be in Tien Giang Province. (Photograph: GM Gurr).

Rice was in an early stage of vegetative growth stage. This was one of three rice crops typically grown each year in the region in contrast to the two usually grown in other regions of the country.

The overall impression was of a well set up research site with a good level of cooperation from the owner farmers. This was evident in the interactive session held on site when cooperating farmers were present and their views expressed (via an interpreter) (Figure 6). The leader of the farmer group reported that they had been encouraged to implement ecological engineering because pesticides were considered expensive and hard work to apply. Mortality of fish and chickens had also been a problem.



Figure 6. Workshop participants meet cooperating farmers at the ecological engineering site at Cai Be in Tien Giang Province. (Photograph: GM Gurr).

Other than sampling related issues that are dealt with in the workshop section of this report, the issues relating to site set up are relatively minor. Access to the site involved crossing a river by boat since there is no direct vehicle access. This constitutes an impediment to its use as a demonstration area.

The earthen bank on which the flowering plants had been sown was reported to have very acid soil and this constrains the range of plant species that could be grown in the setting.

Limited information was available on the plants established on the earth banks. It would be useful for the local leaders to provide a detailed report containing information such as local and scientific name of plants, planting dates etc. This information will be required when the team prepares publications for scientific journals.

ADB 13th RETA 6489 Review and Planning Workshop *Reducing Pest and Postharvest losses in Rice Production* (30 November – 3 December 2009)

The overall aims of this workshop were to review progress to date in the Asian Development bank-funded, IRRI-led project and to plan for 2010 work. Professor Gurr's activities were confined to 'Subcomponent 1' of the project which was concerned with field losses rather than postharvest losses.

Prof Gurr co-convened with Prof Cheng Jia An the outgroup meeting on ecological engineering research methods. Detailed discussions took place involving collaborators from China, Vietnam, Thailand and the Philippines (Figure 7). Major discussion points were: (i) progress to date (ii) how best to handle identification and sorting of sampled arthropods, (iii) achieving consistency of sampling methods for all sites (iv) workload considerations.



Figure 7. Workshopping ecological engineering research methods at the Rex Hotel, Ho Chi Minh City 30 November 2009. (Photograph: GM Gurr).

Since the formal inception of the project in November 2008, ecological engineering sites had been established in eastern and southern China, Vietnam, and Thailand. Work had been broadly as planned in earlier meetings and as set out in a sampling protocol document

(see Interim Reports I and II). Work in Eastern China started in 2008 but that in the other three sites commenced August-September 2009. Data from the Chinese sites at Jinhua and Lingui showed that arthropod biodiversity in the ecological engineering areas planted with soybean and sesame were higher than those in control areas. Results also indicated that natural enemy numbers were significantly enhanced by ecological engineering. Though density of rice planthoppers were significantly lower than those in control areas, overall numbers were low so further work is required.

There was general agreement that there was a need to standardize methodology for data analysis, including form format and the parameters to be used. More specific issues raised were that it was difficult to use sweep nets to take samples at the precise spatial intervals defined in the sampling protocol document. It was suggested that sampling positions be defined as ranges such as 0-1m, 9-10m and 14-15m.

There was concern about the overall level of labour involved and discussion explored scope for rationalizing sampling methods. Vacuum sampling was considered important and most participants considered sweep net, yellow pan, trap plants (with eggs) and light trapping were also good. Some considered sticky traps and pitfall traps not necessary. Others thought these important for understanding movement patterns.

Identifying all specimens was reported to be difficult and time consuming. Discussion considered what level of taxonomic identification was necessary. Prof Gurr recommended that morphospecies be used. Thus individual arthropods were identified as, for example, Braconidae 3 or Salticidae 5. A voucher specimen of each of these morphospecies is kept available when identifying and counting further samples. Analysis can be done on such count data and only later is there a need to use specialist taxonomic services to name each of the significant morphospecies. IRRI staff considered that their existing insect collection, taxonomic skills and identification aids would be a useful resource and agreed to run an identification workshop for participants from each country in 2010. There was also extensive discussion about the intensity of sampling. A prevailing view was that investigations of population dynamics of each planthopper species require sampling at 5-7 day intervals for the whole season.

Subsequent to the workshop Prof Gurr assisted Prof Cheng in developing a revised sampling protocol document (Appendix 1).

Conclusions

There are relatively minor issues to be resolved in relation to the ecological engineering site at Cai Be in Tien Giang Province.

