Biological evaluation of pesticidal plants

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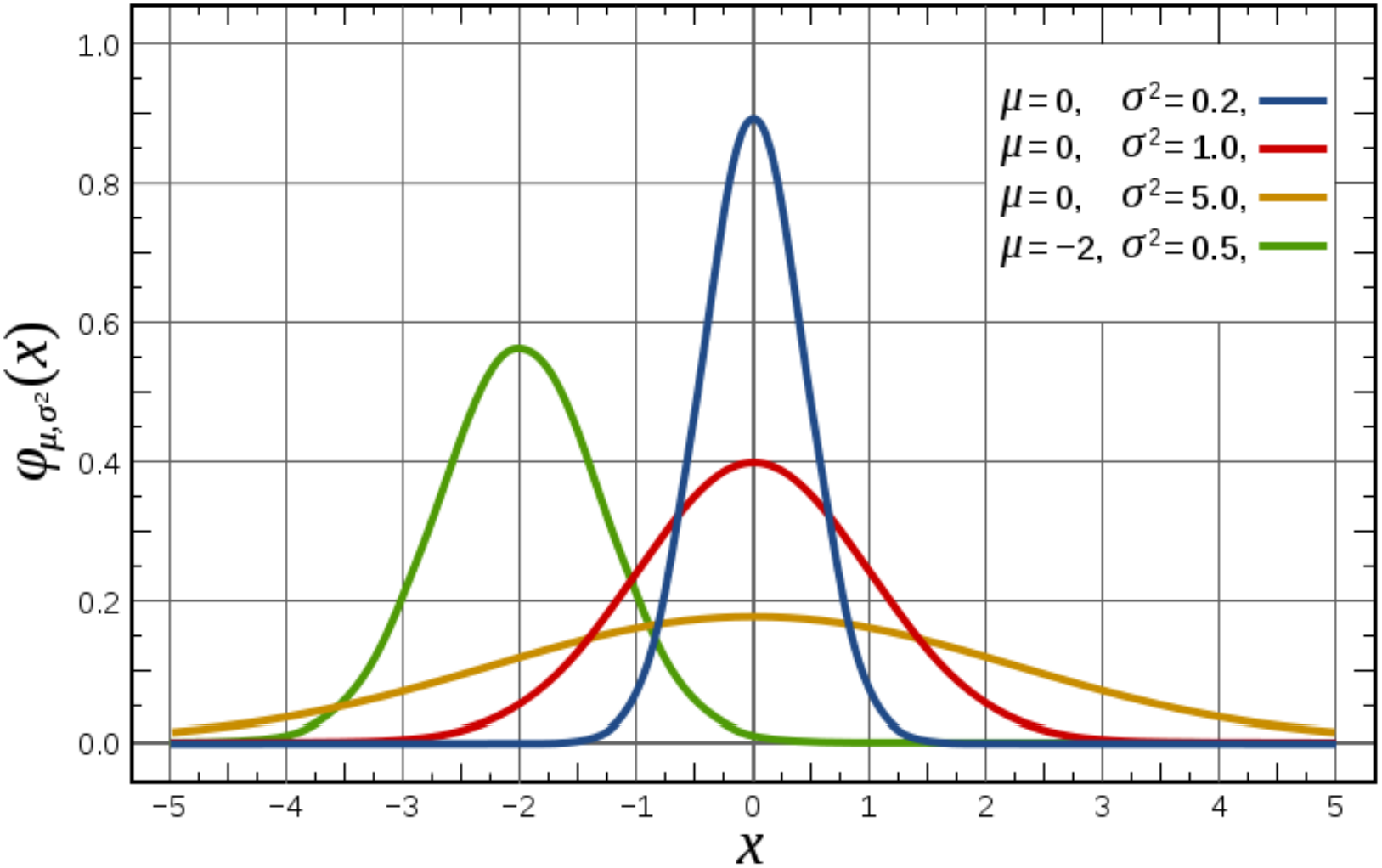
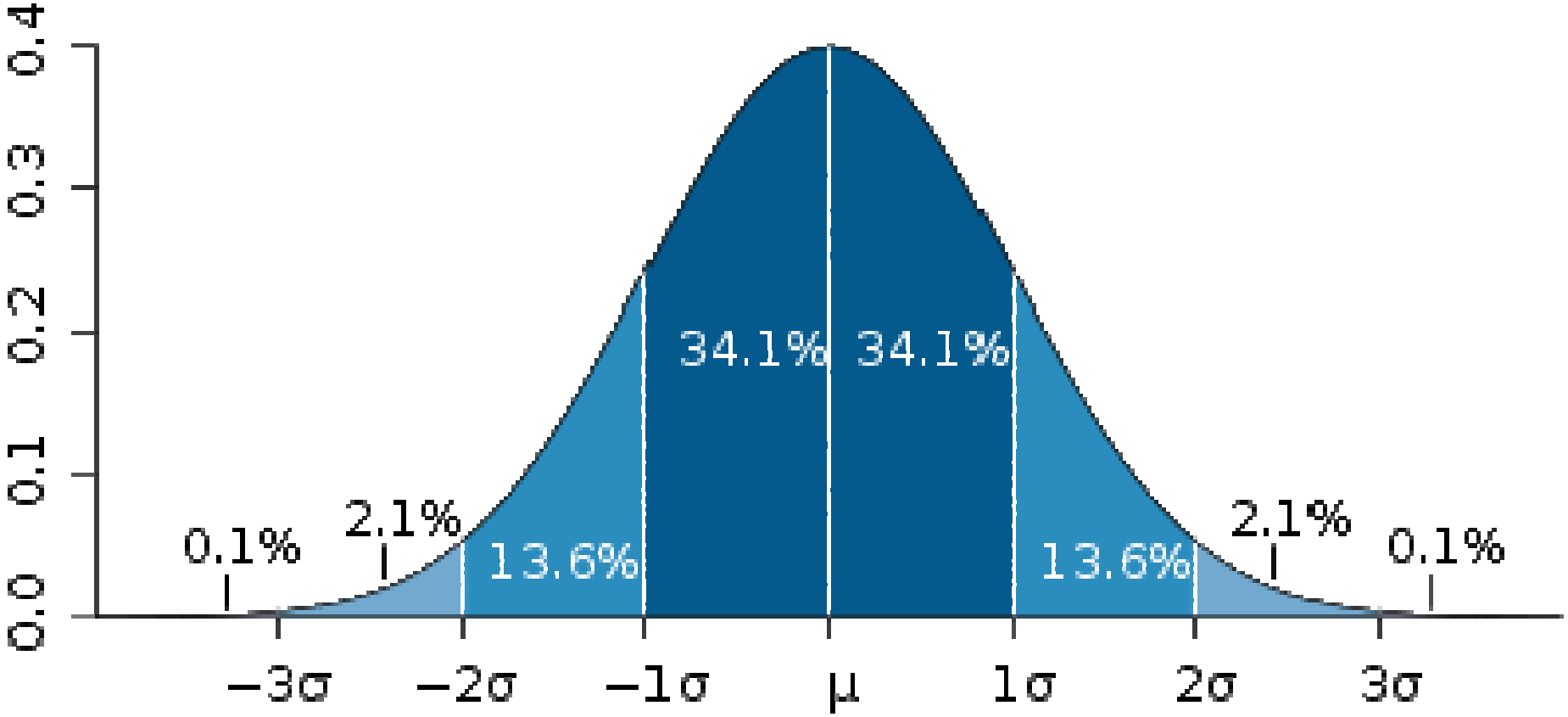
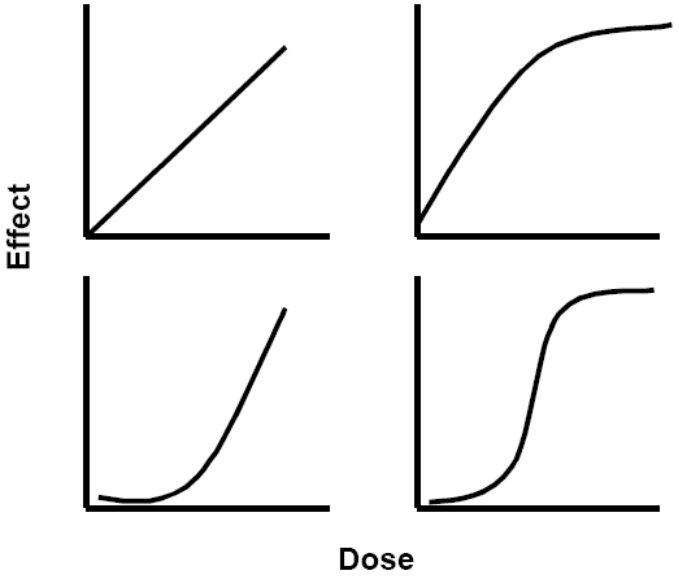
Biological variation

Not all individuals are affected in the same way



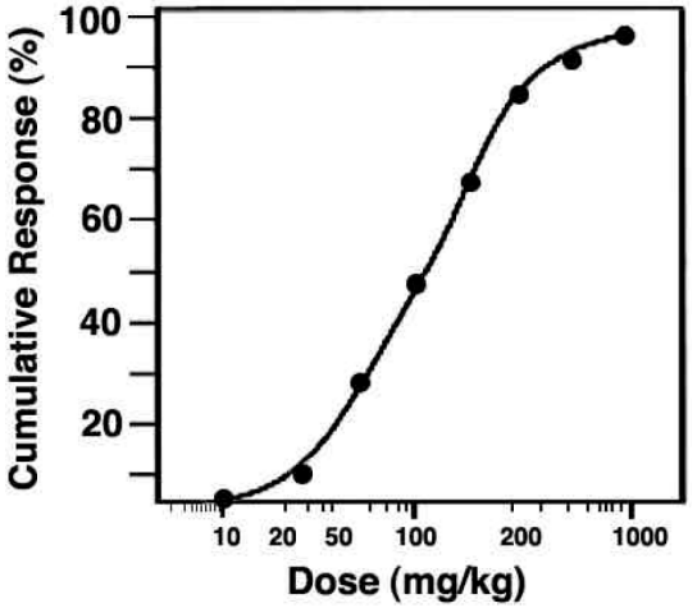


Dark blue is less than one standard deviation from the mean. For the normal distribution, this accounts for about 68% of the set, while two standard deviations from the mean (medium and dark blue) account for about 95%, and three standard deviations (light, medium, and dark blue) account for about 99.7%.

