

Appendix 1: Amendments to working document suggested by GM Gurr 11 July 2009.

Sampling protocols for studying arthropod biodiversity-ecological engineering for rice pest suppression in irrigated tropical rice-ecosystem

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 recognisable taxonomic unit). This means that specimens are sorted into categories in which all individuals are identical. For example 'Ichneumonid #1 or Coccinellid # 3). Specimens should be preserved to allow follow up identification. Scope for DNA barcoding is being investigated. Specimens can be pinned in the normal manner of preserved in 100 ethanol. Bulk, unsorted samples are best stored in a deep freeze until sorted. Scrupulous attention needs to be paid to labeling such bulk samples and individual specimens (eg, date collected, site, exact position or plot number if from within an experiment, collector's name are the minimum) to species.

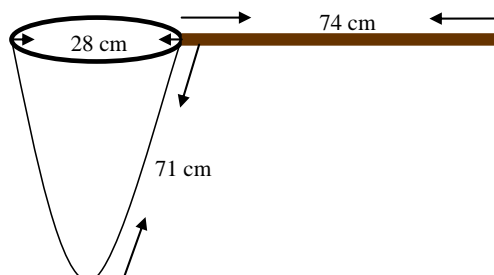
Several sampling techniques are to be used in the overall IRRI/ADB project may be used.

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Insect sweep net

The use of sweep net is a simple and inexpensive way to monitor the presence of a variety of arthropods/insects in the ecosystem. If sampling effort is consistent (eg 20 sweeps or 30 seconds sweeping whilst walking slowly through vegetation) samples can also be used to infer relative abundance of arthropods within a vegetation type. The sweep net is ~~It is~~ a funnel-shaped net, which is made-up of a ~~cotton mesh~~ 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- handled wooden frame. 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.

Comment [g1]: Should we provide to collaborators a number of suitable nets to ensure comparability and quality?



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 10 inches/25cm below the top of the plants during sampling.

Sampling arthropods by a sweep net

1. Sample arthropods ~~weekly or~~ once a week starting from the tillering stage (30 DAT) until the flagleaf stage (80 DAT) of the rice crop.
2. Sampling must be done at 10 am in the morning when all the morning dews ~~has~~ evaporated. Avoid sampling in wet weather.
3. Start to sweep from the end corner of the field towards???. Do twenty sweeps, which corresponds to one sample.
4. The second twenty sweeps going to the center of the field and the third twenty sweeps going to the other end corner of the field.
5. A total of sixty sweeps (three samples) along a diagonal pattern will be done in one-fourth hectare.
6. 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.
7. Close the net by gripping the mid section by the palm.
8. Invert the net and put the collected insects in plastic bags and label with tags.
9. Transfer the collected insects in 3 labeled vials with 100/70% ethanol/alcohol.

Comment [g2]: We need to discuss the underlying research question. Is it simply what is the arthropod fauna in a given crop? If so a zig zag pattern in random parts of paddy is best. We might want to also ask the question what is the fauna in the crop at various distances from edge vegetation of different types.

Suggest pic be replaced or deleted as this is not proper sweep net technique – such arm's length posture is more typical of trying to catch a specific specimen.

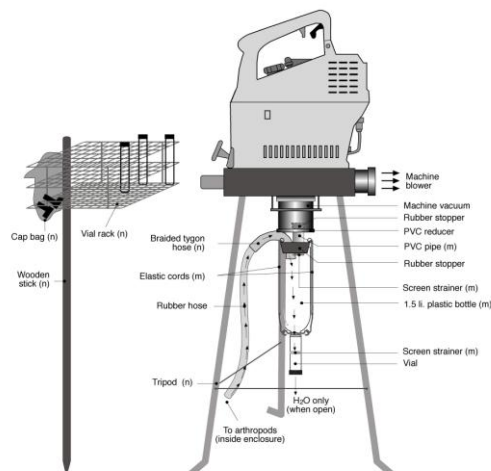


Each passage of the net is considered one sweep.

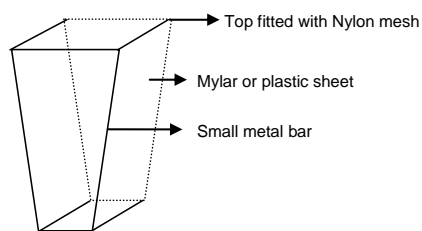
The efficiency of a sweep net may vary depending on many factors. Different weather conditions, wind speed, air temperature, and intensity of solar radiation may affect the number of insects in the area while sweeping. Different habitats, especially the height of the plants, time of day, reflecting different 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 modified enclosure made of metal.