Progress in the ecological engineering objective of the *Reducing Rice Crop Vulnerability to Planthopper Outbreaks* project has been good. All four of the planned sites have been established and data collection commenced. Sampling has been broadly as set out in the previously agreed protocol document. Preliminary results from China look extremely encouraging. A high level of good will and cooperation is evident within the multi-agency, international research team. Workload issues and refinement of field sampling and arthropod identification need to be addressed but agreement was reached at the workshop in relation to the necessary action.

One broader issue has been highlighted in email discussions after the Ho Chi Minh workshop. The team might consider a 'contingency plan'; to assure a good test of ecological engineering in case things do not go smoothly with the multi-country experiment. The overall plan to have an eco-engineering site and control site in each of four locations (Jinhua, Guilin, Thailand and Vietnam) gives four replicates each of the two treatments. Though the number of replicates is quite small, biometrical advice is that this is adequate given that we are expecting major differences between the two treatments for measures like numbers of natural enemies, numbers of pests, parasitism rates etc. If significant effects are evident, that work will be publishable in a top journal like *PANS* or even *Science/Nature*. The attraction of this design is that it will allow us to capture the effects that are a consequence of landscape scale effects rather than local effects and this is a very important current theme in pest ecology. A simple example of this is that under eco-engineering natural enemies may build up and have a greater impact on pest populations at a scale of hundreds of meters rather than at the scale of individual fields. There is a downside, however, of this design: it is critical to have comparable data from each of the four sites. If just one site does not collect comparable data (or has a mishap like spray drift/drought) it lessens our chances of a robust statistical comparison and publication.

A contingency plan would involve initiating additional experiments. This needs to occur remote from the current eco-engineering and control plots (eg >500m). It should employ a simple randomised block design and - ideally - the following treatments: (i) control (normal farmer practice), (ii) low pesticide/zero pesticide (depending on what can be realistically achieved), (iii) eco-engineering (sesame, wild rice etc), (iv) low pesticide AND eco-engineering combined. Each treatment should be replicated once in each of at

least 4 areas. In Appendix 2 this is illustrated schematically for 5 replications. Experimental fields should not be adjoining, rather be separated by at least one standard/normal field.

If this were not achievable then a comparison of the control versus ecological engineering plus low pesticide (ie the same two treatments as are used in our multi-country experiment) would be useful. At least five replications would be appropriate for this option.

Other experiments could test hypotheses relating to the utility of ecological engineering at still smaller scale. Laboratory/growth chamber/greenhouse studies could test the longevity and realised fecundity of a few parasitoid species when provided with the nectar from a range of candidate bund plants (eg sesame) following the methods of Baggen and Gurr (1998). The next step up in scale is to use small plots of different flower species laid out beside a single field in a replicated block design. At a larger scale, 4-6 fields could be established with eco-engineering along one edge and have the opposite edge as a control. It is recommended that the team discuss such options at the next planning workshop scheduled for March 2010 in Bangkok.

References

- Baggen, L. R. & Gurr, G. M. (1998). The influence of food on *Copidosoma koehleri*, and the use of flowering plants as a habitat management tool to enhance biological control of potato moth, *Phthorimaea operculella*. *Biological Control*. 11: 9–17.
- Gurr, G.M. 2009. Prospects for ecological engineering for planthoppers and other arthropod pests in rice. Pp 371 - 389. In Heong, K.L. and Hardy, B. (eds.) Planthoppers – New threats to the sustainability of intensive rice production systems in Asia. International Rice Research Institute, Los Banos, Philippines.
- Gurr G. M., Wratten, S. D. & Alfieri M. A. (eds). (2004). *Ecological Engineering: Advances in Habitat Manipulation for Arthropods*. CSIRO Publishing, Melbourne (Australasian publisher)/ CABI International, Wallingford (European Publisher)/ Cornell University Press, Ithaca (America's publisher). 244 pp. ISBN 0643090223.

Acknowledgements

Prof Cheng Jia An is thanked for making available his notes on workshop discussions. He also took the lead role in revising the sampling protocol document (Appendix 1) to reflect changes agreed in Ho Chi Minh.

DRAFT

Appendix 1:

Sampling protocols for studying ecological engineering for rice pest suppression in irrigated tropical rice

Overview

As in all rigorous research, the methods to be used are determined by the hypothesis to be tested. Accordingly it is worth making this hypothesis explicit:

H⁰: Implementing ecological engineering improves biological control of pests by enhancing biodiversity compared with farmer's practices using insecticides (control).