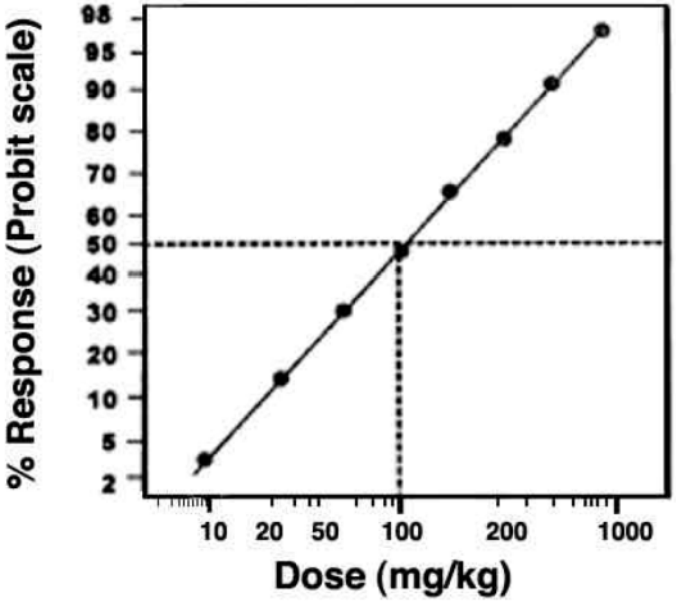


"All substances are poisons: there is none which is not a poison. The right dose differentiates a poison and a remedy."

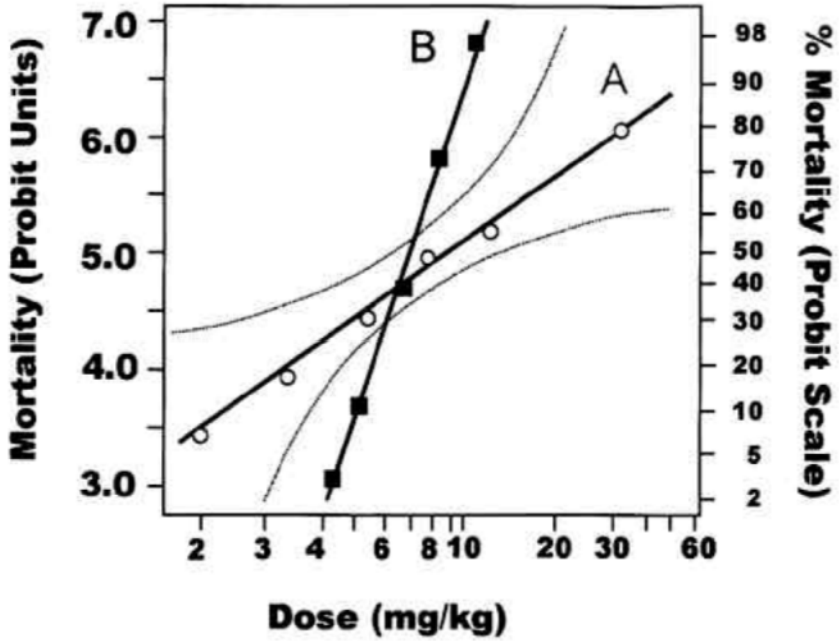
Paracelsus (1493-1541)



Inverse Log



Comparison of dose-response relationship for two different chemicals plotted on a log dose-probit scale



Confidence intervals (CI) are related to standard deviation and tell us how confident we can be that the true population will respond similarly to the experimental population, based on the variability encountered. Note that CI's are tightest closest to the data line nearest the 50% probit response, closest to the mean - response variability increases as we move away from the mean response. The dose-response line for Chemical B is steeper than Chemical A. Which chemical is safer to use?

"The problem with toxicology is not the practicing toxicologists, but chemists who can detect, precisely, toxicologically insignificant amounts of chemicals"

Rene Truhaut, University of Paris (1909- 1994)

Current technology allows us to accurately measure trace quantities of chemicals. Yet, the biological significance of the presence of these extremely small amounts of these chemicals is sometimes not clear (e.g., carcinogens). Just because a drug or environmental contaminant is measurable, does that mean that it poses a threat?

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 e.g. dose response
- **Response variables (dependent variables)** – the random outcomes of the experiment e.g. mortality, repellency
- **Explanatory variables (independent variables)** – measurable characteristics of the stimulus that causes the response (the "treatment") e.g. pesticidal plant species applied

From *Bioassays With Arthropods, 2nd Ed.*, J.L. Robertston 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

Important points to consider

- 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%

Sources of variability

Insects

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

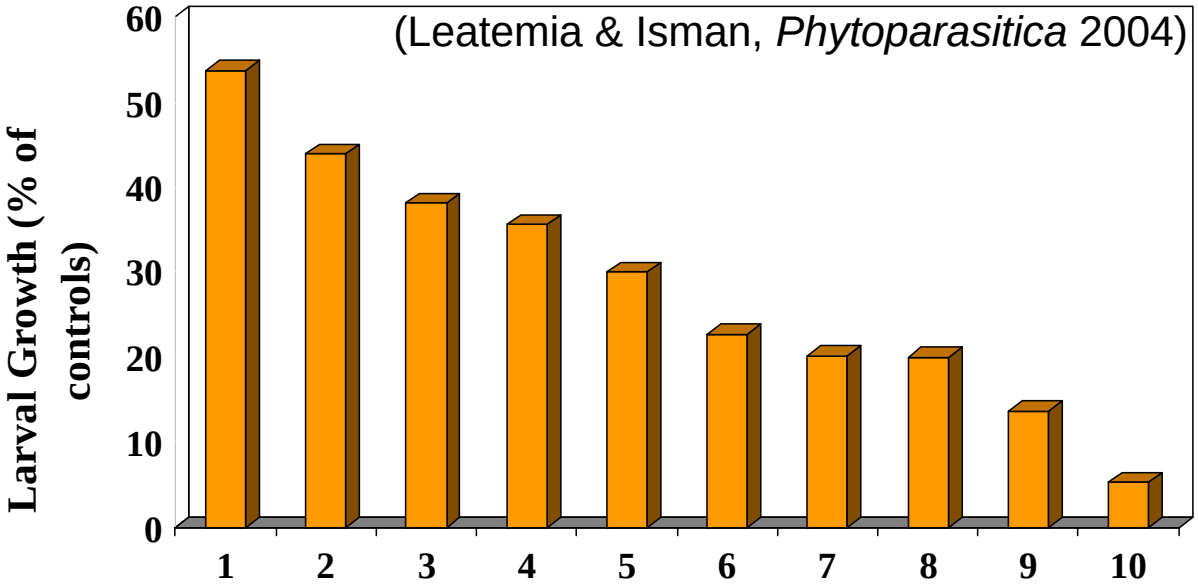
Plants

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

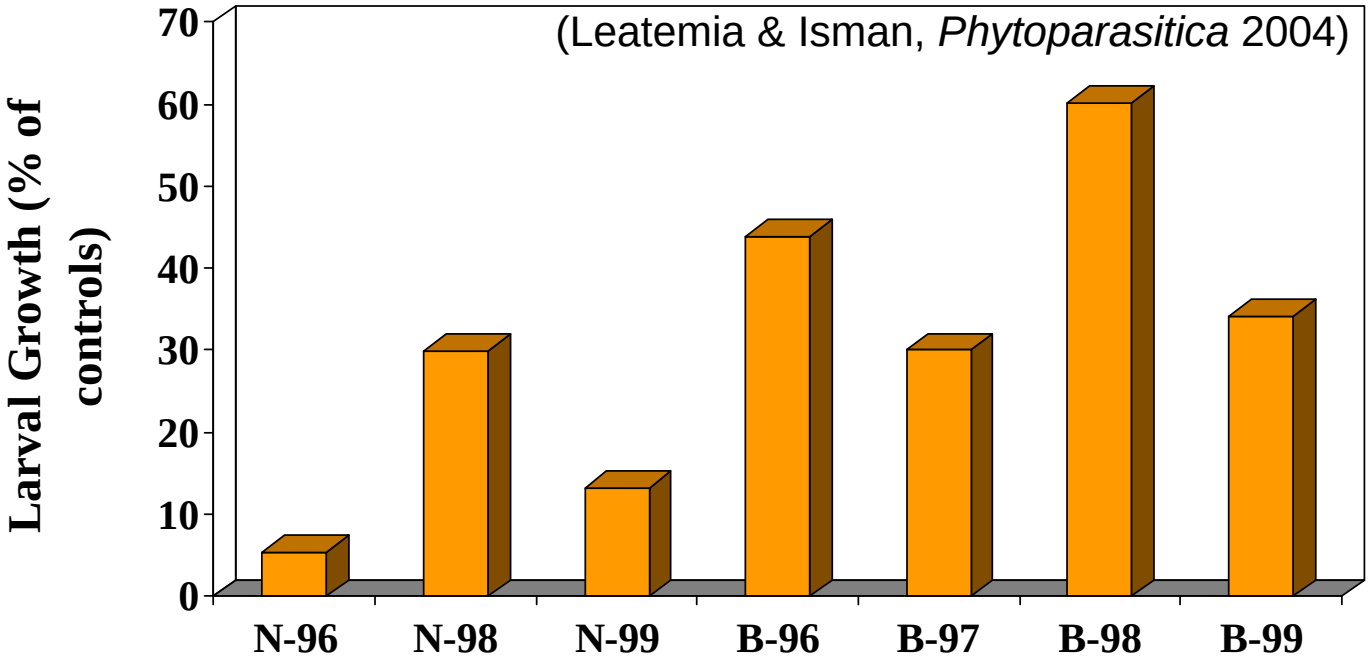
Geographic variation in potency of *Annona squamosa* seed extracts to *Spodoptera litura*



