INSECT BIOASSAY WORKSHOP

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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. Robertston et al., CRC Press, 2007

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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**

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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

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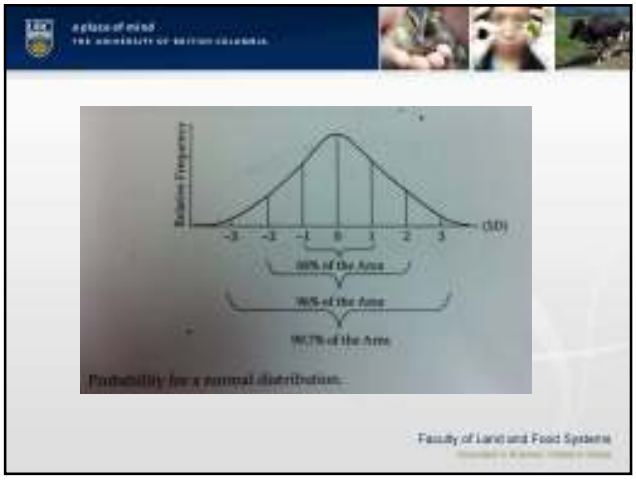

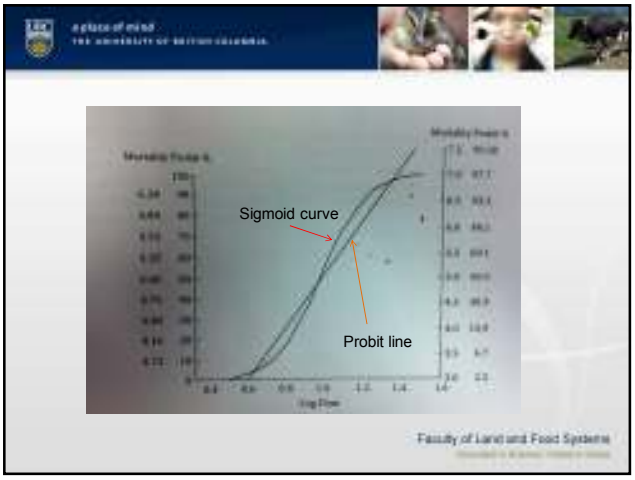

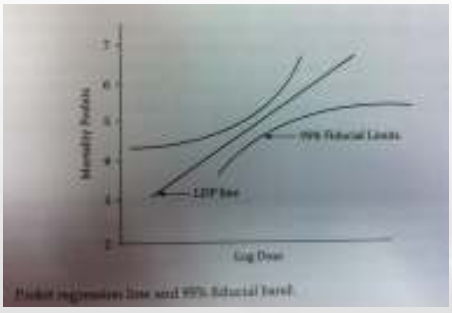

What can be measured?

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

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- Design your bioassays around a specified endpoint
- Include a "positive" control whenever possible
- Your "negative" control should duplicate test conditions and application methods, lacking only the "treatment"
- Maximize numbers of observations and replication, not the number of insects per observation
- For data where a percentage response is measured, aim for a dose/concentration that will produce a 50% response, not 100%

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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

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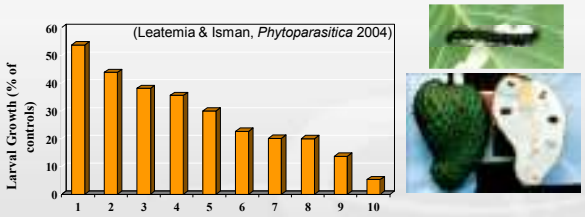
Geographic variation in potency of *Annona squamosa* seed extracts to *Spodoptera litura*



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)

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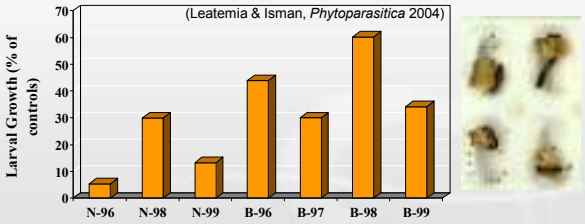
Annual variation in potency of *Annona squamosa* seed extracts to *Spodoptera litura*



N = Namlea (1996-1999)
B = Batugantung (1996-1999)