The following sections that set our sampling protocols are organized according to our collection of data to test this key hypothesis. It is important that data collection is careful and that the agreed protocols are followed because of the following reasons: Our experimental design is one, multi-site (Thailand, Vietnam, China (Guilin), China (Jinhua)) experiment with each site having a single replicate of each of the two treatments: ecological engineering versus *farmer's practices using insecticides (control)*. Accordingly, if any site uses different methods or fails to collect data for any given aspect the whole experiment is compromised. Note that although it is critical to collect data from the ecological engineering and the control areas on all sites in a consistent manner the integrity of the design does not require us to use identical ecological engineering methods on all sites. There is no problem with sites using different flower species, sowing dates, varieties etc. Ecological engineering is about implementing management practices that are appropriate for a given locality and acceptable to farmer practice.

So remember: okay to implement locally applicable ecological engineering practices but MUST use the agreed sampling methods.

Some sites may wish to test supplementary hypotheses. This is encouraged provided that they do not compromise the testing of our key hypothesis. What does this mean? It would be a bad idea to have replicated plots of sprayed and unsprayed plots/fields in the ecological engineering and insecticide spraying (control) areas. Doing this would lead to increased numbers of natural enemies in the insecticide spraying (control) area and decreased numbers in the ecological engineering area. Accordingly, the insecticide spraying (control) area on each of our four sites should be managed according to conventional farmer practice whereby insecticide use is the mainstay of pest management. It would be okay to test supplementary hypotheses that do not compromise the key hypothesis. Examples of these are:

- Sow small, replicated plots of different flower species in one part of the eco-engineering area and sample these to see which attracts the most natural enemies.

- Laboratory bioassays to measure effects of different flower species on parasitoid fitness (see Ricehoppers site for detail on this)
- Mark natural enemies in flowers with rubidium chloride and follow the movement of these into rice.
- Analyze gut contents of predators to identify prey species consumed (see Ricehoppers site for detail on this).

We plan to seek additional funding to develop molecular (DNA ‘barcoding’) identification methods for natural enemies captured in this project so you will notice later sections specify use of 100% ethanol for specimen storage. This will maximize DNA quality and is especially important for samples collected by sweep net and for parasitoids reared from egg trap plants because these specimens can be placed immediately into preservative without any degradation.

Sampling methods for key hypothesis

H⁰: Implementing ecological engineering improves biological control of pests by enhancing biodiversity compared with farmer’s practices using insecticides (control).

Testing this hypothesis will require staff at each of the four sites to collect data from their ecological engineering area and the *farmer’s practices using insecticides (control)* area in following manner.

Sampling in the two areas should be with the same equipment, using the same staff and as far as possible on the same day and approximately the same time of the day so that any differences between the two experimental treatments is attributable to the treatments and not an artifact of sampling.

The sampling methods outlined below should be used in the *approximate centre of rice fields (i.e. at least 10m from the field margin) and near the rice bunds (i.e. about 1m from the bunds) and from 10 fields in the ecological engineering and 10 fields in the farmer practice with insecticide spray (control) areas*. Sampling by different methods should not be done on the same point, which was sampled before using another method (refer to sampling lay out). If different varieties have been sown in each of these areas (or there is some other difference such as fertilizer use/ sowing date/ irrigation etc) then fields should be matched as far as possible so that there is a ‘like for like’ field in the ecological engineering and farmer practice with insecticide spray (control) areas.

For 10 fields in the ecological engineering and 10 fields in farmer practice with insecticide spray (control) take sample at the seedling, tillering, booting and milking stages of rice growth (i.e. samples at each of the four major growth stages) (refer to the general rice growth stages illustrated below) using the following methods:

- Yellow sticky trap (three traps mounted on wooden stakes to be just above the crop canopy with 5 meters distance between traps installed at the center of the rice field and near the rice bund, collected after 24 hr). There will be one hundred twenty samples for each sampling period.
- Yellow pan trap (three traps mounted on stand at approximately the same height of vegetation or just below the canopy level with 5 meters distance between traps at the center of the rice field and near the rice bund, left in place for 24 hr). There will be one hundred twenty samples for each sampling period.
- Blower-vac sampler (single sample from an undisturbed part of the center of the field and near the rice bund). There will be forty samples for each sampling period.
- Sweep net (30 sweeps whilst walking slowly through an undisturbed part of the center of the field at approximately 0.5 m/sec and 30 sweeps on the rice hills at least 1m from the rice bunds). There will be forty samples for each sampling period.

All the sampling methods should be used at the center part of the rice fields as well as close to (about 1 m) the rice bunds.