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*

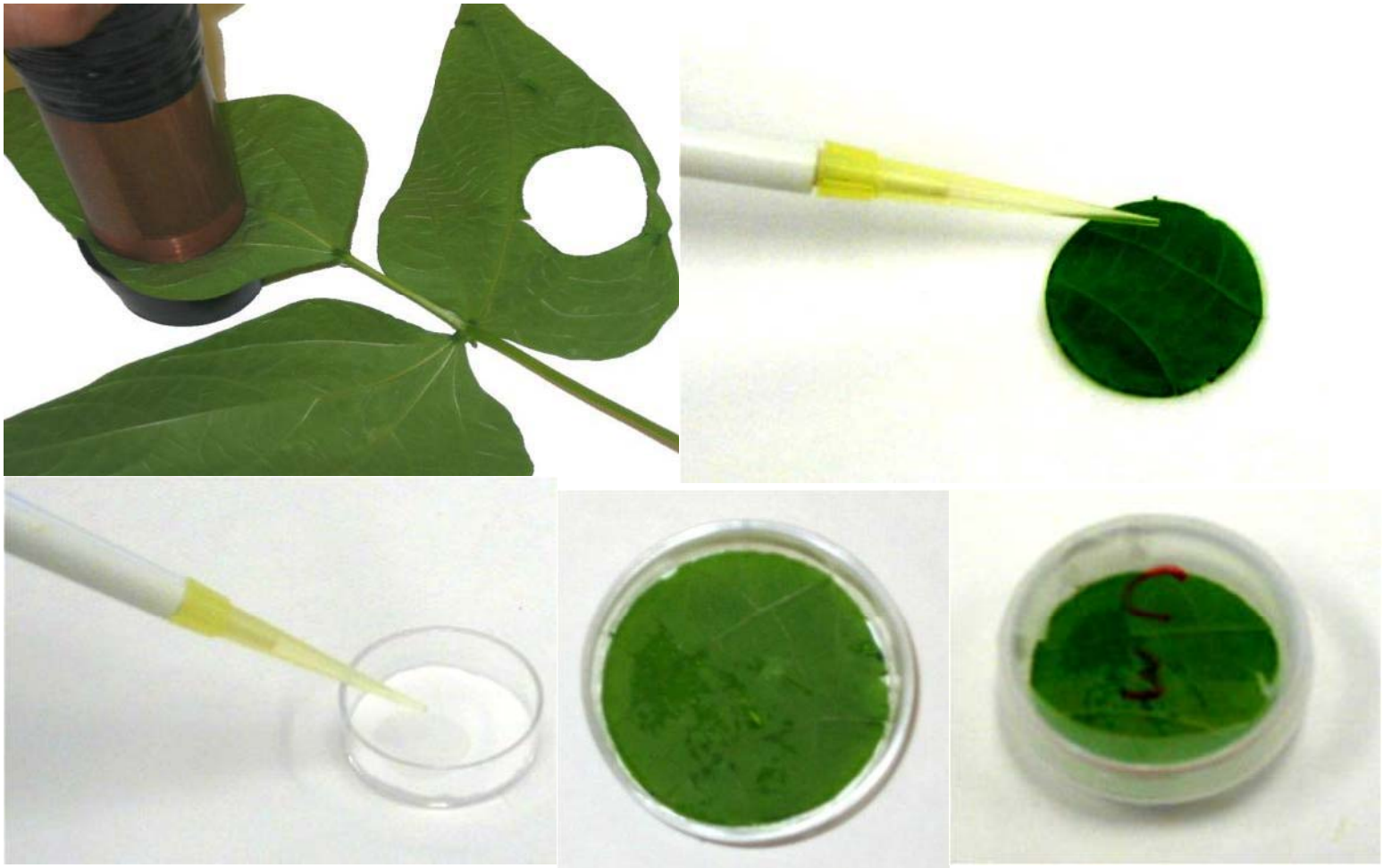


N = Namlea (1996-1999)
B = Batugantung (1996-1999)

Modes of Administration

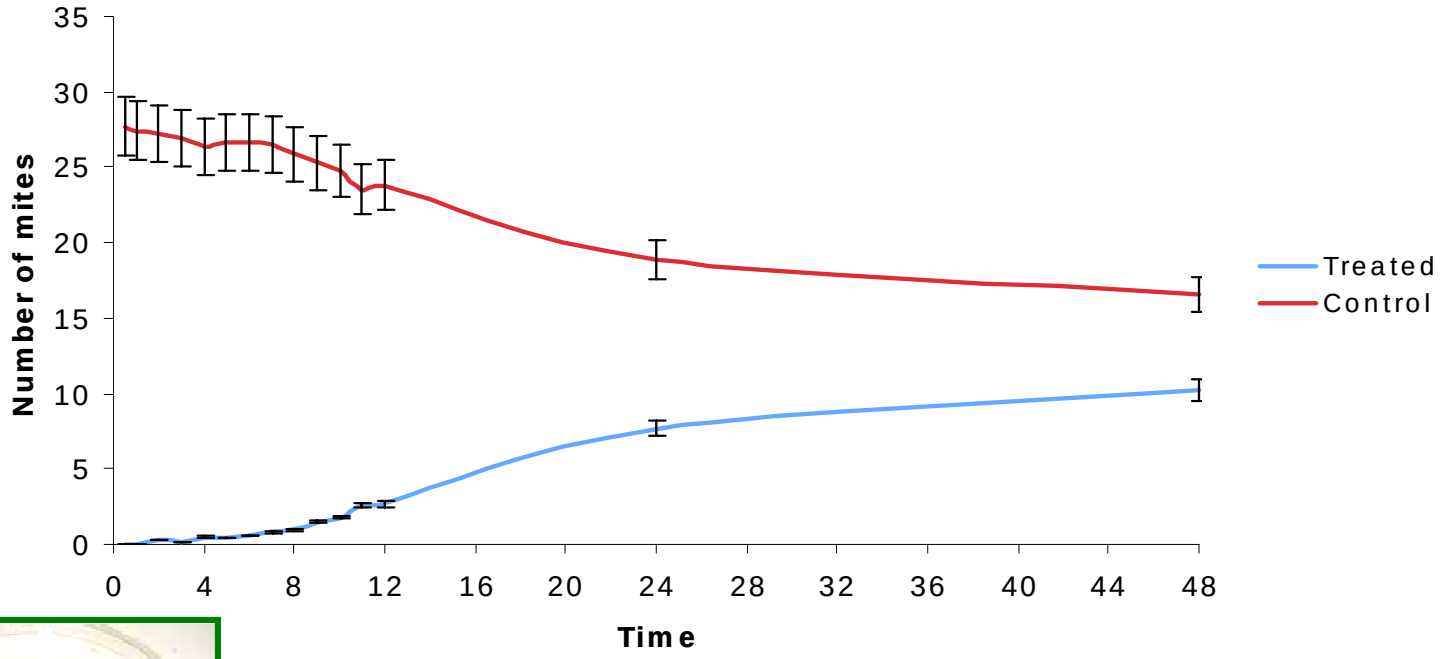
- Direct: topical (fixed dose - precise) or spray (concentration – less precise)
- Surface contact, i.e. applied to substrate (concentration, least precise)
- Residual contact – same as above, but insects introduced at specified times after application of treatment to substrate
- Fumigation – closed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

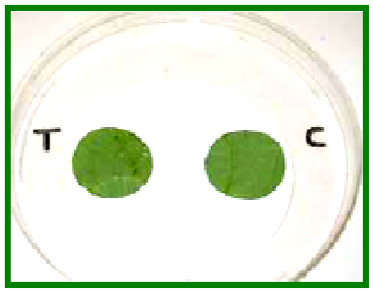
Deterrence & repellence



Repellence of spider mites by rosemary oil

Chioce test bioassay with twospotted spider mite on treated (1% rosemary oil) and non-treated leaf discs