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 modified enclosure made of metal (3 cm x 16 cm) and transparent mylar or plastic sheet (with 42 cm diameter top and 42 cm diameter bottom) with the bottom open and the top fitted with 1 meter nylon mesh cloth to prevent escape of moving arthropods.
Sampling of arthropods by blower-vac machine

1. Sample arthropods biweekly or every two weeks starting from the tillering stage (30 DAT) until the flagleaf stage (80 DAT) of the rice crop.
2. Biweekly sampling will consist of 10 Blower-Vac samples at every sampling date.
3. Sample arthropods from 10 randomly selected hills within the rice field.
4. To sample using the Blower-Vac, drop the enclosure over the rice plant to cover 1 hill.
5. 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).
6. Place the collected insects in labeled vials with ~~70% alcohol~~ 100% ethanol.

Comment [g3]: Sounds fine but we should discuss the possibility of more sophisticated sampling (see comment on sweep net above).



A blower-vac machine in action in the field.

Comment [g4]: Will we provide the equipment? I think this s important.

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.

Option 1

1. Use 500 ml bowl. Deeper bowls experience less evaporation in hot climates.
2. Cut holes 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 yellow UV paint (e.g. Sparvar Leuchtfarbe, Spray-Color GmbH, Merzenich, Germany or <http://www.guerrapaint.com/tandc.html> USA).
4. Place bowls at approximately the same height of vegetation (50-100 cm) using a wire frame.
5. Add a mixture of 400 ml water and sodium benzoate preservative.
6. Place the first bowl in the vegetation bordering the crop/ on the bund at field edge and the others in a transect ~~or and~~ at distances of 0, ~~then at~~ 1 m, 2 m, 4m, 8m, 16m, etc into the centre of the paddy.
7. Cover each bowl with a coarse wire mesh to prevent scavanging of insects by birds.
8. Leave out for 48 hrs at a time.
9. Repeat a minimum of 6 times throughout the rice-growing season.
10. Use an aquarium net or fine sieve to collect the insects and place in ~~100~~99% ethanol.

Comment [g5]: Can we provide the painted bowls?

Comment [g6]: Add concentration as per my earlier email



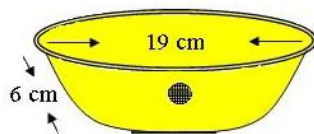
Yellow pan traps at flowering stage of the rice crop.

Option 2.

1. Use yellow pans.
2. Cut holes near top of pan and cover with nylon mesh.
3. Support the bowls with wire and wooden stick.
4. Add a mixture of water and salt and oil or a mixture of water and picric acid.
5. Place the yellow pan just below the canopy of the rice plants supported by a wire frame and wooden stick.
6. Set-up the yellow pan traps on the bunds and inside the rice field 5 meters apart from each other at 21 DAT, 45DAT, and 65 DAT, and 85 DAT (seedling stage, vegetative stage, flowering or reproductive stage, and ripening stages).
7. Collect the pans after 24 hours.
8. Collect the insects by a fine sieve net and place in ~~70% alcohol~~ 100% ethanol.

Comment [g7]: Add concentrations

Add info from above re transect



Yellow pan



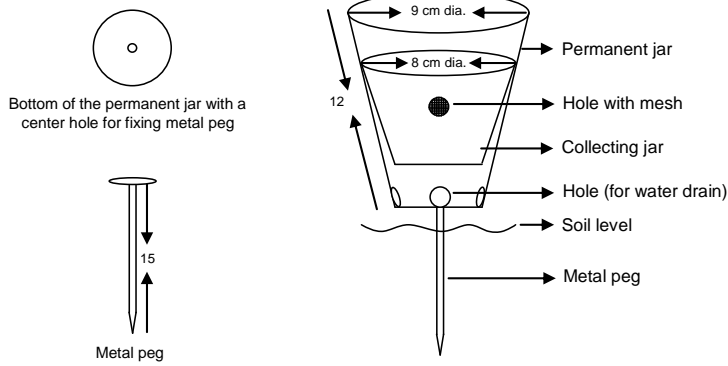
Yellow pan trap at vegetative stage of the rice crop.

Pitfall trap

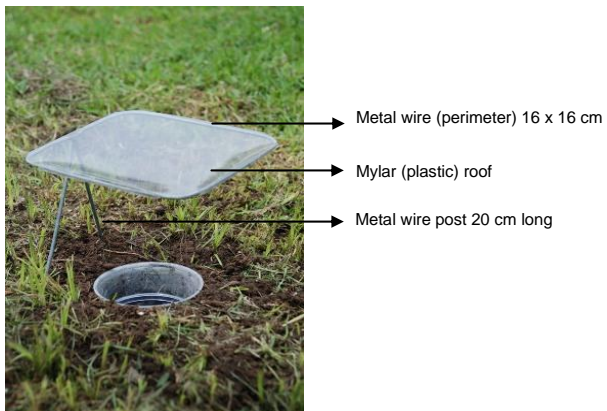
Pitfall traps are often used to sample crawling or ground-dwelling insects. They are placed below the ground with the rim of the container below the surface of the soil. Detergent may be added to reduce the surface tension allowing insects to sink into the liquid.