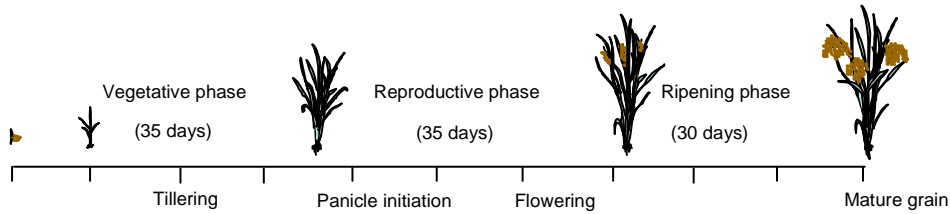
In addition to sampling on different growth stages of the rice crop, take sample of parasitoids during peak abundance of planthoppers (this will be determined based on the population of hoppers from blow-vac and sweep net samplings) using bait trap method.

- Bait traps for egg parasitism (one bait plant with BPH eggs on the center and one near (1 m) from the rice bund for each field) will be carried out in 5 fields in the ecological engineering and 5 fields in farmer practice with insecticide spray (control) at peak time of each generation of rice planthoppers. The trappings will be done 2-3 times for each site. There will be twenty samples for each sampling day.

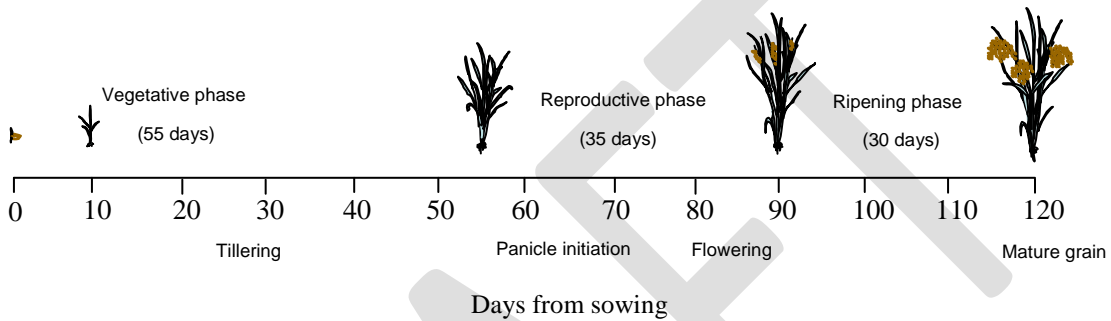
Additional method is the use of sticky plate to count the number of hoppers per unit area. This will be done once every week for the 10 ecological engineering fields and 10 fields in farmer practice with insecticide spray (control).

Further detail on each of these methods is set out later in this document.

100-day variety



120-day variety



General rice growth stages

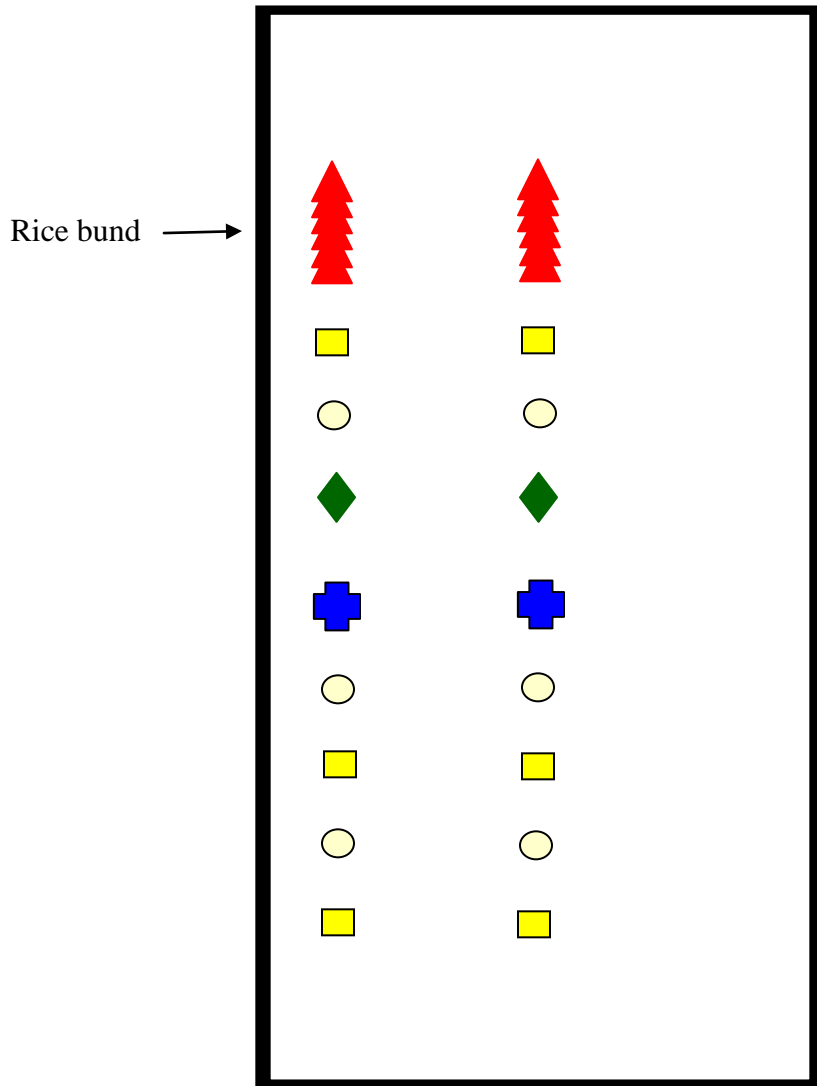
Sampling schedule:

1. Direct seeded rice






Seedling stage: 2-3 weeks after seeding
Tillering stage: 5-6 weeks after seeding
Booting stage: 8-9 weeks after seeding
Milking stage: 10-12 weeks after seeding

2. Transplanted rice

Seedling stage: about 2 weeks after transplanting
Tillering: 4-5 weeks after transplanting
Booting: 8-9 weeks after transplanting
Milking: 11-14 weeks after transplanting



Sampling lay out of different methods in one field

-  Sweep net (which is about 15 m long covering 30 sweeps)
-  Yellow sticky trap
-  Yellow pan trap
-  Egg bait trap
-  Blow-vac machine

Taxonomy and identification

Arthropod biodiversity may be studied through sampling, counting and identifying the specimens. Wherever identification to *named* species is not possible, individuals should be identified to 'morphospecies' (otherwise known as recognizable taxonomic unit). This means that specimens are sorted into categories in which all individuals are identical. For example 'Ichneumonid #1 or Coccinellid # 3). Bulk, unsorted samples are best stored in 70-100% alcohol allow follow up identification. (Scope for DNA barcoding is being investigated?). All the predator specimens should be sorted as early as possible and preserved in 100 % ethanol for further testing. Scrupulous attention needs to be paid to labeling such bulk samples and individual specimens (e.g. date collected, site, exact position or plot number if from within an experiment, collector's name are the minimum).

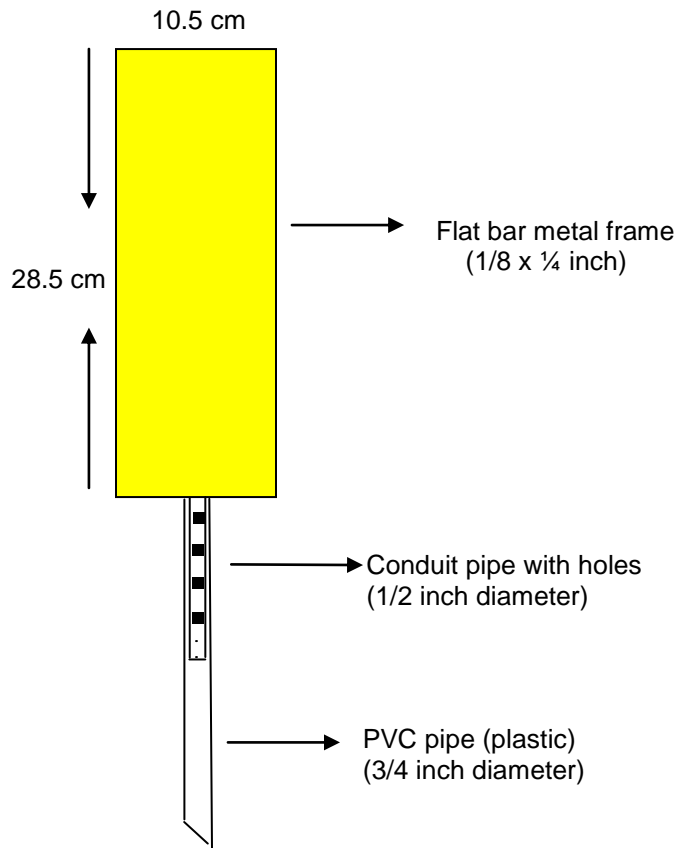
Several sampling techniques are to be used in the overall IRRI/ADB project.