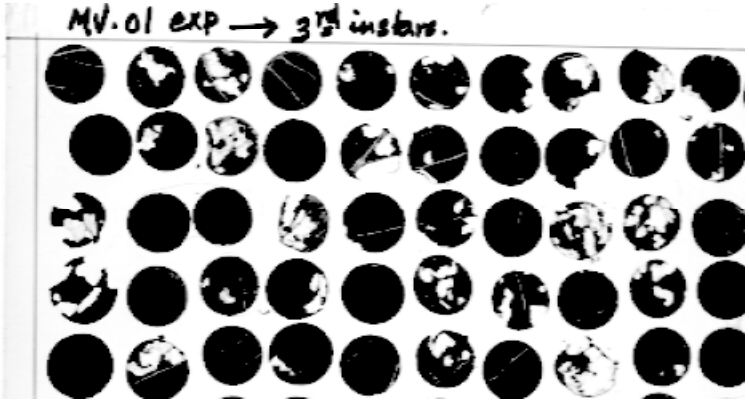


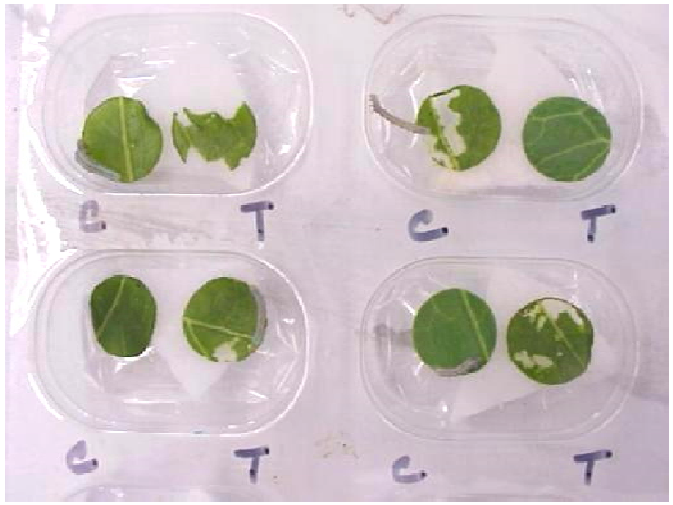
Leaf Disc Choice Bioassay

Feeding deterrence:

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

C= control T= treated leaf discs



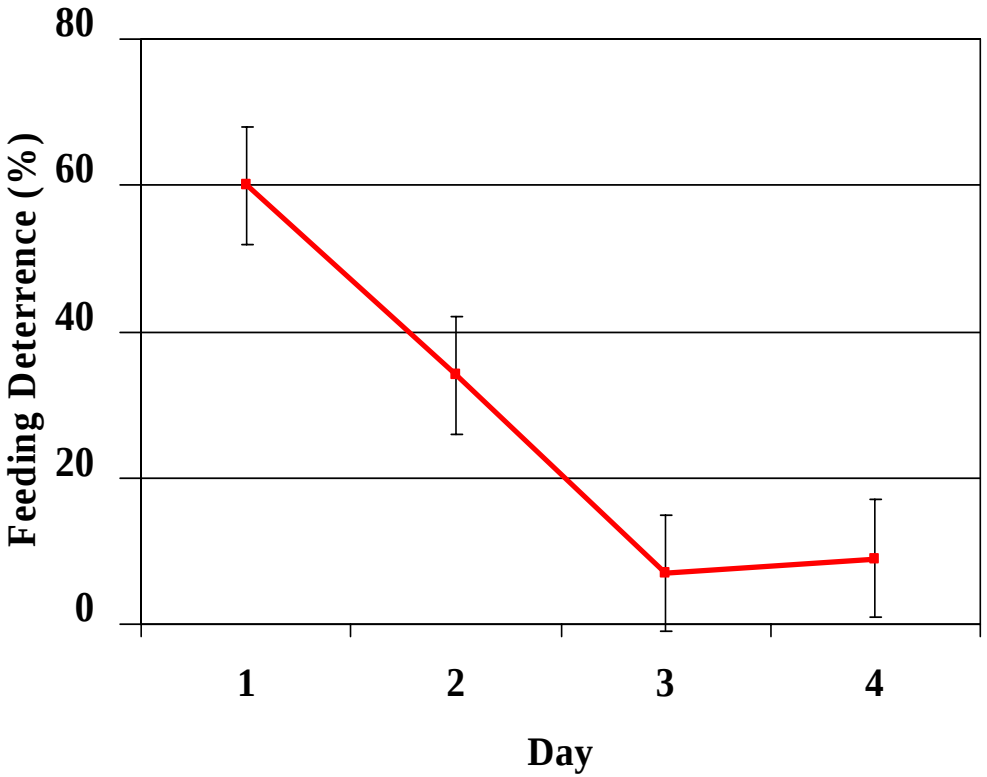


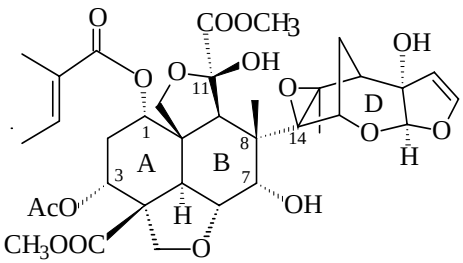
Leaf area measured

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





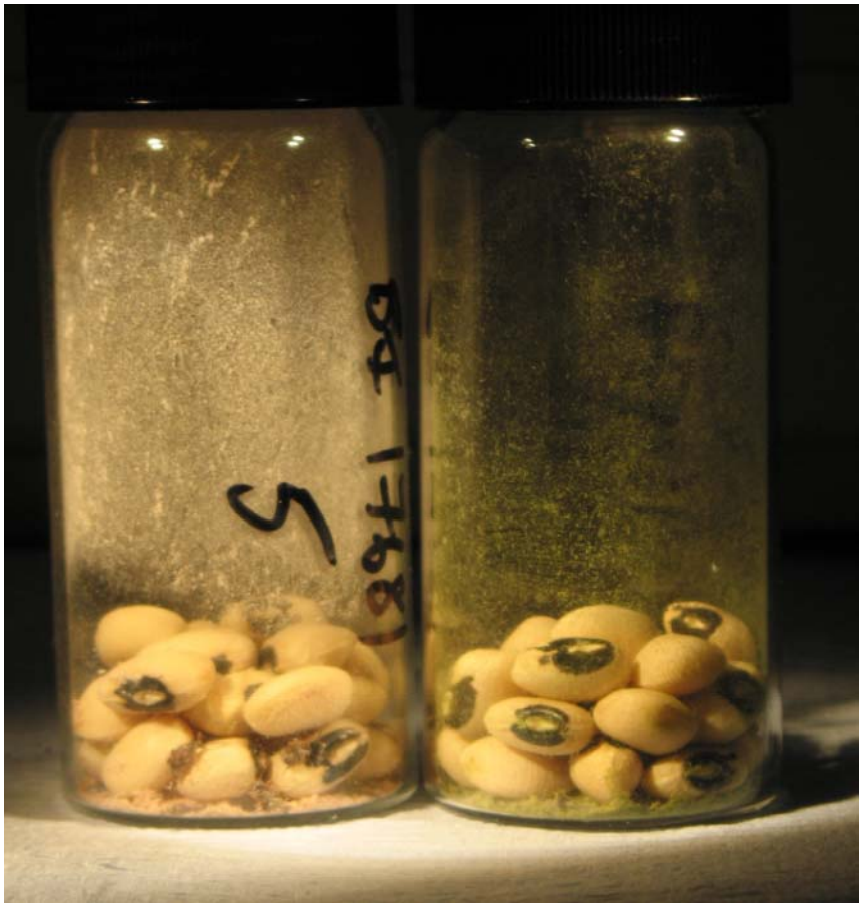
Azadirachtin

1.3 ng/cm²

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

Using small amounts of plant material

Treatments need to be small to enable small quantities of plant chemical to be tested



Insect numbers need to be proportionate to the amount of host material

Diluting an extract

Making a series of dilutions for testing at different concentrations

1 L crude extract, e.g. 5% w/v
1 ml crude into 10 ml
0.1 ml crude into 10 ml
0.01 ml crude into 10 ml

1 L crude extract, e.g. 5% w/v
1 ml crude into 10 ml
1 ml of above into 10 ml
1 ml of above into 10 ml

Both methods will make up the same concentrations.
Which is better?

Using a Gilson pipette

1L	=	1000ml
1ml	=	1000µl
0.1ml	=	100µl

http://www.youtube.com/watch?v=uEy_NGDfo_8

Making solutions of known concentration

Q. You have 3.24mg of a compound of molecular mass 423. How do you make a 1mM stock solution, and then make 400µl of a 370µM solution?

A. Dissolve 3.24mg in 7.66ml solvent to make a 1mM stock solution, (3.24/423 x 1000) then take 148µl and dilute with 252µl to make a 370µM solution (desired conc/present conc x desired final volume)

http://chemistry.about.com/od/lecturenotesl3/a/concentration.htm

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Sample concentration

You have an extract or fraction, how do you concentrate it? Research these sample concentration methods and find out their advantages and disadvantages

