Bioassays – some definitions

- **Bioassay** – an experiment in which a living organism is used as a test subject
- **Quantal response bioassay** – where the intention is to estimate the relationship between the response and the quantity or intensity of the stimulus
- **Response variables (dependent variables)** – the random outcomes of the experiment
- **Explanatory variables (independent variables)** – measurable characteristics of the stimulus that causes the response (the “treatment”)

From Bioassays With Arthropods, 2nd Ed., J.L. Robertson et al., CRC Press, 2007

Types of response variables:

- **Binary** – yes or no, e.g. alive or dead
- **Continuously variable** – spectrum of responses, e.g. weight increase, food consumed
- **Binary model with multiple explanatory variables** – produces a “dose-response” curve

More definitions:

- **Experimental unit** – the entity actually receiving the treatment
- **Replication** – repetition of the bioassay at a different time but under the same conditions (as much as possible)
- **Subsets within a replication = pseudo-replication**

Properties of a Good Bioassay

1) Reproducibility (the “litmus test” of science)
2) Results easily observed and measured
3) Relatively low cost
4) Preferably of short duration (less opportunity for confounding factors); more replication
5) Linear dose-response
What can be measured?

**Physiological responses**
1) Mortality (fixed time period or within developmental stage)
2) Larval growth (weight gain)
3) Development (time to pupation, time to eclosion); longevity
4) Fecundity

**Behavioural responses**
1) Feeding deterrence
2) Oviposition deterrence
3) Repellence
Important Points to Consider

- Field efficacy of conventional insecticides is most closely linked to mortality. However, behavioural effects should not be overlooked.
- Delayed mortality can be important, e.g. azadirachtin (neem), rotenone. IGRs, protein synthesis inhibitors and mitochondrial poisons often take >48 hours to kill insects.
- For prolific species with fast generation times, fecundity can be an important criterion.

Sources of variability

Insects
1) Age and/or life stage
2) Hunger and/or nutritional status

Plants
1) Location collected, tissue harvested, phenological age
2) Extraction method (solvent, volume, time)

Application method
1) Solvent, emulsifier(s)
2) Substrate (leaf, filter paper, glass)
3) Container: open/closed, humidity

Geographic variation in potency of Annona squamosa seed extracts to Spodoptera litura

Leatemia & Isman, Phytoparasitica 2004:

1 Negeri Lama 2 Batugantung-a 3 Semarang 4 Tantui 5 Batugantung-b 6 Blora 7 Latuhalat 8 Kudamati 9 Kate-Kate 10 Namlea (all seeds collected 1996)
Annual variation in potency of *Annona squamosa* seed extracts to *Spodoptera litura*

(Laakonia & Isman, *Phytoparasitica* 2004)

**Modes of Administration**

- Direct: topical (fixed dose - precise) or spray (concentration – less precise)
- Surface contact, i.e. applied to substrate (concentration, least precise)
- Residual contact – same as above, but insects introduced at specified times after application of treatment to substrate
- Fumigation – closed container; ideally insects should not be able to have direct contact with treatment
Repellence of spider mites by rosemary oil

Leaf Disc Choice Bioassay

Problems/Issues with Feeding Deterrence Bioassays

- Choice or no-choice: which is more appropriate?
  - Binary choice tests are more sensitive than no-choice tests
  - Which most accurately reflects the situation in the field?
- Minimize the duration. Feeding bioassays should be as short as possible (i.e., one or two feeding bouts). “Feeding” tests that take 24 hours (or more) are easily confounded by post-ingestive (physiological) effects.
- Insects can habituate to feeding deterrents, sometimes rapidly
- Avoid using groups of insects – social facilitation can influence results

Deterrence to azadirachtin in *Spodoptera litura*: sequential feeding leads to habituation
Influence of rosemary oil on whitefly oviposition

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<th>Control</th>
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<td>30</td>
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<td>96hrs</td>
<td>80</td>
<td>90</td>
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Number of eggs counted

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<td>20</td>
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Oviposition cage with four cells

Control and treated leaves

Influence of rosemary oil on whitefly oviposition

Repellence of twospotted spider mites by volatile monoterpenes emanating from rosemary oil over 1 hour

Repellence of obliquebanded leafrollers (1st instar) by volatile monoterpenes emanating from rosemary oil over 2+ hours
Screening plant extracts in artificial diets for new insecticides: Inhibition of Spodoptera litura larval growth by crude twig extracts from Costa Rican Trichilia species

All extracts added to artificial diet at 1000 ppm (0.1%) fwt


Effect of spraying cabbage plants in the laboratory with crude twig extract of T. americana on weight gained and leaf area eaten by 4th instar Spodoptera litura larvae over 24 hours


Mortality of Plutella xylostella larvae on cabbage plants sprayed with 0.5 – 2% aqueous emulsions of ethanolic seed extracts of Annona squamosa or dusted with rotenone (1%) in a greenhouse trial (Leatemia & Isman, Intl. J. Pest Mgmt. 50: 129 (2004))

Summary

• Plan your bioassay endpoints first (what do you want to measure?)
• Maximize the number of observations (experimental units)
• Remember the importance of controls: negative control under identical conditions minus the treatment, positive control as a benchmark
• Reduce variability in preparations, methods, pests and conditions as much as you can. BUT, the closer your experimental conditions are to real conditions (“the field”), the more variability you will introduce