Yellow sticky trap

Yellow sticky trap is designed to attract a variety of insects. The yellow color of the trap attracts the insects and the sticky coating captures them. Its dimension is 28.5 cm x 10.5 cm. A flat bar metal frame holds the yellow sticky strap and its base is attached to a conduit pipe, which is then inserted into a rounded PVC pipe. Both pipes are with holes for easy adjusting during the different growth stages of the rice plant.

Sampling by yellow sticky trap

1. Install the yellow sticky trap just above the rice canopy for 24 hours.
2. Retrieve the trap after 24 hours.
3. Cover the sticky trap with a transparent plastic sheet after retrieval.
4. Slice or cut the trap longitudinally into 2-3 parts for easy handling under the microscope.
5. Identify the natural enemies that stucked on the traps.
6. Keep the yellow sticky trap refrigerated if identification is to be delayed. Do the sampling on seedling, tillering, booting, and milking stages of the rice crop.
7. The standardized sticky trap will be provided by Dr Lv.
8. Do the sampling on seedling, tillering, booting, and milking stages of the rice crop.



Yellow pan trap

Many small day-active insects are attracted to the color yellow. Yellow pan traps collect insects that are attracted to the color. They are inexpensive and simple means of passively sampling insects in an area. This trapping method uses small pans filled with a mixture of water and liquid detergent. The pans are then placed on the ground in conspicuous places in the morning. When flying insects land on the surface of the water they rapidly sink and drown. At the end of the day or after 1-2 days, the water is strained through a fine sieve and the specimens are collected.

Sampling arthropods by yellow pan trap

1. Use 500 ml circular plastic 'take away food container' (with 17 cm diameter and 5 cm depth). Deeper bowls experience less evaporation in hot climates.
2. Cut one hole near top of bowl and cover with mesh. In excessive rain this allows water to flow out of the bowl without losing any samples.
3. Paint with two top coats of yellow UV paint (e.g. RJ London acrylic spray paint).
4. Place bowls at approximately the same height of vegetation (50-100 cm) or just below the canopy level using a wire frame.

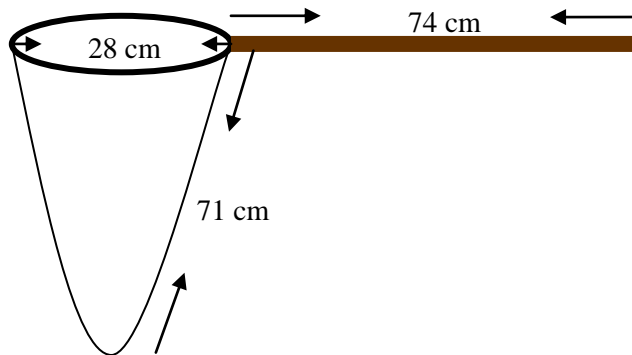
5. Add a mixture of 400 ml water and 1.2 g of sodium benzoate preservative and one drop of liquid detergent (washing-up liquid).
6. Cover each bowl with a coarse wire mesh to prevent scavenging of insects by birds.
7. Leave out for 24 hrs at a time.
8. Use an aquarium net or fine sieve to collect the insects and place in 100 % ethanol.



Yellow pan trap at vegetative stage of the rice crop.

Insect sweep net

The use of sweep net is a simple and inexpensive way to monitor the presence of a variety of arthropods in the ecosystem. If sampling effort is consistent (e.g. 30 sweeps whilst walking slowly through vegetation) samples can also be used to infer relative abundance of arthropods within a vegetation type. The sweep net is a funnel-shaped net, which is made-up of a nylon or similar synthetic fabric. It is important that the net is mounted on a rigid metal ring rather than wire. This allows the net to be swept through dense vegetation, dislodging arthropods. The net's ring is attached to a long wood or metal handle. A standard sweep net has a diameter of 28 cm with a length of 71 cm long. The stick handle is about 74 cm long.



A typical sweep net

How to use a sweep net

1. Hold the sweep net near the end of the handle with the hoop end nearest to the ground in front of you.
2. Swing the net from side to side in a full 180° arc or forming a semicircle. Keep the circular frame of the open end of the net perpendicular to the ground and pointing to the direction of the swing.
3. Sweep one stroke per step as you casually walk through the field or down the row. Do not swing the net up and down.
4. In short vegetation, swing the net as deeply as possible.
5. In taller vegetation, sweep only deeply enough to keep upper edge of the sweep net opening even with the top of the plants.
6. The net should not go more than 25 cm below the top of the plants during sampling.