Heating block

Freeze-dryer

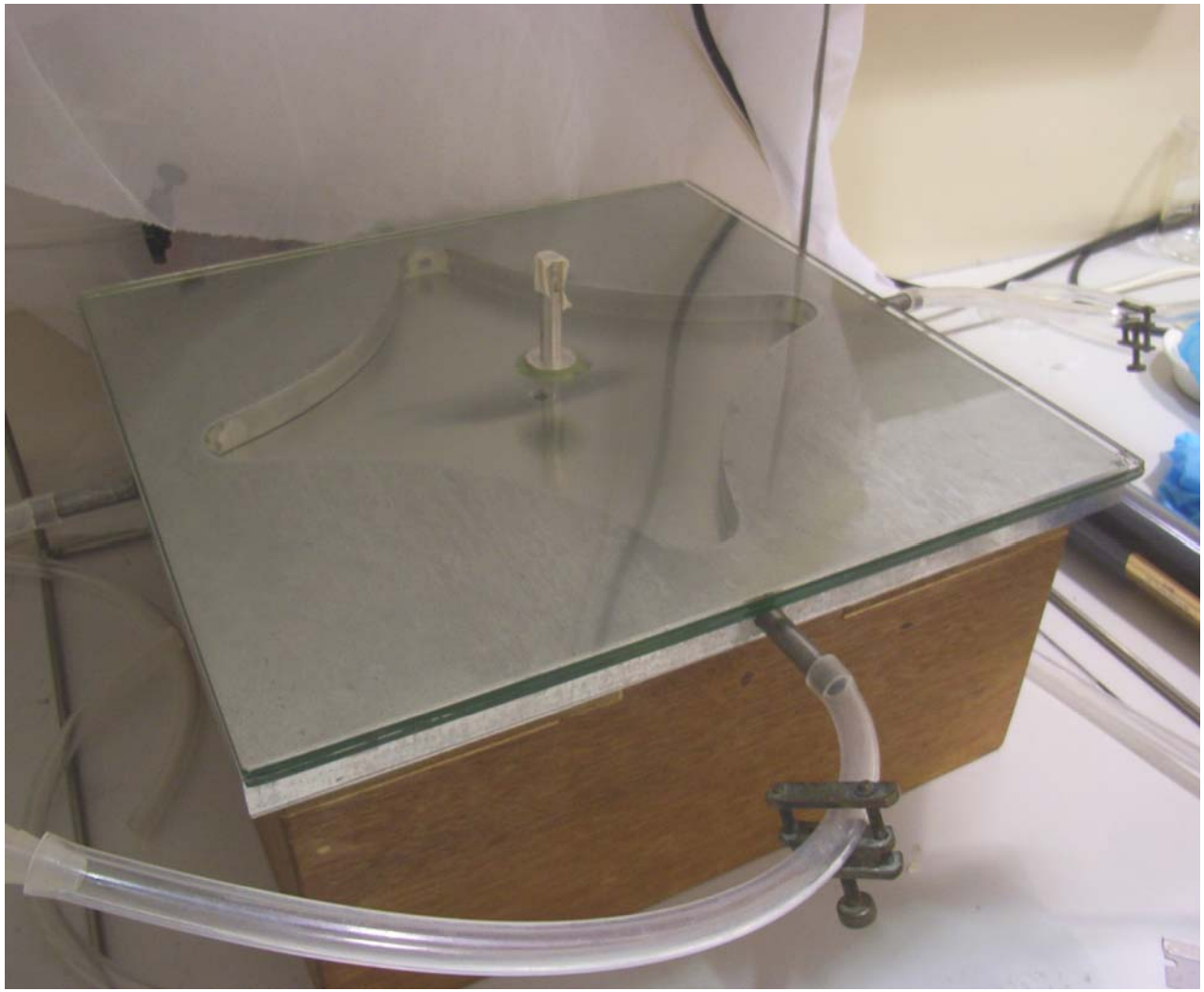
Rotary-evaporator

http://www.youtube.com/watch?v=dKQIdSDso5E

Weighing compounds

Use the balance on the left to weigh out gram quantities, use the one on the right to weigh out mg quantities.

Olfactometers



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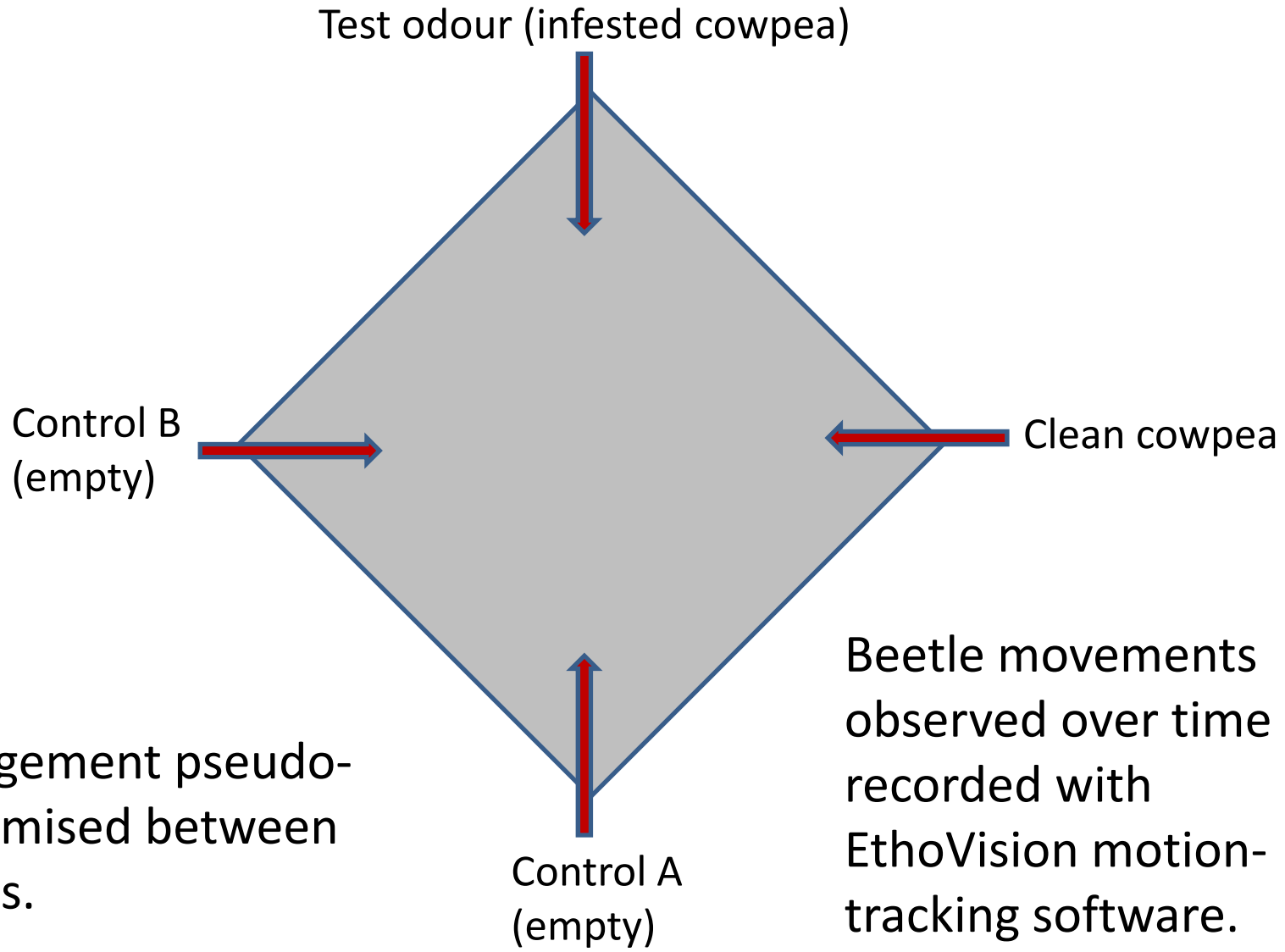
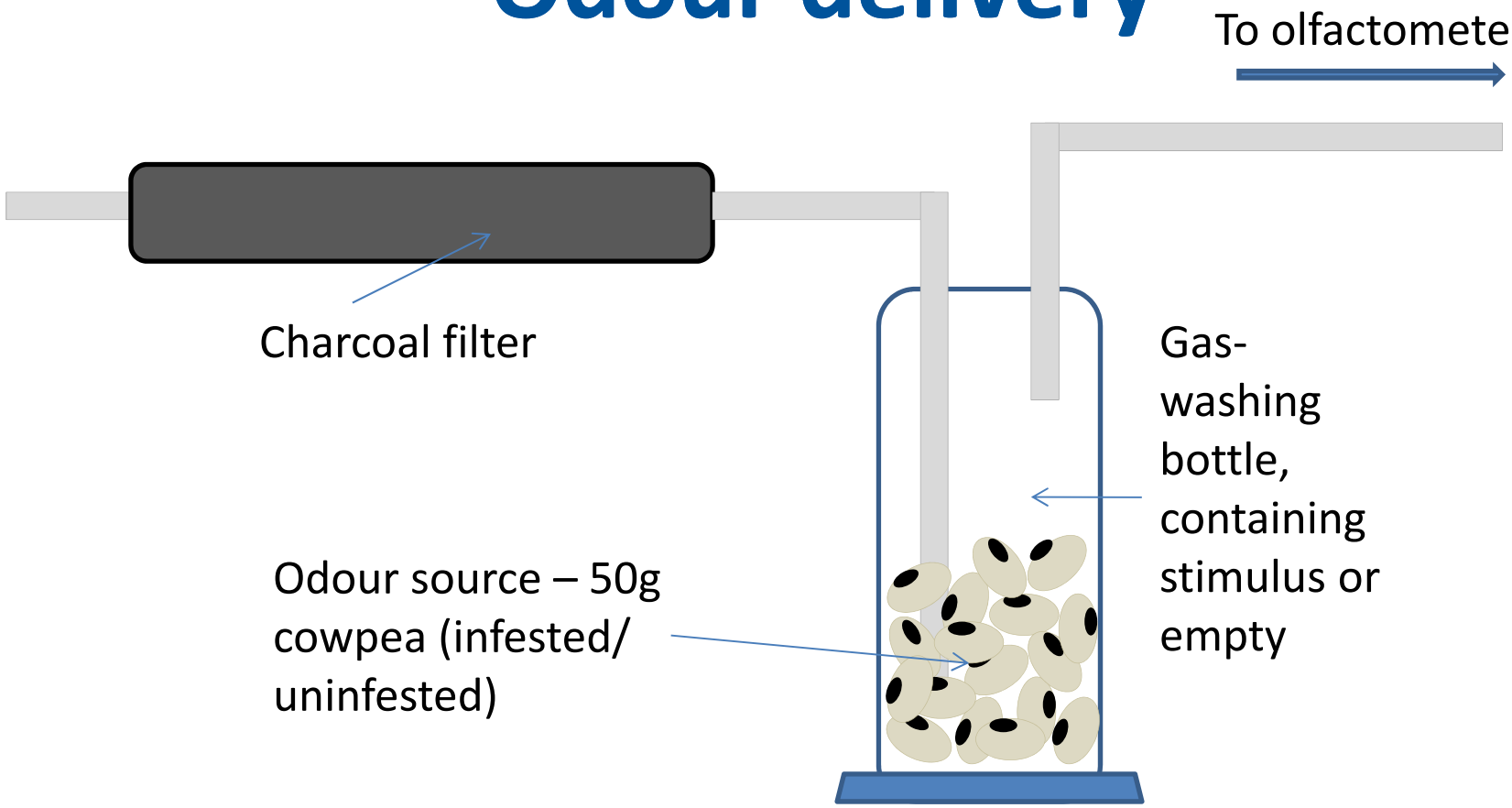
Olfactometers