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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

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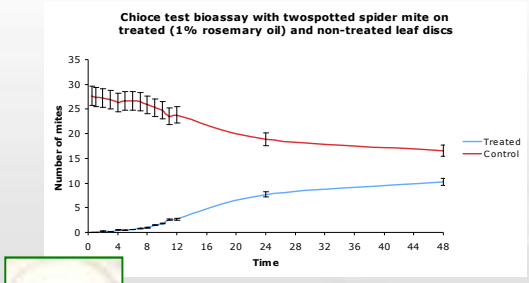
Repellence of spider mites by rosemary oil



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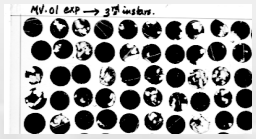
Leaf Disc Choice Bioassay

2. Feeding deterrence:

FD = 100* [(C-T)/(C+T)]

C= control T= treated leaf discs



1. Leaf area measured

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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

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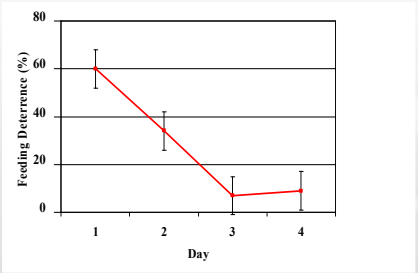
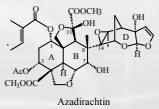
Deterrence to azadirachtin in *Spodoptera litura*: sequential feeding leads to habituation