Sampling arthropods by a sweep net

1. Sampling must be done when all the morning dew has evaporated. Avoid sampling in raining and wet weather.
2. Do thirty sweeps on the center of the field and another thirty sweeps on the rice hills next to the bunds or about 1 m from the bunds.
3. Swing the net as hard as possible after the last sweep. This will allow the insects to be deposited at the funnel end of the net.
4. Close the net by gripping the mid section by the palm.
5. Invert the net and put the collected insects in plastic bags and label with tags.
6. Transfer the collected insects in labeled zip loc or plastic bag with 100% ethanol.
7. Bring the zip loc to the laboratory and transfer into labeled vials maintaining the 100% ethanol. Record the time from sampling to transferring into labeled vials.
8. Identify all the insects based on guilds.
9. Do the sampling on seedling, tillering, booting, and milking stages of the rice crop.



Each passage of the net is considered one sweep.



Hold firmly the end of the net after the last sweep.



Invert the net and transfer the insects in labeled zip loc or plastic bag.



Transfer all collected insects.



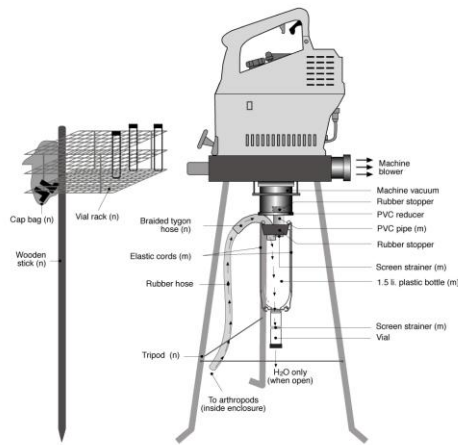
Close the zip loc.

The efficiency of a sweep net may vary depending on many factors. Different weather conditions, wind speed, air temperature, and intensity of solar radiation may affect the

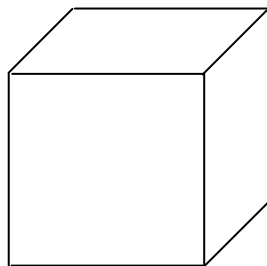
number of insects in the area while sweeping. Different habitats, especially the height of the plants, time of day, reflecting different cycles of behavior of the species, and different styles of sweeping are also factors to be considered.

Blower-vac machine

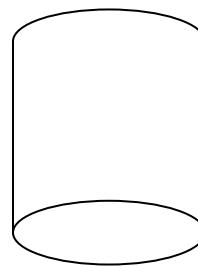
Blower-vac machine may be used for more quantitative studies of insects in rice. It is operated by a gasoline-powered motor. The machine sucks the insects from rice plants by vacuum pressure. This machine is similar to that described by Arida and Heong (1992). However, instead of a plastic bucket, it will use a plastic bin.



A modified blower-vac apparatus for sampling arthropods. Arrows indicate the flow of air, water and arthropods through the apparatus. Symbols: (n) new or (m) modified part from the original blower-vac apparatus.



A



B

(A) A square and (B) a circular sampling enclosure, which can enclose (A) 4 hills or 0.25 m² for transplanted rice and (B) 0.1 m² for direct seeded rice, as shown in the above picture, should be prepared for sampling.

Sampling of arthropods by blower-vac machine

1. To sample using the blower-vac, drop the plastic bin enclosure over the rice plants.
2. Suck the arthropods from the nylon net sleeve, the air column, the plant surfaces and finally the water surface. The suction time will depend until all the insects are collected (suction time will later be prolonged as rice crop matures).
3. Place the collected insects in labeled vials with 70% ethanol.
4. Sort and identify all the insects based on guilds as early as possible and all the predator specimens should be kept in vials with 100% ethanol and store at -18 to 20°C.
5. Do the sampling on seedling, tillering, booting, and milking stages of the rice crop in the morning. Avoid sampling during the afternoon.



A blower-vac machine in action in the field.

Bait traps for egg parasitization

Egg trap is special trap used for investigating natural enemies related to egg stage.

1. Use about 30-day-old rice plants susceptible to BPH.
2. Thin the rice plants to 5 tillers each pot.
3. Introduce five gravid female adults to the rice plants for oviposition in the morning.
4. Remove the adults after 24 hours.
5. Bring the plants with newly laid eggs in the morning to the rice field and expose for 72 hours.
6. Retrieve the rice plants with eggs after 72 hours and bring to the greenhouse or laboratory and cover with a mylar cage for another 3 days.
7. Use a black cloth to cover the cage, but leave a hole with glass tube and light at top to attract parasitoids after their emergence.