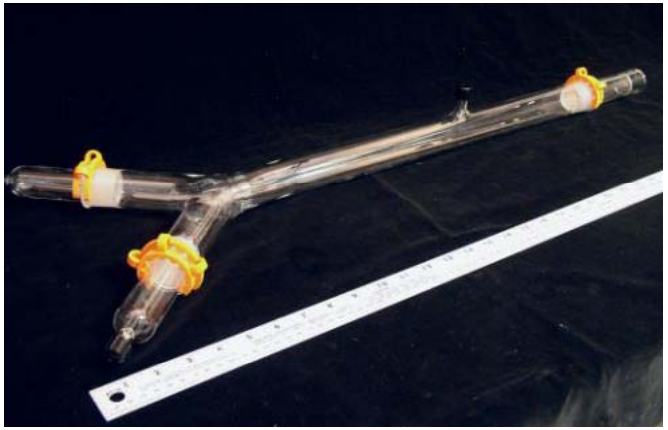
Arrangement pseudo-randomised between insects.

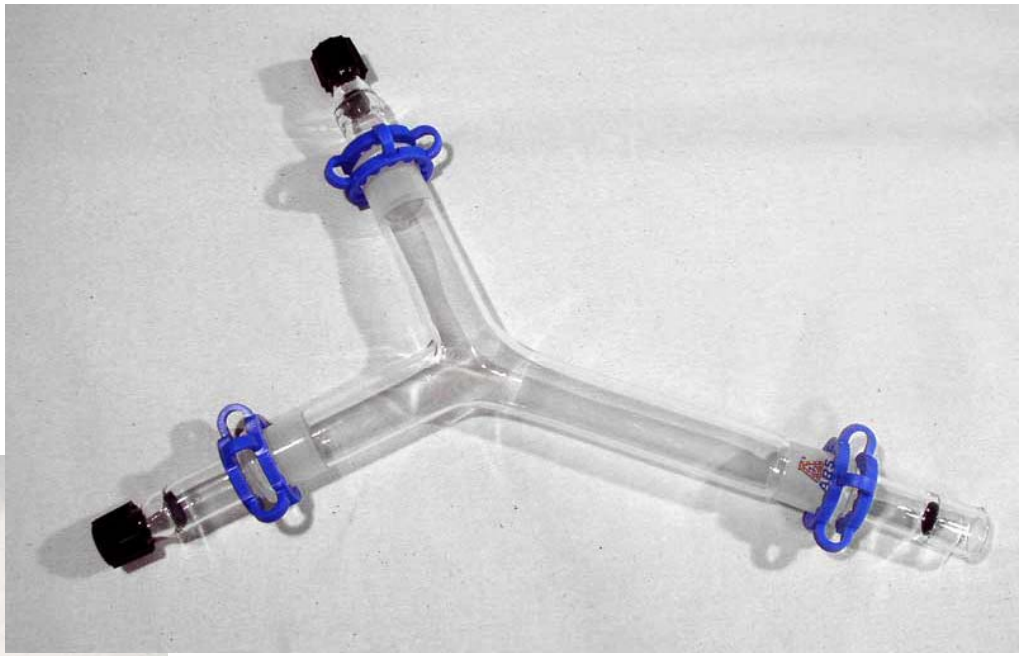
Beetle movements observed over time or recorded with EthoVision motion-tracking software.

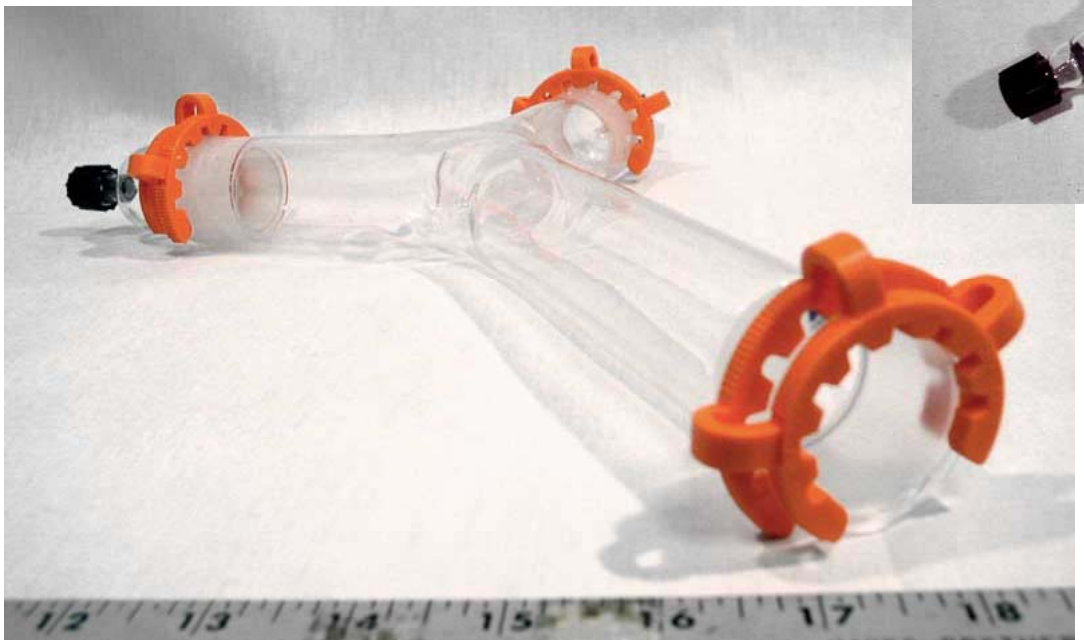
Odour delivery



Y-tube olfactometers



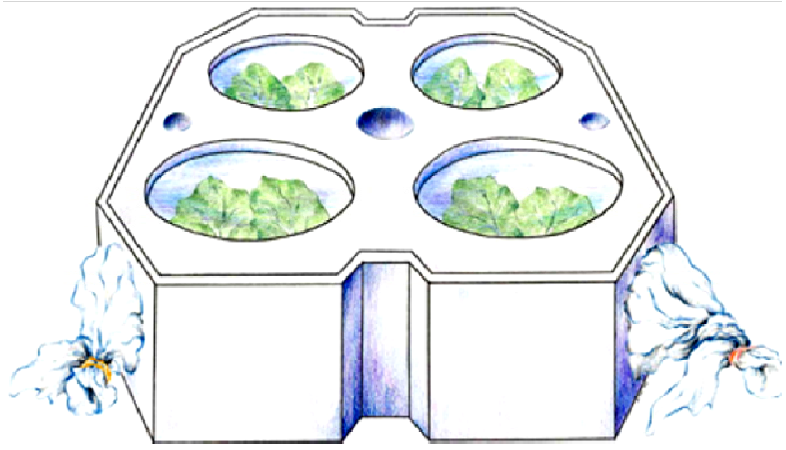




Behavioural bioassays have more inherent biological variability. More replicates are needed to statistically account for natural variation

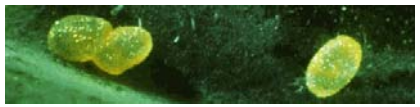
Oviposition

Adults released

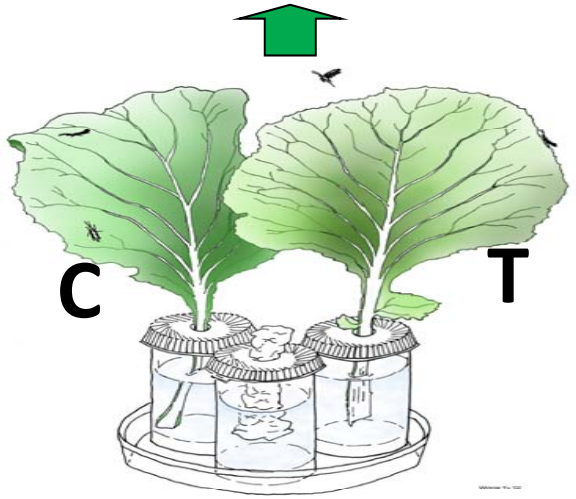


Oviposition Cage with four cells

ODI = 100 (C-T)/(C+T)