Azadirachtin

1.3 ng/cm²

Bomford & Isman (1996), *Entomol. exp. appl. 81: 307-313.*

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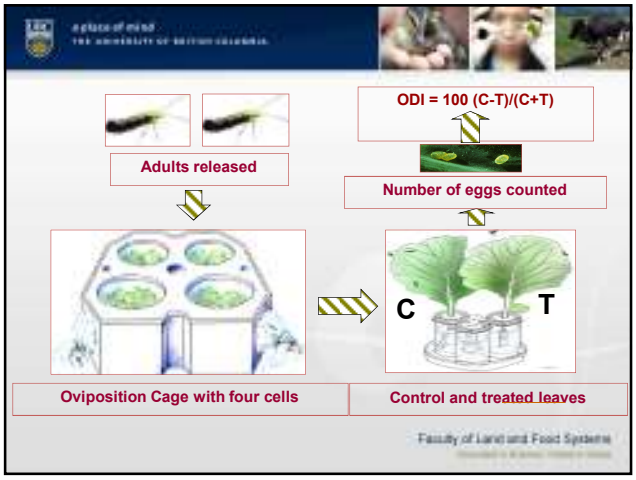
ODI = 100 (C-T)/(C+T)
Adults released
Number of eggs counted
C
T
Oviposition Cage with four cells
Control and treated leaves
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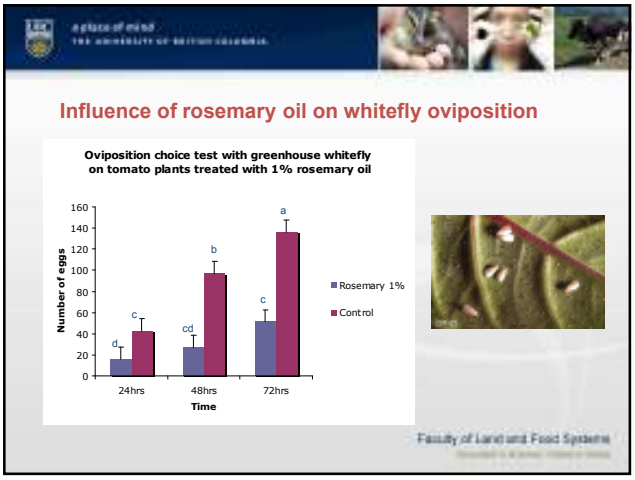
Influence of rosemary oil on whitefly oviposition
Oviposition choice test with greenhouse whitefly on tomato plants treated with 1% rosemary oil
Number of eggs
Time
24hrs
48hrs
72hrs
Rosemary 1%
Control
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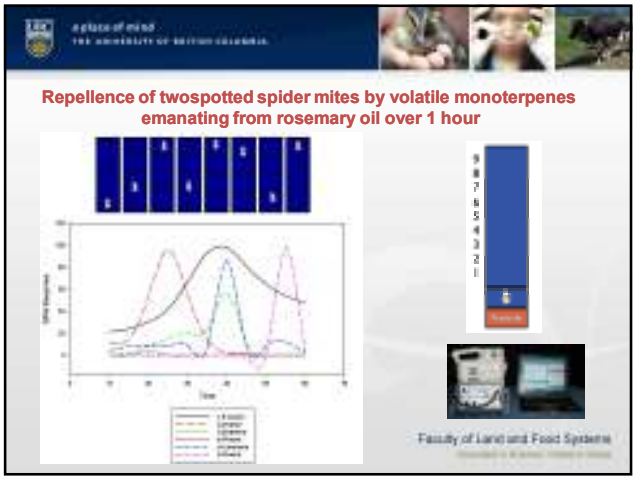
Repellence of twospotted spider mites by volatile monoterpenes emanating from rosemary oil over 1 hour
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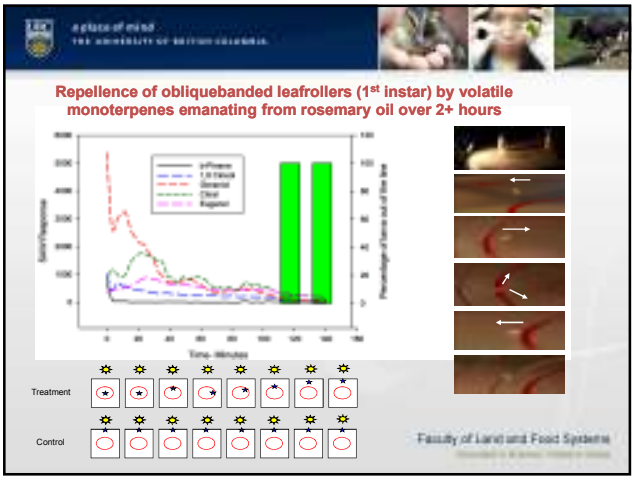
Repellence of obliquebanded leafrollers (1st instar) by volatile monoterpenes emanating from rosemary oil over 2+ hours
Treatment
Control
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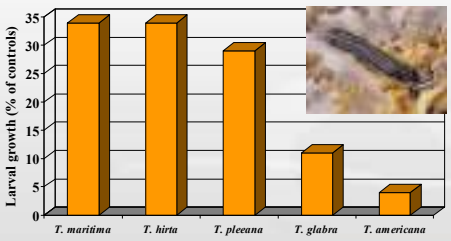
Screening plant extracts in artificial diets for new insecticides: Inhibition of *Spodoptera litura* larval growth by crude twig extracts from Costa Rican *Trichilia* species

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



Wheeler *et al.*, *Biochem. Syst. Ecol.* 29: 347 (2001)

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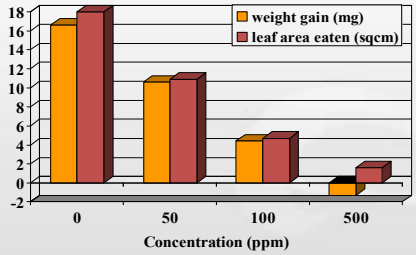
Effect of spraying cabbage plants in the laboratory with crude twig extract of *T. americana* on weight gained and leaf area eaten by 4th instar *Spodoptera litura* larvae over 24 hours



Wheeler & Isman, *J. Chem. Ecol.* 26: 2791 (2000)

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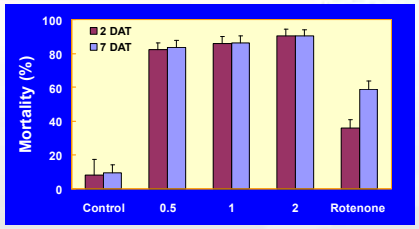

Mortality of *Plutella xylostella* larvae on cabbage plants sprayed with 0.5 – 2% aqueous emulsions of ethanolic seed extracts of *Annona squamosa* or dusted with rotenone (1%) in a greenhouse trial (Leatemia & Isman, *Intl. J. Pest Mgmt.* **50**: 129 [2004])

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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

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