8. Check daily the glass tube for parasitoids and count the number of planthopper nymphs that emerge. Identify the species and take records.
9. Calculate parasitism rate for each pot, as well as the mean parasitism rate for each area.
10. Do the bait traps at the peak time for each planthopper generation.



Rice plants with eggs ready for field exposition



A wooden stick is pegged on the bait trap in the field



The rice plant is enclosed with a mylar cage after field exposition.



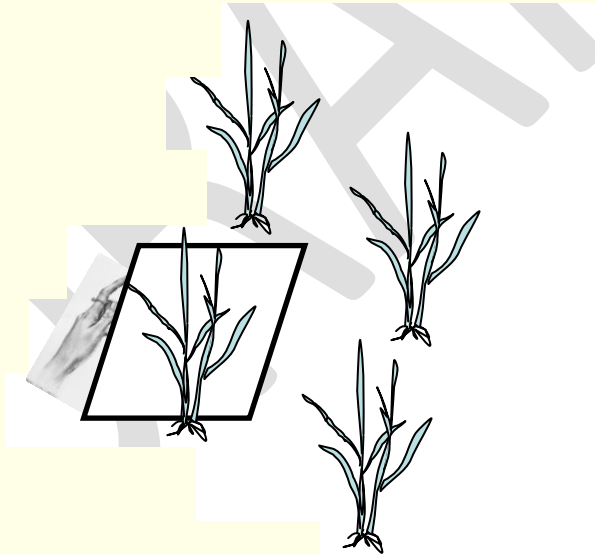
A black cloth covers the mylar cage with a glass tube on top for parasitoids.

Counting of hoppers/m²

White enamel plates coated with sticky substance can be used to count the number of hoppers per hill.

Sampling of hoppers by hill

1. Use a white enamel plate with 30 x 45 cm dimension.
2. Spread kerosene (or petroleum jelly) or anything sticky on the plate. (Leave a small space uncoated for easy handling).
3. Position the plate close enough to the base of the rice hills or plants carefully to avoid agitating the planthoppers and tap the hills several times with the hand to collect the hopper.
4. For transplanted fields, tap 2 hills at a time and sample 5 times for each field. For direct seeded fields, use 0.05 m² for each sampling and sample 5 times for each field.
5. Bring the plate to the laboratory and count the number of hoppers based on age (young nymphs from 1st – 3rd instars and mature nymphs from 4th – 5th instars) and forms (short-winged or brachypterous and long-winged or macropterous) that stick to the board.
6. Do the sampling at 7 day intervals.



Sampling of hoppers using an enamel plate.

Evaluating predation quantitatively using triplex RT-PCR

The triplex RT-PCR could be used to evaluate species-specific predation by all the predators qualitatively and compare relative predation quantitatively for particular predator species among sites. All the samples taken by the sampling methods mentioned above could be used for evaluation by the RT-PCR method if the samples could be kept in 100% ethanol.

1. Samples (mainly predators) taken by ***Blower-vac machine and Insect sweep net*** (samples taken each time should be put into a nylon stocking with a label (field number and sampling date), then keep in bottle with 70% ethyl alcohol in fields as soon as possible (the longer the samples are kept without ethyl alcohol, the greater the predation will be and DNA quality will also decline).
2. All the samples should be sorted as early as possible (within a week) and all the predators are transferred into labeled vials with 100% ethanol by species or groups after sorting or identification.
3. The vials with predators should be kept in freezer at minus 18-20°C.
4. Testing. All the samples will be collected before November next year and will be tested by ZJU.

Identification of arthropod samples from all sampling techniques

1. All the samples should be sorted out first as early as possible (within 1-2 weeks after sampling) as first step for further identification.
2. All the predator specimens should be kept in labeled vials with 100% ethanol and should be stored in minus 18-20°C (for PCR analysis later).
3. Sort, count and identify the collected arthropods to species level (if possible).
4. Group the sampled arthropods based on guilds (predators/omnivores and parasitoids/parasites) described by Moran and Southwood (1982).

Data analysis

1. The raw data will be entered into Excel file using a standardized data sheet (refer to attachment).
2. Analysis will follow.

References:

Arida GS, Heong KL. 1992. Blower-Vac: a new suction apparatus for sampling rice arthropods. *International Rice Research Newsletter* 17(6):30-31.

Moran VC, Southwood TRE. 1982. The guild composition of arthropod communities in trees. *J. Animal Ecol.* 51:289-306.