Sampling by pitfall traps

1. Install the pitfall traps two to three times a week before dibble and at seedling stage until harvest time.
2. Dig holes with the same size as the traps.
3. Dig holes every 5 meters within the bunds and at the center of the field forming a straight line.
4. Make sure the rim of the trap is just below the surface of the soil.
5. Put some mixture of water and teepol on the trap. Ten percent picric acid can also be mixed with water to preserve the insects.
6. To keep rainwater out of these traps, a cover or a mylar roof supported by a metal wire can be placed above the opening of the trap.
7. Leave the traps for 1 day.
8. Retrieve the traps after 1 day.
9. To empty the traps, take the inside cup and turn it over in a labeled plastic bag.
10. Transfer the collected insects in labeled vials with ~~70% alcohol~~ 100% ethanol.
11. Add info from above re transect

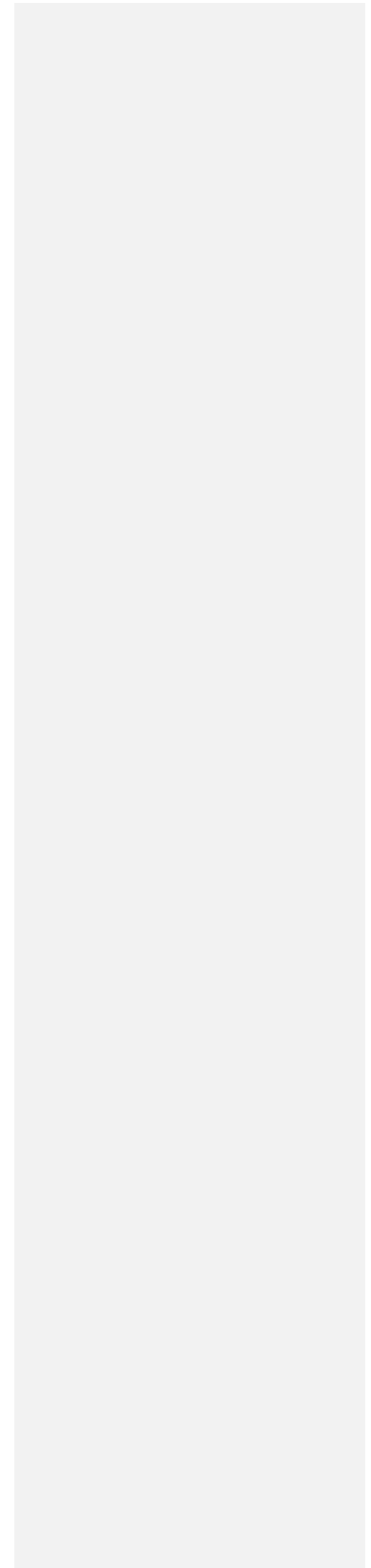


Plastic containers for pitfall trap



Pitfall trap with a mylar roof and plastic container below the ground

Light traps



Light traps are simple methods for collecting large numbers of insects attracted to light. At the bottom is a collecting pan with alcohol or a mixture of water and detergent.

Sampling by light traps

1. Install a single fluorescent bulb light trap 250 m away from the field.
2. Traps should run once a week between dusk and dawn.
3. Collect the insects at 7am in the morning.
4. Transfer the insects in labeled vials with ~~70% alcohol~~ 100% ethanol.



A dry fluorescent light trap

Bait traps for egg parasitization

1. Use 30-day-old rice plants.
2. Thin the rice plants to 5 tillers each pot.
3. Introduce five gravid female adults to the rice plants for oviposition.
4. Remove the adults after 24 hours.
5. Bring the plants with newly laid eggs to the rice field and expose for 48 hours.
6. Retrieve the rice plants with eggs after 48 hours and bring to the greenhouse or laboratory.
7. Remove the tillers with eggs and transfer to petri dishes lined with moistened filter paper.
8. Check daily for parasitoids emergence.



Comment [g8]: Fine but again we need to discuss underlying question and the resultant layout/design to be used.

Rice plants with eggs ready for field exposition



A wooden stick is pegged on the bait trap in the field
Identification of arthropod samples from all sampling techniques

1. Place the collected samples in labeled vials with 70% ethyl alcohol.
2. Sort, count and identify the collected arthropods to species level (if possible).
3. 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.
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.

Barrion AT. Personal communication.

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