Number of eggs counted



Control and treated leaves

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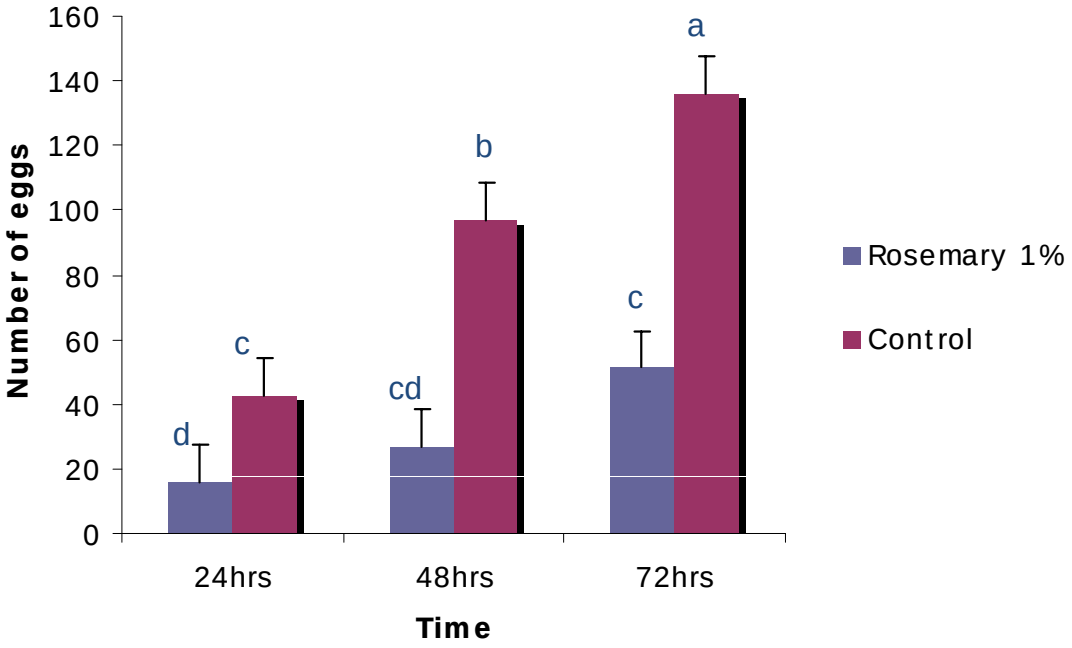
Influence of rosemary oil on whitefly oviposition

Oviposition choice test with greenhouse whitefly on tomato plants treated with 1% rosemary oil



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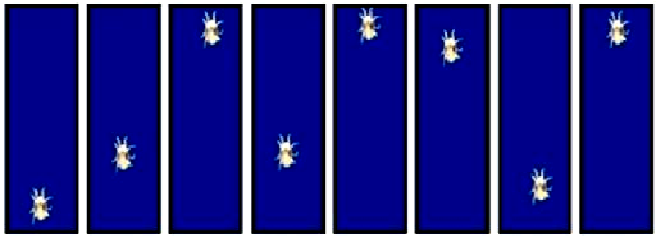
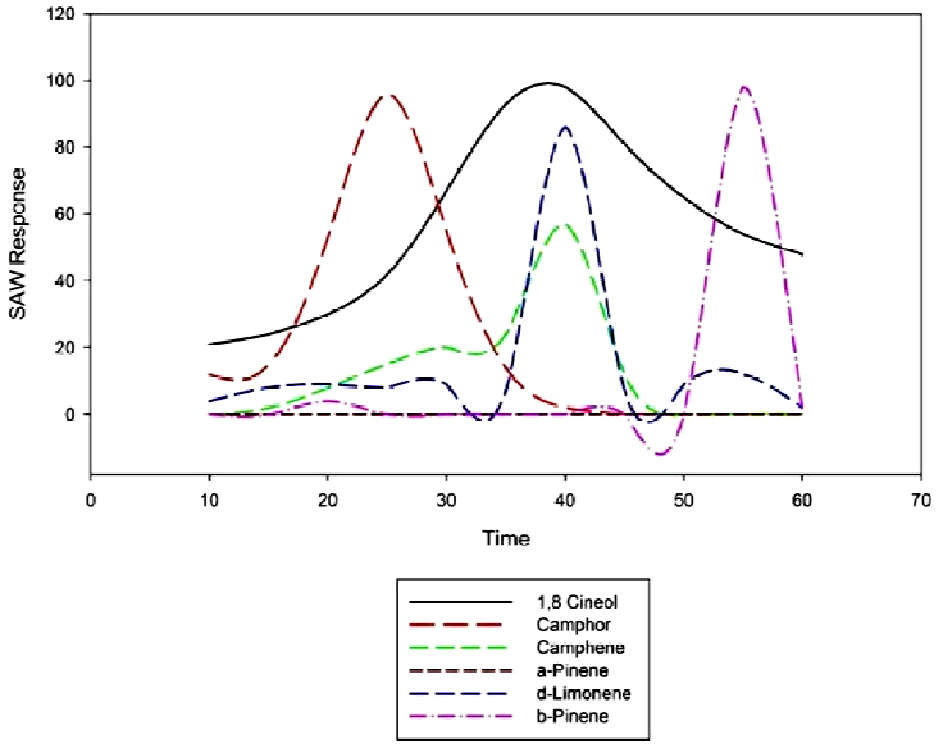
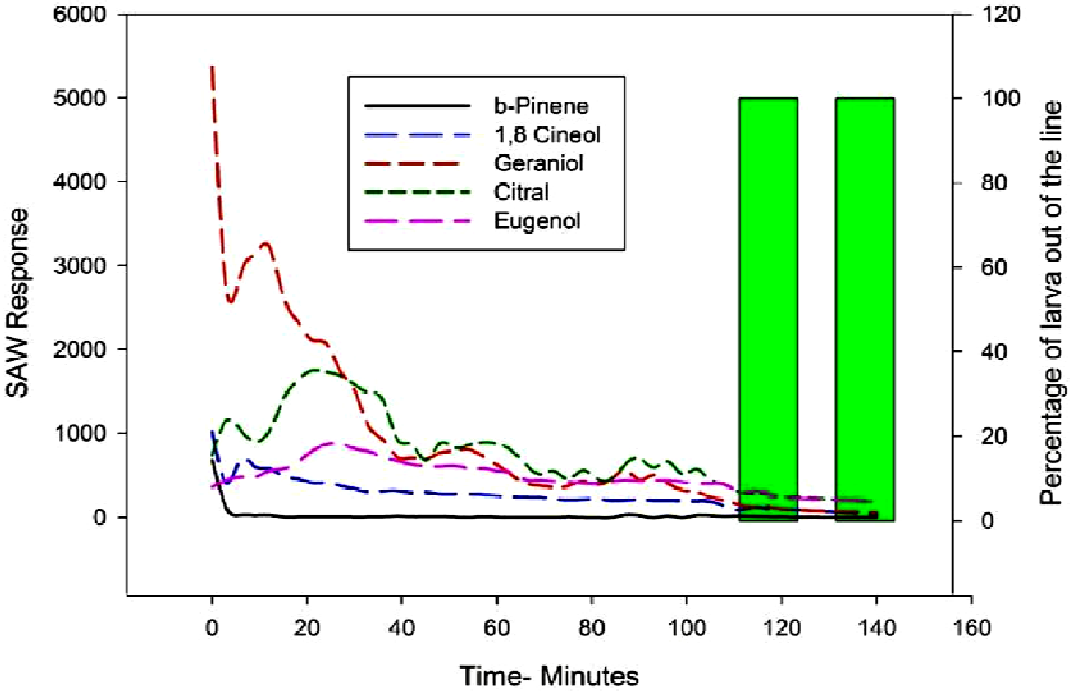
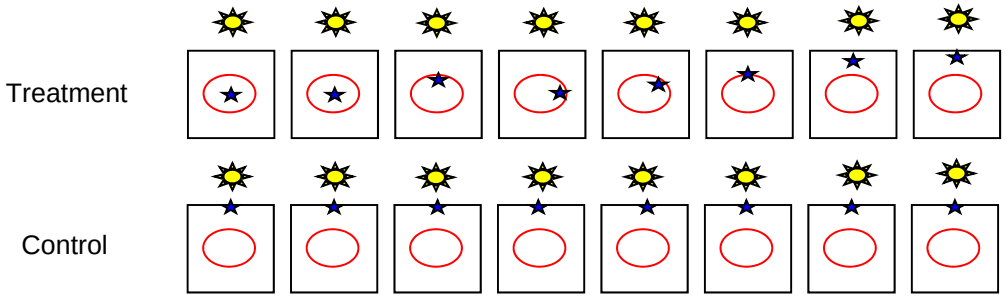
Repellence of two-spotted spider mites by volatile monoterpenes emanating from rosemary oil over 1 hour

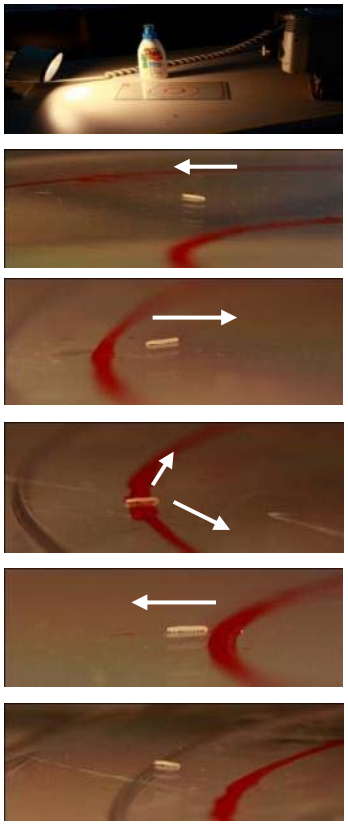




Repellence of oblique banded leafrollers (1st instar) by volatile monoterpenes emanating from rosemary oil over 2+ hours

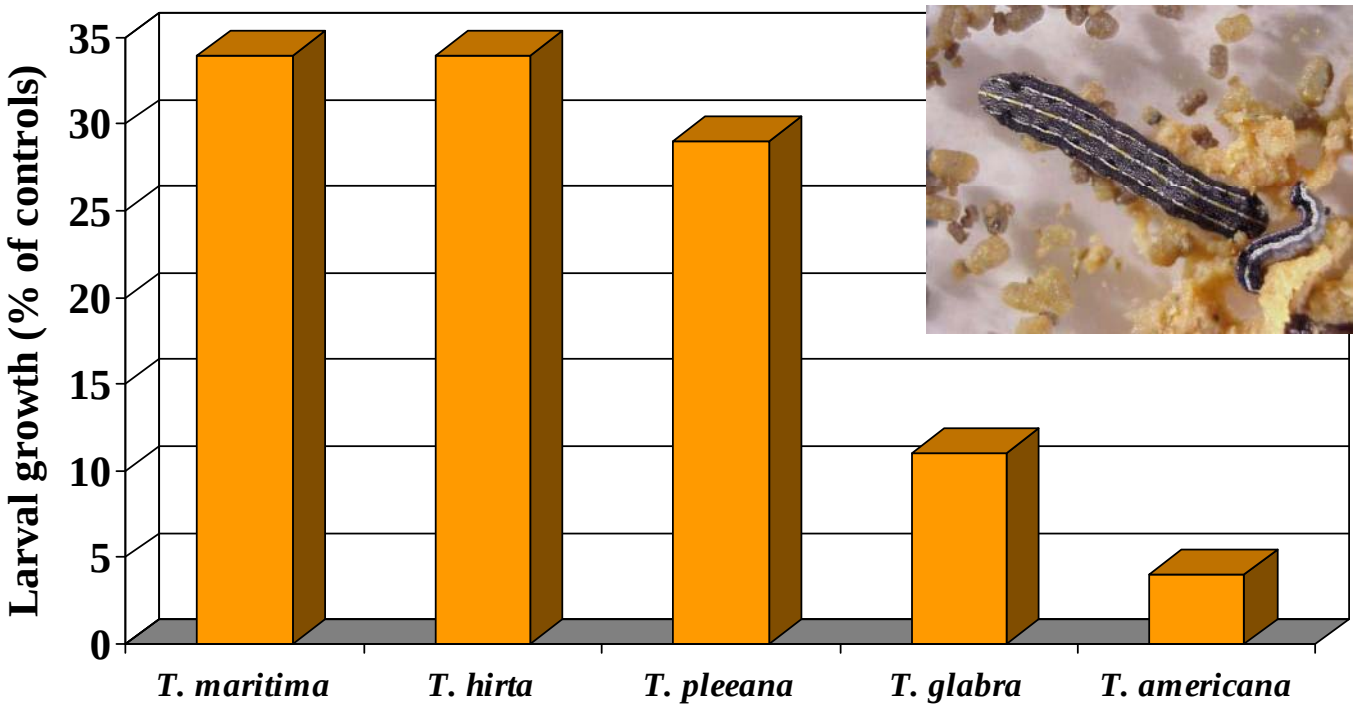






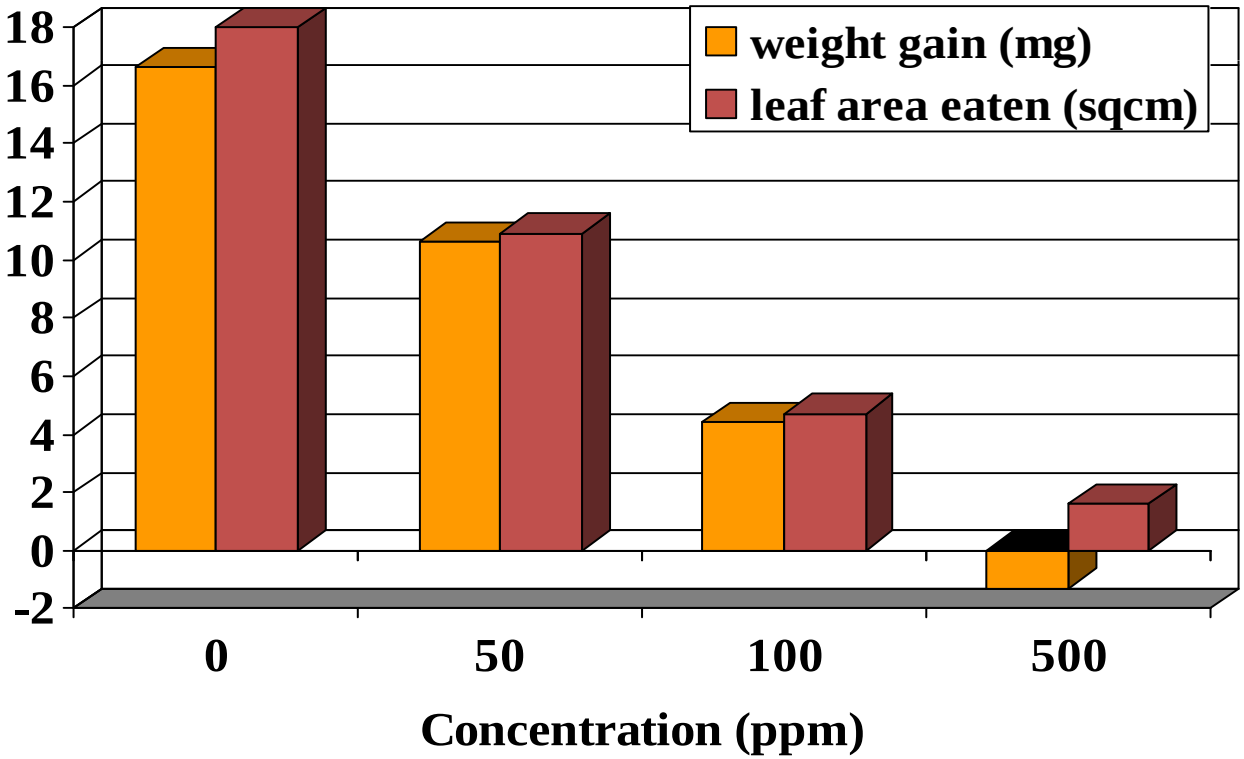
Screening plant extracts in artificial diets for new insecticides: Inhibition of *Spodoptera litura* larval growth by crude twig extracts from Costa Rican *Trichilia* species

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

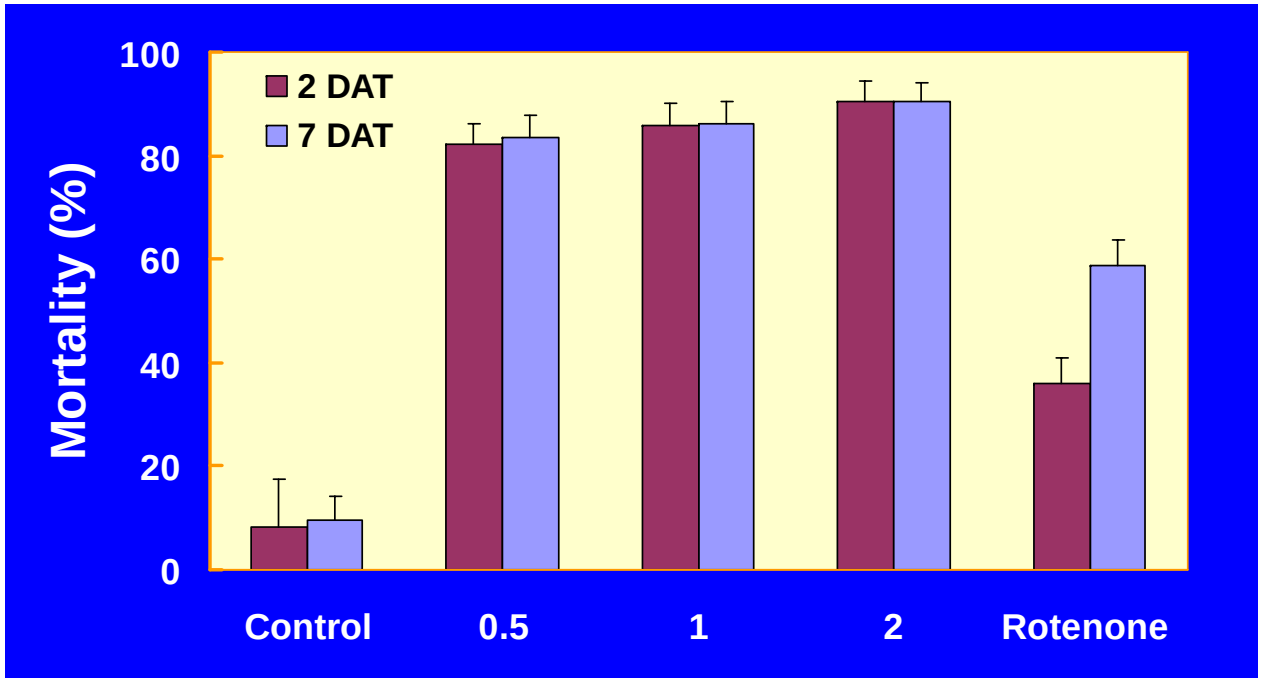


Wheeler *et al.*, *Biochem. Syst. Ecol.* 29: 347 (2001)

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)



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)
- Avoid pseudo-replication - must repeat at different times
- 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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Thank you